TURKISH JOURNAL OF VETERINARY RESEARCH

Vol: 6 No:2
Year 2022
E-ISSN: 2602-3695

TJVR
http://dergipark.gov.tr/tjvr

TURKISH JOURNAL OF VETERINARY RESEARCH

E-ISSN:2602-3695

Year	Volume	Issue / Number
2022	6	2

All articles in this journal are available free of charge from

https://dergipark.org.tr/en/pub/tjvr

TJVR is published biannually

Abstracting & Indexing:

The Turkish Journal of Veterinary Research (TJVR) is abstracted & indexed by the following indexes and platforms (Alphabetical order); *Academic Keys, Asos Index, BASE, CABI Abstract, Crossref, EBSCO, Embase, Entire WEB, EuroPub, Genamics, Google Scholar, Ideal Online, Index Copernicus, IP Indexing, J-Gate, Journal TOCs, Open Access Library, Open AIRE, Pmi to Cite, Research BIB, Scholar Index, TRDizin, Turkiye Atıf Dizini, Udl Edge, Ulrichs Web, World Cat*

TURKISH JOURNAL OF VETERINARY RESEARCH
E-ISSN:2602-3695

Year	Volume	Issue / Number
2022	6	2

ORIGINAL ARTICLES

CASE REPORT

REVIEW

Original Article https://doi.org/10.47748/tjvr.1061413

TJVR 2022; 6 (2): 37-42

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Investigation of the effects of N-acetylcysteine on asprosin hormone activity and liver tissues in rats with experimentally-induced diabetes

Hasan Aydin[1] Alper Yalçin[2] Ahmet Türk[3] Orhan Özdiller[4] Atilla Yoldaş[5] Hikmet Keleş[6] Muhammad Nasir Bhaya[6]

[1] *Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Adıyaman University, Adıyaman, Türkiye*
[2] *Department of Histology and Embryology, Faculty of Medicine, Kahramanmaraş Sütçü Imam University, Kahramanmaraş, Türkiye*
[3] *Department of Histology and Embryology, Faculty of Medicine, Adıyaman University, Adıyaman, Türkiye*
[4] *IVF Center, Department of Obstetrics and Gynecology, Private Medical Park Elazığ Hospital, Elazığ,, Türkiye*
[5] *Department of Anatomy, Faculty of Medicine, Kahramanmaraş Sütçü Imam University, Kahramanmaraş, Türkiye*
[6] *Department of Pathology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye*

Correspondence: *Hasan Aydin: (hasanaydin72@hotmail.com)*

Received: 11.02.2022 Accepted: 27.03.2022

ABSTRACT

Objective: To investigate the possible effect of N-acetylcysteine (NAC) treatment on rat diabetes-induced liver damage and immune reactivity of asprosin hormone in the liver.

Material-Method: Twenty-eight Wistar albino male rats were used in the study. They were separated into 4 groups as Control (n=7), Diabetes (n=7), Diabetes+NAC (n=7), and NAC (n=7). The rats in all groups were dissected after the treatment, and liver tissues were taken for pathological examination. Tissue sections were stained with immunohistochemistry for detecting asprosin immunoreactivity, hematoxylin-eosin and picrosirius red staining were performed to determine the changes in the tissues.

Result: In the microscopical examination of hematoxylin-eosin-stained sections normal histological hepatic tissues were seen in the Control and NAC groups. Pathological examination of liver tissue from diabetic rats showed marked dissociation, fibrosis, degeneration, inflammation, necrosis, Kupffer cells activation, bile duct proliferation, and congestion. A significant decrease in these lesions was observed in the DM+NAC group. Immunohistochemical studies showed that asprosin immunoreactivity was increased in the DM group in a significant manner. Asprosin expression was observed to be significantly reduced in the DM+NAC group in comparison to the DM group.

Conclusion: Our findings show that NAC administration reduces liver damage in diabetic rats and can be used to reduce/eliminate the negative effects of diabetes in rat liver tissue..

Keywords: *Asprosin, Diabetes mellitus, Liver, N-acetylcysteine, Rat*

INTRODUCTION

Nowadays, Diabetes mellitus (DM), which is reported to be a risk factor for Covid-19 disease (Lim et al., 2021; Sarkar et al., 2021; Shang et al., 2021), is common all over the world (WHO, 2021). DM is defined by an increase in blood sugar level (hyperglycemia) caused by insulin hormone not being synthesized or insufficiently synthesized. Insulin deficiency and resistance to insulin production also cause DM and, accordingly, changes in protein, carbohydrate, and lipid metabolism can occur (Hasselbaink et al., 2003). Hyperglycemia causes an increase in hepatic production of glucose and a decrease in peripheral

use of muscle and fat cells due to insufficient glucose intake and an increase in blood glucose levels (Yaman and Doğan, 2016). This situation causes oxidative stress by increasing reactive oxygen species (King and Loeken, 2004). It has been reported that as a result of oxidative stress caused by hyperglycemia, marked swelling, chromatin concentration, apoptotic bodies, and necrosis occur in hepatocytes (Manna et al., 2010). In other words, DM causes different structural and functional disorders that influence the metabolism of glycogen and lipid in the liver (Sanchez et al., 2000). Degenerated hepatocytes in form of cloudy swelling, vacuolar or fatty changes were observed in rats with STZ-induced diabetic liver injury during histopathological examination (Al-Ani et al., 2017; Sharkawi et al., 2020). In addition, severe dilatation and congestion of the central vein along with diffuse Kupffer cells proliferation (Sharkawi et al., 2020), and inflammatory cell infiltration (Begum and Mahboob, 2020) were observed. These changes can lead to liver fibrosis and are similar to the modifications observed in the human liver. Asprosin is a fasting-induced glycogenic protein that targets the liver to increase glucose release and plasma glucose levels synthesized from the C-terminal portion of pro-fibril. The main release site of asprosin is white adipose tissue and it circulates in the plasma at nanomolar levels. Studies on this newly discovered hormone have shown that asprosin increases the insulin resistance in humans and mice, and is thought to be linked to diabetes and metabolic syndrome (Romere et al., 2016). It has been observed that the balance between the antioxidant defense system and free radicals is disrupted by the effect of pro-oxidant and oxidant substances, it has paved the way for the development of oxidative stress in patients with diabetes (Rahal et al., 2014). N-acetylcysteine (NAC) is a mucolytic drug that contains a sulfhydryl group. By interacting with disulfide bonds in mucus, NAC breaks down mucoproteins and reduces the viscosity of mucus. Mucolytic drugs cause an increase in the amount of cysteine in the cell. It has been discovered that the increased cysteine in the cell has antioxidant properties by increasing glutathione synthesis over time (Ivanova et al., 2020; Muftakhov and Shchukin, 2020). Some antioxidants have been used against the negative effects of diabetes on various tissues and positive results have been reported at different levels (Bajaj and Khan, 2012).

In the present study, considering the positive effects of antioxidants, the effects of NAC to reduce oxidative damage and possible release sites of asprosin hormone other than white adipose tissue have been investigated.

MATERIALS and METHODS

The ethical guidelines for the care of laboratory animals (Kahramanmaraş Sütcü İmam University (KSÜ) Faculty of Medicine) Animal Experiments Local Ethics Committee (Ethics committee dated December 06, 2017, session number 2017/05, decision number 02) were followed throughout the experimental period. The rats were kept at 22-25°C room temperature for 12 hours of light and 12 hours of darkness and were fed in specially constructed cages. Standard rat food was given to all groups and add-libitum water was supplied, and the animals were cared for on daily bases by cleaning their bottoms. Twenty-eight male Albino rats (Wistar strain), 8-10 weeks old, weighing 200-210 g, were separated into 4 groups with 7 rats in each group.

Preparation of Streptozotocin (STZ)

STZ was dissolved in distilled water and then added to one drop of 0.1 M citrate buffer to obtain STZ solution with a pH of 4.5 and stored at 4°C.

Groups

Group I (Control group); No action was taken throughout the experiment period of 8 weeks. Glucose levels and body weights were measured and noted at the beginning and end of the study.

Group II (Diabetic group); A single dose of streptozotocin (STZ) at 50 mg/kg (Gajdosik et al., 1999) was administered intraperitoneally (i.p.). Those with blood sugar levels above 250 mg/dL in blood taken from the tail vein after 72 hours were approved to have diabetes, and glucose levels and body weights were measured and recorded at the beginning and end of the study (Shanmugam et al., 2011).

Group III (Diabetes+NAC group); A single dose of STZ at 50 mg/kg was administered i.p. After 72 hours, those with a blood sugar level above 250 mg/dL in the blood were taken from the tail vein was approved as diabetic. After inducing experimental diabetes, 100 mg/kg NAC i.p. was administered (Hong et al., 2009) every day for 8 weeks (Mahajan et al., 2020).

Group IV (NAC group); rats were treated with NAC at 100 mg/kg (i.p.) on daily basis for 8 weeks. Glucose levels and body weights were measured

and recorded at the beginning and at the end of the study. Rats in all groups were anesthetized by i.p. administration of ketamine (75 mg/kg)+xylazine (10 mg/kg) and decapitated.

Histopathological Method

The liver tissues were collected and fixed with 10% buffered formaldehyde, followed by histological follow-up series and embedded in paraffin. Tissue sections of 5 μm thickness were obtained from paraffin blocks and were stained with hematoxylin-eosin, picrosirius red, and also immunohistochemical techniques were applied for asprosin.

The histoscore, that reflects the prevalence of asprosin expression on the liver tissue was calculated according to Yalcin et al. (2017). Rating scale: 0.1, < 25%; 0.4, 26–50%; 0.6, 51–75%; 0.9, 76–100%, and intensity of expression: 0, unstained; 0.5, little staining; 1, some staining; 2, moderate staining; 3, strong staining. The histoscore = prevalence x intensity.

Immunohistochemical Analyzes

Sections taken from paraffin blocks were used to determine the immune reactivity of asprosin. For this purpose, 5 μm thick sections were transferred to Poly-L-Lysine slides. These slides were deparaffinized with xylene and cleared with graded alcohol. Endogenous enzyme activity was quenched by treating the tissues with 10% hydrogen peroxide solution for 10 minutes. After that, the tissues were boiled in a microwave oven (750W) for 7+5 minutes for antigen retrieval. It was incubated with primer (anti-asprosin antibody, FNab09797, Fine Test, China) for 60 minutes after treatment with Ultra V Block solution to prevent background stain. Slides were treated with secondary antibody (30 minutes), Streptavidin Alkaline Phosphatase (TS–125-HR, Lab Vision Corporation, USA) (30 minutes), and Fast Red Substrate System. The tissues that were counterstained with Mayer's hematoxylin were passed through PBS (Phosphate Buffered Saline) solution and distilled water and covered with a suitable sealing solution (Su-Ming et al., 1981). The slides were evaluated and photographed under a light microscope.

Statistical Methods

Statistical analysis of histopathological and immunohistochemical findings was performed using the SPSS 25.0 version (SPSS for Windows®) package program. Normal distribution analysis of the obtained data was performed using the Kolmogrov-Smirnov test. In addition, the homogeneity of variances was controlled by the Levene test. Normally distributed data were first evaluated using the one-way ANOVA test. Next, post-hoc Duncan analysis was performed to determine the differences between groups. Results were reported as mean±standard error ($\bar{X}$ ± SE) and $p<0.05$ was approved significant (Özdamar, 2004).

RESULTS

Histopathological Findings

During the microscopical examination of hematoxylin-eosin (HE) stained sections normal histological hepatic tissues were seen in the Control (Figure 1a) and NAC (Figure 1b) groups. In comparison to the Control group, markedly dissociated Remark cords, congestion, degenerated and necrotic hepatocytes, kupffer cell activation, fibrosis, scarce inflammation, and bile duct proliferation were observed in the DM group (Figure 1c). In comparison to the DM group, a marked decrease of these lesions was observed in the DM+NAC group (Figure1d) ($p<0.001$) (Table 1).

Table 1. Histopathological findings *

	Control	DM	NAC	DM+NAC
Congestion	0.33 ± 0.21^{a}	2.66 ± 0.21^{c}	0.83 ± 0.16^{ab}	1.33 ± 0.21^{b}
Fibrosis	0.16 ± 0.16^{a}	2.16 ± 0.16^{c}	0.33 ± 0.21^{a}	1.33 ± 0.21^{b}
Degeneration	0.16 ± 0.16^{a}	2.00 ± 0.00^{c}	0.33 ± 0.21^{a}	1.33 ± 0.21^{b}
Inflammation	0.16 ± 0.16^{a}	1.66 ± 0.21^{c}	0.50 ± 0.22^{ab}	0.83 ± 0.16^{b}
Necrosis	0.00 ± 0.00^{a}	1.33 ± 0.21^{b}	0.33 ± 0.21^{a}	0.33 ± 0.21^{a}
Kupffer cell activation	0.16 ± 0.16^{a}	2.33 ± 0.21^{c}	1.83 ± 0.16^{bc}	1.33 ± 0.21^{b}
Bile duct proliferation	0.16 ± 0.16^{a}	2.66 ± 0.21^{c}	0.50 ± 0.22^{a}	1.66 ± 0.21^{b}
Dissociation	0.33 ± 0.21^{a}	2.00 ± 0.25^{c}	0.83 ± 0.16^{ab}	1.33 ± 0.21^{b}
	p<0.001	p<0.001	p<0.001	p<0.001

* The values represent the mean ±SE; $^{a-c}$ Values in rows without common superscripts differ significantly, $p<0.01$ (One-way ANOVA post-hoc Duncan Test).

Table 2. Immunohistochemical findings *

	Control	DM	NAC	DM+NAC
Asprosin	0.33±0.21[a]	2.66±0.21[c]	0.83±0.16[a]	1.50±0.22[b]
	p<0.001	p<0.001	p<0.001	p<0.001

* The values represent the mean ±SE; [a-c] Values in rows without common superscripts differ significantly, p<0.01 (One-way ANOVA post-hoc Duncan Test).

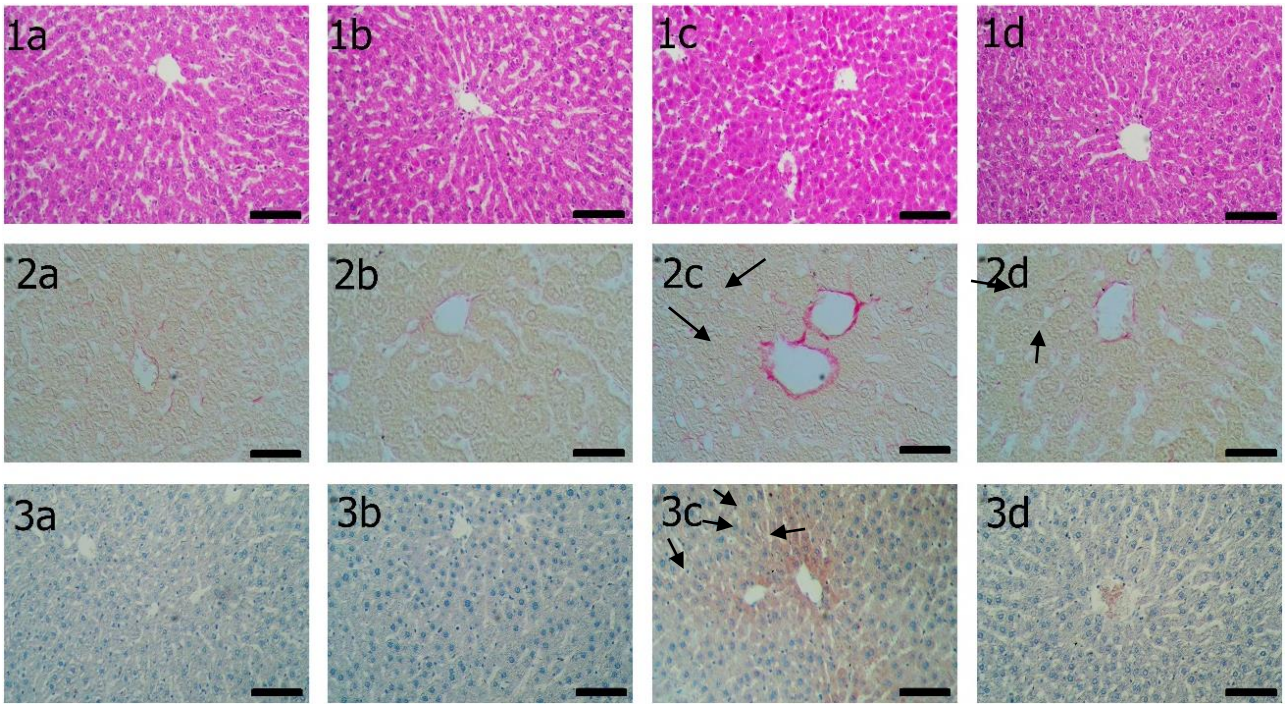

Figure 1. 1a-d; HE stained liver. Scale bars represent 200 μm. 1a- Control group, normal histological appearance of the liver. 1b- NAC group, normal histological appearance of the liver. 1c- DM group, dissociated Remark cords, degenerated hepatocytes, and inflammatory cells in the liver. 1d- DM+NAC group, compared to the DM group, pathological lesions are significantly less. **2a-d;** Picrosirius red staining for collagen in liver tissue. Scale bars represent 200 μm. 2a- Control group, no collagen staining. 2b- NAC group, no collagen staining. 2c- DM group, specific collagen staining (arrows). 2d- DM + NAC group, less specific staining of collagen (arrows). **3a-d;** Asprosin expression in liver tissue. Scale bars represent 200 μm. 3a-Control group, no immunoreactivity of asprosin. 3b-NAC group, no immunoreactivity of asprosin. 3c- DM group, highly pronounced immunoreactivity of asprosin (arrows). 3d- DM + NAC group, indistinct immunoreactivity of asprosin.

Collagen Content

In picrosirius red-stained liver tissues examination there were no histopathologic findings in the Control (Figure 2a), and NAC (Figure 2b) groups in terms of collagen content (p>0.05). Notably, significantly increased collagen fibers staining was determined in liver tissue of the DM group (Figure 2c) (p<0.05). In the DM+NAC group (Figure 2d), significantly decreased collagen content was observed in comparison to the DM group (p<0.05). These findings provide compelling evidence for the therapeutic use of NAC in DM-induced liver fibrosis.

Immunohistochemical Findings

Similar asprosin expressions were determined in the Control (Figure 3a) and NAC (Figure 3b) groups. Asprosin expression was significantly upregulated in the DM group (Figure 3c) in comparison to the Control group (p<0.001) while significantly reduced in the DM+NAC group (Figure 3d) in comparison to the DM group (p<0.001) (Table 2).

DISCUSSION

In the current study, DM led to severe histopathological damage in liver tissues described as dissociation and hemorrhages, degenerated

hepatocytes, and inflammatory cells infiltration. These findings are consistent with the previously reported data (Alqasim et al., 2017; Atta et al., 2020; Samadi-Noshahr et al., 2021) on liver injury induced by DM. In our study, the pathological examination of liver tissue from diabetic rats clearly indicates an association between liver damage and DM.

Oxidative stress has been reported to cause diabetic pathology, including diabetic liver injury (Arthur, 2000). The hepatocytes are injured and Kupffer cells are activated during oxidative stress. The inflammatory cells along with platelets release growth factors and cytokines that lead to fibrogenesis (Friedman, 2000). Fibrosis is the accumulation of connective tissue by the liver in response to liver injury (Komolkriengkrai et al., 2019). It has been reported that collagen accumulation is an initiating factor that triggers the formation of fibrosis in the liver tissue (Mabuchi et al., 2004). Concerning diabetic experiments, diabetes has been reported to increase collagen deposition in the liver tissue (Lo et al., 2011; Yang-en et al., 2016; Komolkriengkrai et al., 2019; Samadi-Noshahr et al., 2021). In our study, to evaluate fibrosis development in the liver, tissue sections were stained with picrosirius red. In line with previous reports (Lo et al., 2011; Yang-en et al., 2016; Komolkriengkrai et al., 2019), DM led to a significant increase in liver collagen content. However, collagen was observed to reduce after NAC administration in diabetic rats, implying that the liver tissue reorganization regained its normal histologic appearance. This restorative effect of NAC on liver tissue could be attributed to its anti-oxidative (Lei et al., 2012; Yalçın and Gürel, 2021) and anti-fibrotic effects (Morsy et al., 2012; Nagai et al., 2014).

Previous research has shown a linear link between blood glucose levels and asprosin hormone levels in experimental studies on animals (Romere et al., 2016). Accordingly, in our experimental study on animals, asprosin levels were found to be below in the livers of the control and NAC-treated groups compared to the DM group in immunohistochemical analyzes. In the experimental study conducted by Kocaman and Kuloğlu (2020), tissue asprosin hormone expressions decreased in group livers that developed diabetes with streptozotocin, unlike our study. This difference is because of the cytoprotective feature of asprosin. We think that the amount of asprosin increases in order to protect the tissue in the early stages of diabetes and may decrease in the late period of diabetes (after the 8 months) because of burnout. Therefore, long-term experimental studies are required to indicate the effect of asprosin on the liver with diabetes. Rosa et al. (2018) reported the beneficial effect of NAC on the liver due to its anti-oxidative properties against DM, it is thought that asprosin expression was decreased in the liver tissue of the DM+NAC group in the current study.

CONCLUSION

Our findings show that NAC administration reduces liver damage in diabetic rats and can be used to reduce/eliminate the negative effects of diabetes on rat liver tissue.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

Author's Contributions: HA, and AY designed the study. AY, AT, OÖ and AY performed experimental animal study. HA performed statistical analysis. HK, and MNB performed pathological analysis. HA, AY AT, AY, HK, and MNB participated in drafting and revising the manuscript.

HA: Hasan AYDIN, AY: Alper YALÇIN, AT: Ahmet TÜRK, OÖ: Orhan ÖZDİLLER, AY: Atilla YOLDAŞ, HK: Hikmet KELEŞ, MNB: Muhammad Nasir BHAYA

REFERENCES

Al-Ani IM, Abired AN, Mustafa BE, Wahab EN, Azzubaidi MS. Effect of flaxseed extract on the liver histological structure in streptozotocin induced diabetic rats. Int Med J Malaysia. 2017; 16(1):91-98.

Alqasim AA, Noureldin EE, Hammadi SH, Esheba GE. Effect of melatonin versus vitamin D as antioxidant and hepatoprotective agents in STZ-induced diabetic rats. J Diabetes Metab Disord. 2017; 16(1):1-8.

Arthur MJ. Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. Am J Physiol Gastrointest Liver Physiol. 2000; 279(2):245-249.

Atta AH, Saad SA, Atta SA, *et al*. Cucumis sativus and Cucurbita maxima extract attenuate diabetes-induced hepatic and pancreatic injury in a rat model. J Physiol Pharmacol. 2020; 71(4):507-518.

Bajaj S, Khan A. Antioxidants and diabetes. Indian J Endocrinol Metab. 2012; 16(2):267-271.

Begum Q, Mahboob T. Silver nanoparticles protects streptozotocin-induced hepatotoxicity: A biochemical and histopathological approach Int J Nano Rech. 2021; 4(1):1-9.

Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem. 2000; 275(4):2247-2250.

Gajdosik A, Gajdosikova A, Stefek M, Navarova J, Hozova R. Streptozotocin-induced experimental diabetes in male Wistar rats. Gen Physiol Biophys. 1999; 18:54-62.

Hasselbaink DM, Glatz JF, Luiken JJ, Roemen TH, Van der Vusse GJ. Ketone bodies disturb fatty acid handling in isolated cardiomyocytes derived from control and diabetic rats. Biochem J. 2003; 371(3):753-760.

Hong RT, Xu JM, Mei Q. Melatonin ameliorates experimental hepatic fibrosis induced by carbon tetrachloride in rats. World J Gastroenterol. 2009; 15(12):1452-1458.

Ivanova OM, Drozhzhyna GI, Troichenko LF, Vit VV, Abramova AB, Lotosh TD. Efficacy of topical N-acetylcysteine as a part of multicomponent treatment for severe dry eye syndrome. Oftalmol Zh. 2020; 494(3):3-8.

King GL, Loeken MR. Hyperglycemia-induced oxidative stress in diabetic complications. Histochem Cell Biol. 2004; 122(4):333-338.

Kocaman N, Kuloğlu T. Expression of asprosin in rat hepatic, renal, heart, gastric, testicular and brain tissues and its changes in a streptozotocin-induced diabetes mellitus model. [published online ahead of print June 5, 2020]. Tissue Cell. doi: 10.1016/j.tice.2020.101397.

Komolkriengkrai M, Nopparat J, Vongvatcharanon U, Anupunpisit V, Khimmaktong W. Effect of glabridin on collagen deposition in liver and amelioration of hepatocyte destruction in diabetes rats. Exp Ther Med. 2019; 18(2):1164-1174.

Lim S, Bae JH, Kwon HS, Nauck MA. COVID-19 and diabetes mellitus: from pathophysiology to clinical management. Nat Rev Endocrinol. 2021; 17(1):11-30.

Lei S, Liu Y, Liu H, Yu H, Wang H, Xia Z. Effects of N-acetylcysteine on nicotinamide dinucleotide phosphate oxidase activation and antioxidant status in heart, lung, liver and kidney in streptozotocin-induced diabetic rats. Yonsei Med J. 2012; 53(2):294-303.

Lo L, McLennan SV, Williams PF, *et al.* Diabetes is a progression factor for hepatic fibrosis in a high fat fed mouse obesity model of non-alcoholic steatohepatitis. J Hepatol. 2011; 55(2):435-444.

Mabuchi A, Mullaney I, Sheard P, *et al.* Role of hepatic stellate cells in the early phase of liver regeneration in rat: formation of tight adhesion to parenchymal cells. Comp Hepatol. 2004; 3(1):1-4.

Mahajan MS, Upasani CD, Upaganlawar AB, Gulecha VS. Renoprotective effect of co-enzyme Q10 and n-acetylcysteine on streptozotocin-induced diabetic nephropathy in rats. Int J Diabetes Clin Res. 2020; 7(2):1-12.

Manna P, Das J, Ghosh J, Sil PC. Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, IκBα/NF-κB, MAPKs, and mitochondria-dependent pathways: prophylactic role of arjunolic acid. Free Radic Biol Med. 2010; 48(11):1465-1484.

Morsy MA, Abdalla AM, Mahmoud AM, Abdelwahab SA, Mahmoud ME. Protective effects of curcumin, α-lipoic acid, and N-acetylcysteine against carbon tetrachloride-induced liver fibrosis in rats. J Physiol Biochem. 2012; 68(1):29-35.

Muftakhov MV, Shchukin PV. Resonance electron capture by Cysteine and n-acetylcysteine molecules. Russ J Phys Chem A. 2020; 94(1):102-109.

Nagai T, Kanasaki M, Srivastava SP, *et al.* N-acetyl-seryl-aspartyl-lysyl-proline inhibits diabetes-associated kidney fibrosis and endothelial-mesenchymal transition. [published online ahead of print March 24, 2014]. Biomed Res Int. doi: 10.1155/2014/696475

Özdamar K. Paket programlar ile istatistiksel veri analizi (çok değişkenli analizler). Eskişehir: Kaan Kitabevi; 2004. s.574.

Rahal A, Kumar A, Singh V, *at al.* Oxidative stress, prooxidants, and antioxidants: the interplay. [published online ahead of print Jan 23, 2014]. Biomed Res Int. doi: 10.1155/2014/761264

Romere C, Duerrschmid C, Bournat J, *et al.* Asprosin, a fasting-induced glucogenic protein hormone. Cell. 2016; 165(3):566-579.

Rosa LR, Kaga AK, Barbanera PO, Queiroz PM, do Carmo NO, Fernandes AA. Beneficial effects of N-acetylcysteine on hepatic oxidative stress in streptozotocin-induced diabetic rats. Can J Physiol Pharmacol. 2018; 96(4):412-418.

Samad-Noshahr Z, Hadjzadeh MA, Moradi-Marjaneh R, Khajavi-Rad A. The hepatoprotective effects of fennel seeds extract and trans Anethole in streptozotocin induced liver injury in rats. Food Sci Nutr. 2021; 9(2):1121-1131.

Sanchez SS, Abregú AV, Aybar MJ, Riera AS. Changes in liver gangliosides in streptozotocin-induced diabetic rats. Cell Biol Int. 2000; 24(12):897-904.

Sarkar S, Das D, Wann SB, Kalita J, Manna P. Is diabetes mellitus a wrongdoer to COVID-19 severity? [published online ahead of print Jul 1, 2021]. Diabetes Res Clin Pract. doi: 10.1016/j.diabres.2021.108936

Shang J, Wang Q, Zhang H, *et al.* The relationship between diabetes mellitus and COVID-19 prognosis: a retrospective cohort study in Wuhan, China. Am J Med. 2021; 134(1):6-14.

Shanmugam KR, Mallikarjuna K, Kesireddy N, Reddy KS. Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats. Food Chem Toxicol. 2011; 49(4):893-897.

Sharkawi SM, El-Shoura EA, Abo-Youssef AM, Hemeida RA. The potential hepatoprotective effects of lovastatin combined with oral hypoglycemic agents in streptozotocin-induced diabetes in rats. Immunopharmacol Immunotoxicol. 2020; 42(2):165-73.

Su-Ming H, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem. 1981; 29(4):577-80.

World Health Organization. Diabetes. Available at: https://www.who.int/health-topics/diabetes#tab=tab_1. Accessed July 13, 2021.

Yalcin A, Yumrutas O, Kuloglu T, *et al.* Hepatoprotective properties for Salvia cryptantha extract on carbon tetrachloride-induced liver injury. Cell Mol Biol. 2017; 63(12):56-62.

Yalçın A, Gürel A. Effects of N-acetylcysteine on kidney tissue, matrix metalloproteinase-2, irisin and oxidative stress in a diabetes mellitus model. Biotech Histochem. 2021; 96(8):616-622.

Yaman T, Doğan A. Streptozotosin ile diyabet oluşturulan sıçanlarda meşe palamudu (*Quercus branti* Lindl.) ekstraktların karaciğer ve pankreası koruyucu etkileri. Dicle Üniv Vet Fak Derg. 2016; (1):7-15.

Yang-en S, Petpiboolthai H, Khimmakthong W, Anupunpisit V. Effect of curcumin on collagen deposition and tissue reorganization in liver of diabetic rats. J Med Assoc Thai. 2016; 99(8):118-129.

Original Article https://doi.org/10.47748/tjvr.1062162

TJVR 2022; 6 (2): 43-52

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Determination of the effects of Chia (*Salvia hispanica L.*) oil and dandelion (*Taraxacum Officinale*) extract on tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) release in liver tissue of diabetic rats

Şükran Yediel Aras[1] Pınar Aksu Kiliçle[2] Sevda Eliş Yildiz[1] Erhan Uluman[2] Buket Bakir[3] Ebru Karadağ Sari[4] Serpil Dağ[5]

[1] *Department of Midwifery, Faculty of Health Sciences, Kafkas University, Kars, Türkiye.*
[2] *Department of Biology, Faculty of Arts and Sciences, Kafkas University, Kars, Türkiye.*
[3] *Department of Histology and Embryology, Faculty of Veterinary Medicine, Namık Kemal University, Tekirdağ, Türkiye.*
[4] *Department of Histology and Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye.*
[5] *Department of Pathology, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye.*

Correspondence: *Şükran Yediel Aras (s.yediel@hotmail.com)*

Received: 09.03.2022 Accepted: 06.06.2022

ABSTRACT

Objective: This study aimed to investigate the effects of Chia (*Salvia hispanica L.*) oil and Dandelion (*Taraxacum Officinale*) extract on Tumor Necrosis Factor-α (TNF-α) and Interleukin 6 (IL-6) release in liver tissue of diabetic rats.

Materials and Methods: Experimental groups were created as control, sham, chia, dandelion, diabetes (DM), diabetes+chia (DC), and diabetes+dandelion (DD). Body weight and blood glucose measurements were taken on the 1st, 3rd, and 17th days of the study and evaluated statistically. A one-way ANOVA test was performed to determine the differences between the groups. The Duncan test was used to compare significant differences between groups. At the end of the study, Masson's trichrome staining and Hematoxylin-Eosin staining were employed for histological examinations of liver tissues, and the distribution of TNF-α and IL-6 was examined by applying the Streptavidin-biotin peroxidase method.

Results: It was determined that body weight and blood glucose measurements were significantly decrease for the DC group compared to other groups. Immunoreactivity of TNF-α and IL-6 was found to decrease in DC and DD groups at close to the control levels.

Conclusion: Based on our results, it was thought that the use of chia and dandelion in diabetes may contribute to the alleviation of disease-related complications by having a positive effect on proinflammatory cytokine levels.

Keywords: *Chia, Dandelion, Diabetes mellitus, IL-6, TNF-α*

INTRODUCTION

Diabetes is a chronic disease characterized by the absence of insulin production (Type 1 diabetes) or the development of insulin resistance (Type 2 diabetes and gestational diabetes) (WHO, 1999). The most common causes of diabetes are hereditary and environmental causes. The most obvious symptom of this disease is that blood glucose levels are higher than normal (Tierney et al., 2002). The World Health Organization (WHO) reported that there were approximately 422 million diabetics worldwide in 2014. It has been noted that most of the increase in the number of diabetes

patients is in developing low- and middle-income countries. WHO data of 2016 show that diabetes ranks fourth in deaths due to 'non-infectious diseases' with a death rate of 1.6 million. It has been reported that diabetes directly caused 1.5 million deaths in 2019 (WHO, 2020; WHO, 2021).

Cytokines are soluble proteins or glycoproteins that regulate the relationships of immune system cells with each other in both the natural and specific immune response (Köklüdağ, 1999; Manuel et al., 1999). Cytokines have been reported to play a role in the development of many chronic complications, including neurological and vascular lesions, in patients with diabetes (Shanmugam et al., 2003). Tumor necrosis factor-alpha (TNF-α), one of the cytokines, is a protein released from alveolar macrophages, monocytes, neutrophils, and lymphocytes (Martinet et al., 1988; Sung et al., 1988; Djeu et al., 1990). Interleukin-6 (IL-6), on the other hand, is a cytokine released by monocytes, alveolar macrophages, endothelial cells, fibroblasts, B and T cells (Kotloff et al., 1990; Zitnik et al., 1993).

Chia is an annual herbaceous plant from the mint family (*Labiatae*). Chia, a plant that grows annually in an area extending from western Mexico to northern Guatemala, grows in the temperature range of 15-30 degrees and needs a high amount of precipitation. Chia use has been reported to have positive effects in cases such as weight loss, obesity, and diabetes (Ayerza and Mealla, 1993; Vuksan et al., 2017). Dandelion is a plant in the genus *Taraxacum* and is a member of the *Asteraceae* family. It has been used as a medicinal plant for many years. Dandelion use is beneficial in relieving type 2 diabetes, blisters, spleen, and liver complaints (Alarcon-Aguilara et al., 1998; Honek et al., 2011).

It is known that cytokines such as TNF-α and IL-6 are associated with the development of complications in patients with diabetes. It is also suggested that chia and dandelion plants may have a positive effect on diabetes. Therefore, the view that alternative treatments can be developed in the treatment of diabetes and its complications forms the basis of this study. This study aims to reveal the effects of Chia (*Salvia hispanica L.*) oil and Dandelion (*Taraxacum Officinale*) extract on the liver tissue of rats with experimental diabetes and on TNF-α and IL-6, which are pro-inflammatory cytokines, by immunohistochemical methods.

MATERIALS and METHODS

Ethical approval was obtained from the Animal Experiments Local Ethics Committee of Kafkas University for the study (Project No: KAU HADYEK/2019-027). The animals used in the study were obtained from the Experimental Animals Unit of Kafkas University.

Materials

This study was designed as a future-oriented experimental study. Forty-nine 3-month-old *Spraque dawley* male rats were used in the study. Rats were housed in standard cages at an ambient temperature of 22±2°C, 12 hours of light, 12 hours of dark environment, and fed as *ad-libitum*. The study was carried out following the principles of the International Declaration of Helsinki. All rats were weighed before experimental rats were grouped. Experimental groups were created from randomly selected rats so that each group had 7 rats.

Methods

Experimental rats were grouped as follows:

1. Control group (n:7): No application was made to rats in this group. They were fed only rat feed.

2. Sham group (n:7): A single dose of sodium citrate solution 50 mg/kg intraperitoneal (i.p.) was administered to rats in this group.

3. Chia Group (n:7): Chia oil (naturaoil, barcode no: 8-697589-643265) was administered as 1ml/ kg by oral gavage for 14 days to rats in this group (Baş et al., 2016).

4. Dandelion Group (n:7): 2.4 g/kg dandelion extract (Kale natural herbal products, serial no: LE 487) was administered to rats in this group by oral gavage for 14 days (Cho et al., 2002).

5. Diabetes group (DM) (n:7): The rats in this group were administered a single dose of streptozotocin (STZ) (50 mL citric acid+40 mL disodium was dissolved in hydrogen phosphate buffer solution and set to pH: 4.5) 50 mg/kg i.p., and rats with a blood glucose value of 200 mg/dL were considered diabetic.

6. Diabetes+Chia Group (DC) (n:7): A single dose of streptozotocin (STZ) (50 mL citric acid + 40 ml disodium hydrogen phosphate was dissolved in buffer solution and set to pH: 4.5) 50 mg/kg i.p. was administered to the rats in this group and diabetes was created. Then 1 mL/kg chia oil was administered by oral gavage for 14 days.

7. Diabetes+Dandelion Group (DD) (n:7): The rats in this group were administered a single dose of streptozotocin (STZ) (50 mL citric acid+40 mL disodium hydrogen phosphate was dissolved in buffer solution and set to pH: 4.5) 50 mg/kg i.p. and diabetes was created. Then the dandelion extract was applied as 2.4 g/kg through oral gavage for 14 days.

At the end of the study, liver tissues were taken from the rats under deep anesthesia and fixed in a 10% solution of formalin for histological and immunohistochemical examinations. It was then blocked in paraffin by undergoing routine histological tissue follow-up procedures.

Body Weight Measurement

The weights of all rats were measured on the first day of the study, 72 hours after STZ administration, and after 8 hours of fasting at the end of the experiment.

Determination of Blood Glucose Levels

To determine fasting blood glucose levels, blood samples were taken from the tail vein of rats before starting the STZ administration and after 72 hours starving period following STZ administration and after 8 hours of fasting at the end of the study and measured with a glucometer (Yasee, GLM-76, Taiwan).

Histological Investigations

Masson's trichrome staining technique and Hematoxylin-Eosin staining were performed to examine the general structure of liver tissue in the sections taken from paraffin blocks.

Immunohistochemistry

Slides in which tissue sections were taken were coated with chromium-alum gelatin, and the Streptavidin-biotin peroxidase method was applied to the sections. PBS (0.1 M, pH, 7.2) buffer was used for all washing operations during the immunohistochemical procedure. The sections were incubated for 15 minutes at 3% H_2O_2 prepared at 0.1 m PBS and boiled in citrate buffer solution at 800 watts in a microwave oven for 10 minutes. It was then incubated for 10 min with a Large Volume Ultra V Block solution. TNF-α (Santa Cruz-sc52746) primary antibody (1/500 dilution) and IL-6 (Biorbty-orb651448) primary antibody (1/200 dilution) were applied to the sections at room temperature and in a humid environment for 1 hour. After that, Biotinylated Goat Anti B Polyvalent solution and Streptavidin Peroxidase solution were applied at room temperature for 30 minutes. DAB-H_2O_2 (Diaminobenzidine-Hydrogen Peroxide) Substrate solution was added for chromogen application. Modified Gill III hematoxylin solution was used for contrast staining. All procedures were performed exactly without adding primary antibodies to the sections held in PBS to determine whether immunoreactivity was specific. For immunohistochemical assessment, staining properties and density of target cells were taken into account. The assessment was carried out by two independent observers. Semi-quantitative scoring was made from 0 to 3 based on no reaction (0), weak reaction (1), moderate reaction (2), strong reaction (3). All prepared sections were evaluated and photographed under a light microscope (Olympus BX51, Olympus Optical Co. Osaka, Japan).

Statistical Analysis

SPSS (20.0) packaged software was used to evaluate the data obtained in the study. A one-way ANOVA test was performed to determine the differences between the groups. The Duncan test was used to compare significant differences between groups. The results were expressed as mean ± standard deviation (SD). Also, a p-value <0.05 was considered statistically significant.

RESULTS

Body Weight Results

According to the body weight measurement days (1, 3, and 17 days), there was no statistically significant difference in the comparison made within the groups ($p>0.05$) (Table 1). A statistically significant difference was found in the comparison between the groups at 1, 3, and 17 days ($p<0.05$) (Table 2, Figure 1). Especially, in the 17-day DC group, it was noted that the weights decreased significantly compared to other groups.

Fasting Blood Glucose Results

A fasting blood glucose assessment was performed on days 1, 3, and 17. It was found that there was a statistically significant difference in the comparison made within the groups in these days ($p<0.05$) (Table 3). Especially, on the 3rd day of the study, it was determined that fasting blood sugar values increased in DM, DC, DD groups. Fasting blood glucose values were compared between groups on days 1, 3, and 17; there was a statistically significant difference in DM, DC, DD groups on day 3, and DM and DD groups on day 17 ($p<0.05$) (Table 4, Figure 2).

Table 1. Evaluation of body weights within the groups.

Days	Control (gr)	Sham (gr)	DM (gr)	Chia (gr)	DC (gr)	Dandelion (gr)	DD (gr)
1st day	257.71±45.04	258.42±21.85	276±34.45	285.57±18,33	230±25.35	280.57±52.95	282±42.22
3rd day	264.28±42.75	264.71±22.10	280.71±42.84	292.57±20,31	214.57±26.04	296.85±48.35	290.14±32.52
17th day	271.71±45.40	272.28±40.38	296.57±40.31	304.85±23,91	207.14±56.81	32757±42.90	265.14±15.39
p	0.842	0.683	0.6	0.246	0.545	0.208	0.351

Table 2. Evaluation of body weights between the groups.

Days	Control (gr)	Sham (gr)	DM (gr)	Chia (gr)	DC (gr)	Dandelion (gr)	DD (gr)	p
1st day	257.71±45.04^{a}	258.42±21.85^{a}	276±34.45^{b}	285.57±18.33^{b}	230±25.35^{a}	280.57±52.95^{b}	282±42.22^{b}	0.07
3rd day	264.28±42.75^{a}	264.71±22.10^{a}	280.71±42.84^{a}	292.57±20.31^{a}	214.57±26.04^{b}	296.85±48.35^{a}	290.14±32.52^{a}	0.001
17th day	271.71±45.40^{a}	272.28±40.38^{a}	296.57±40.31^{ac}	304.85±23.91^{ac}	207.14±56.81^{b}	327.57±42.90^{c}	265.14±15.39^{a}	0.000

a, b, c: There is a statistically significant difference between the means shown with different letters on the same line ($p<0.05$).

Table 3. Evaluation of fasting blood glucose measurements within the groups.

Days	Control (mg/dl)	Sham (mg/dl)	DM (mg/dl)	Chia (mg/dl)	DC (mg/dl)	Dandelion (mg/dl)	DD (mg/dl)
1st day	77.43±6.80	77.86±3.98	79.86±6.26	84.57±11.16	87.86±13.01	77.29±2.98	83.14±5.49
3rd day	96.57±14.06^{b}	96.43±4.86^{b}	406.57±109.07^{b}	95.71±12.63^{a}	300.57±78.02^{b}	78.29±2.50^{a}	465.14±54.91^{b}
17th day	83.71±10.81^{a}	95±8.16^{b}	320.86±76.43^{b}	78.28±6.18^{b}	135.43±28.51^{a}	86.43±13.14^{a}	256.29±80.37^{c}
p	0.01	0.000	0.000	0.018	0.000	0.086	0.000

a, b, c: There is a statistically significant difference between the means indicated by different letters in the same column ($p<0.05$).

Table 4. Evaluation of fasting blood glucose measurements between the groups.

Days	Control (mg/dl)	Sham (mg/dl)	DM (mg/dl)	Chia (mg/dl)	DC (mg/dl)	Dandelion (mg/dl)	DD (mg/dl)	p
1st day	77.43±6.80^{a}	77.86±3.98^{a}	79.86±6.26^{a}	84.57±11.16^{a}	87.86±13.01^{a}	77.29±2.98^{a}	83.14±5.49^{a}	0.1
3rd day	96,.57±14.06^{a}	96,.43±4.86^{a}	406.57±109.07^{b}	95.71±12.63^{a}	300.57±78.02^{c}	78.29±2.50^{a}	465.14±54.91^{b}	0.000
17th day	83.71±10.81^{a}	95±8.16^{ab}	320.86±76.43^{c}	78.28±6.18^{a}	135.43±28.51^{b}	86.43±13.14^{ab}	256.29±80.37^{d}	0.000

a, b, c: There is a statistically significant difference between the means shown with different letters on the same line ($p<0.05$).

Histopathological Results

No pathological findings were found in the liver tissue of rats in the control, sham, chia, and dandelion group (Figure 3). In addition to sinusoidal dilation and hyperemia in liver tissue of DM group rats, necrosis in hepatocytes was observed in some areas, also DC and DD groups had lower necrotic changes in hepatocytes compared to the DM group (Figure 4).

Immunohistochemical Results

TNF-α Immunoreactivity

TNF-α immunoreactivity were determined around the central veins in the liver tissue of rats a weak in control, sham, chia, dandelion groups; strong in the DM group, and moderate in DC and DD groups (Figure 5).

IL-6 Immunoreactivity

IL-6 immunoreactivity was no detected in the control, sham, chia and dandelion groups. Strong IL-6 immunoreactivity in the DM group and moderate in the DC and DD groups were determined around the central veins (Figure 6).

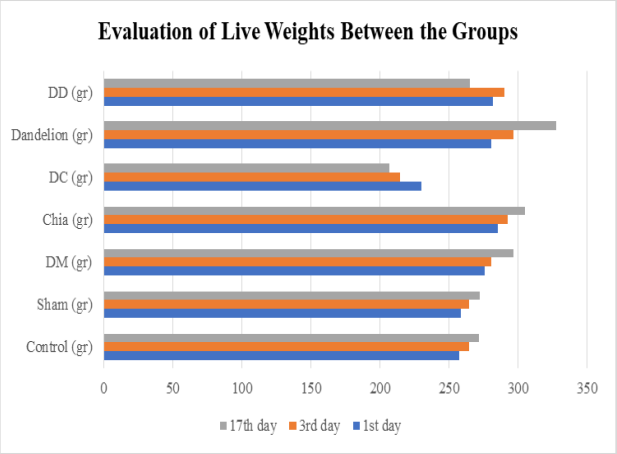

Figure 1. Evaluation of live weights between the groups.

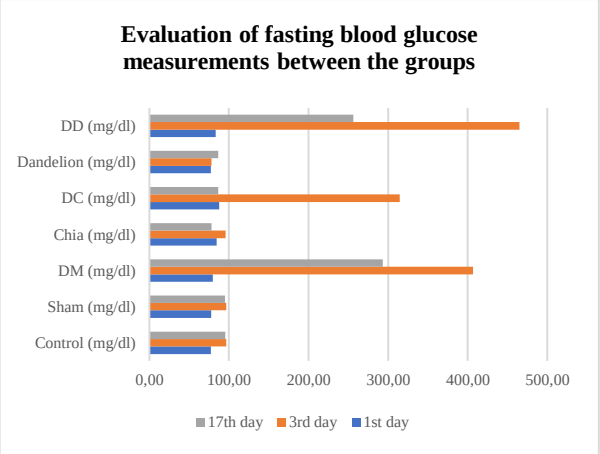

Figure 2. Evaluation of fasting blood glucose measurements between the groups.

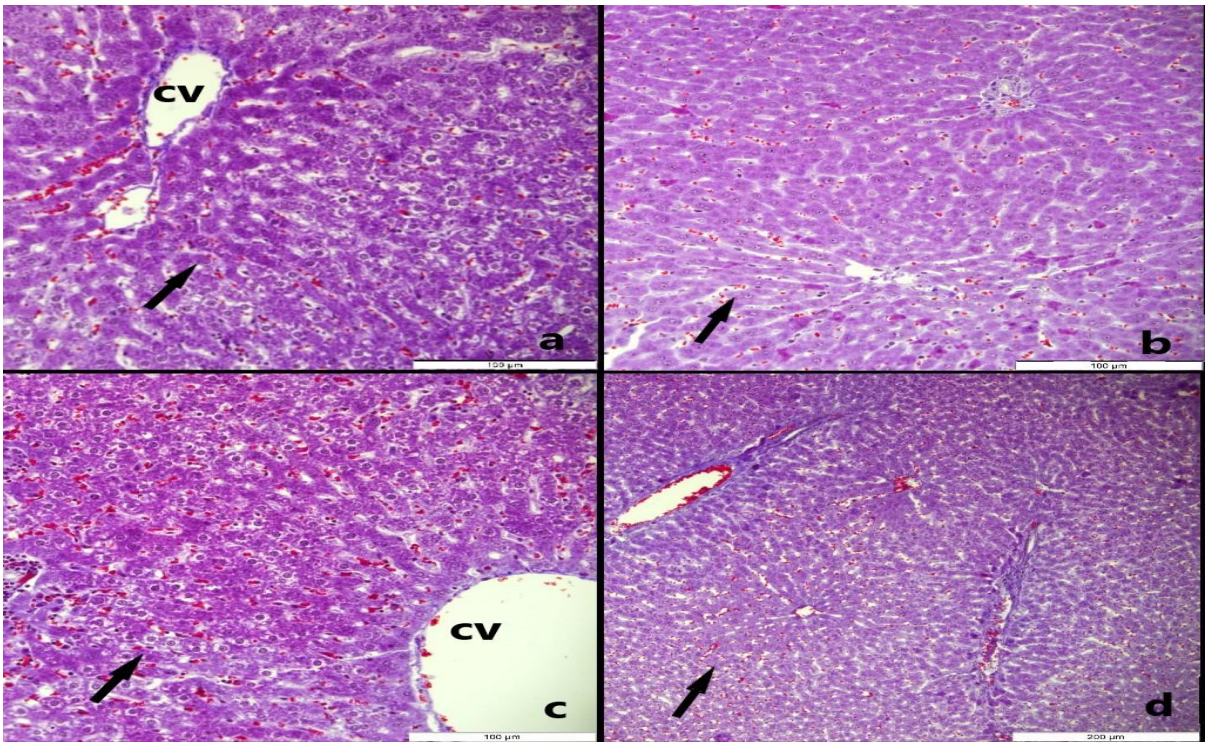

Figure 3. Rat liver tissue. a: Control, b: Sham, c: Chia, d: Dandelion. cv: Central vein, arrow: Hepatocytes. Masson's trichrome staining.

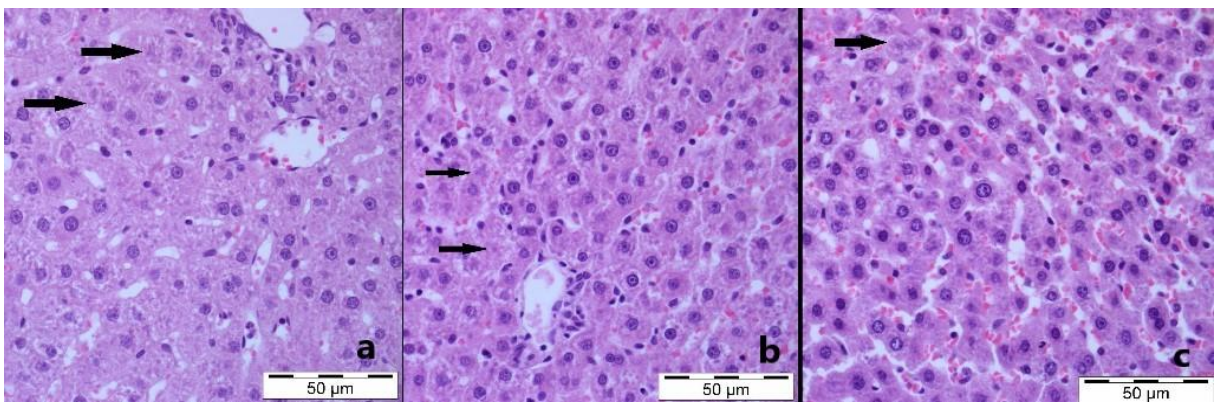

Figure 4. Rat liver tissue. a: Diabetes, b: DC, c: DD. Necrotic changes in hepatocytes (arrows). Hematoxylin-Eosin staining.

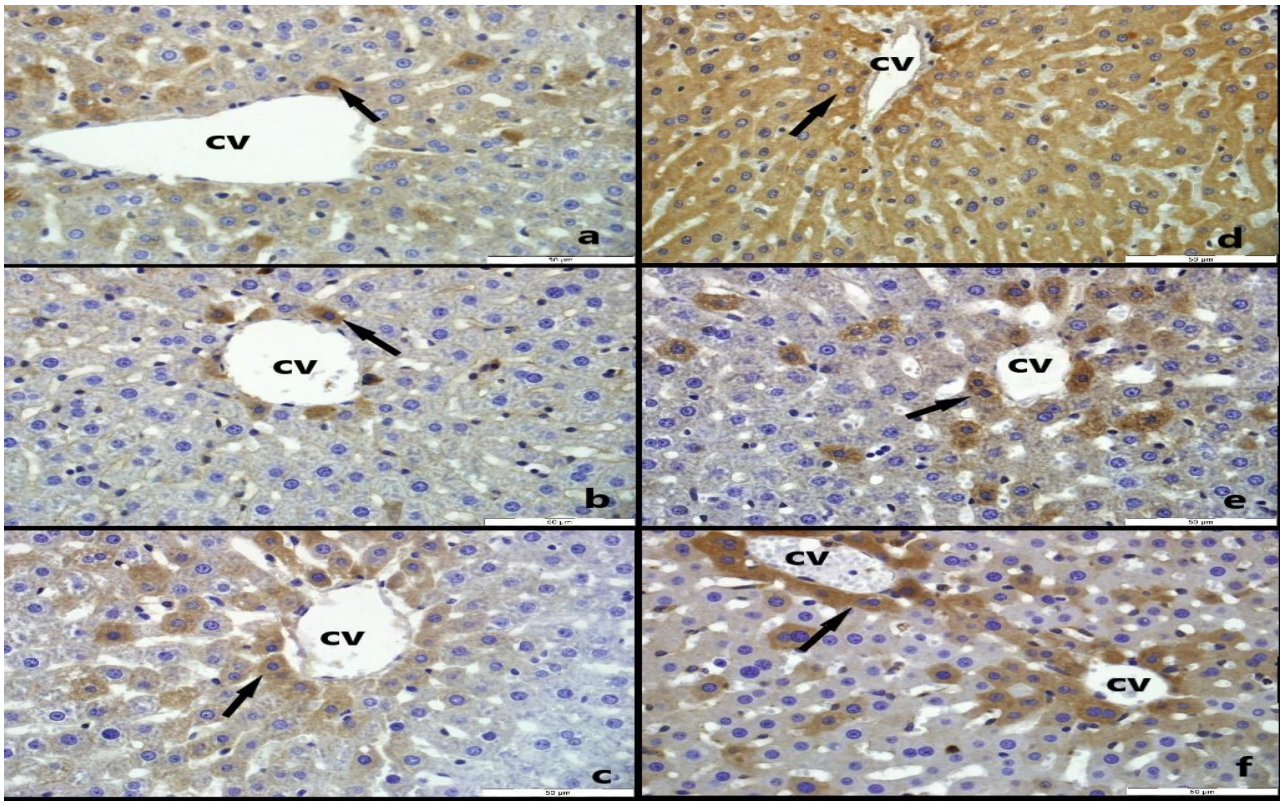

Figure 5. TNF-α immunoreactivity in rat liver tissue. a: Control, b: Chia, c: Dandelion, d: Diabetes, e: DC, f: DD. cv: Central vein, arrow: Hepatocytes. Bar: 50µm.

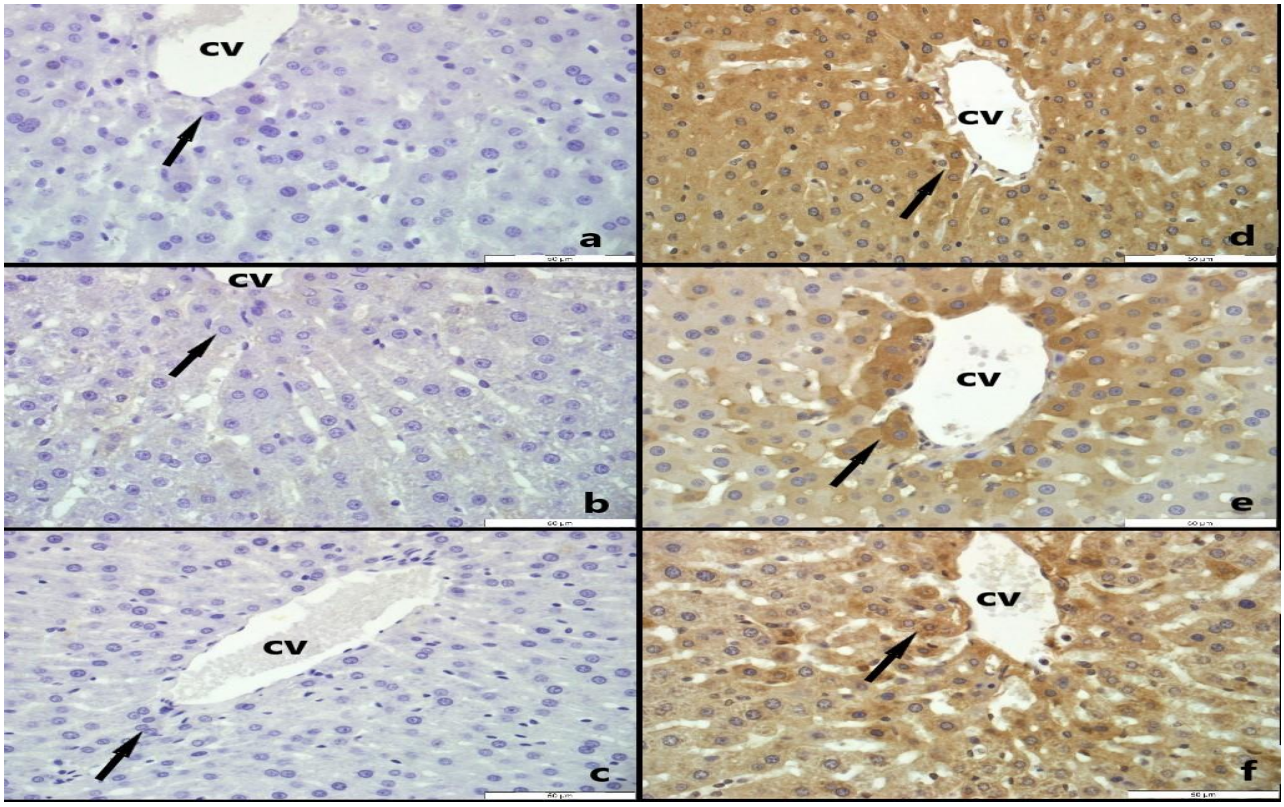

Figure 6. IL-6 immunoreactivity in rat liver tissue. a: Control, b: Chia, c: Dandelion, d: Diabetes, e: DC, f: DD. cv: Central vein, arrow: Hepatocytes. Bar: 50µm.

DISCUSSION

Chia has been reported to have positive effects on decreasing body weight, serum triglycerides, and high blood sugar values, and increasing high-density lipoprotein levels (Ayerza and Coates, 2007; Guevara-Cruz et al., 2012). Long-term use of chia seeds in the diet has been shown to increase bone mineral content, as well as reduce lipid accumulation in the liver, and have a positive effect on intestinal muscle layers and crypt size morphology (Chani et al., 2018). A study conducted on obese rats found that consumption of chia seeds and chia oil did not reduce body weight increase and abdominal fat accumulation, but only improved glucose and insulin tolerance (Marineli et al., 2015a). It has been reported that dandelion administration in diabetic rats reduces cholesterol, triglycerides, malondialdehyde, blood glucose levels, and body weight levels, and increases serum HDL-cholesterol levels. Due to the mentioned characteristics, it has been suggested that dandelion may have positive effects on diabetes (Cho et al., 2002). At the end of the study, it was determined that body weight measurements and blood glucose levels significantly decreased especially in the DC group compared to rats in the DM and DD group. There was no significant difference between DM and DD groups in terms of body weight measurements and blood glucose levels. Our results suggested that Chia administration would have a positive effect, in particular, on controlling high blood sugar levels. In addition, we concluded that the duration of dandelion extract application may have different effects on the results.

Structural and functional disorders occur in the liver due to diabetes, and therefore glycogen and lipid metabolism is influenced (Levinthal and Tavill, 1999; Sanchez et al., 2000). As a result of diabetes, oxidative stress increases in many organs, especially the liver, also, bloating in hepatocytes, chromatin condensation, apoptotic bodies, and necrosis occur (Tolman et al., 2007; Manna et al., 2010). In rats with STZ-induced diabetes, degeneration and necrosis in hepatocytes in liver tissue, inflammatory cell infiltration in portal areas, fibrosis, and bile duct hyperplasia were observed (Yaman and Doğan, 2016). It has been suggested that the use of chia has positive effects on liver tissue in diseases such as non-alcoholic dyslipidemia, non-alcoholic steatohepatitis, and hepatocellular carcinoma (Fernandez-Martinez et al., 2019). The use of chia has been stated to have a positive effect on preventing and normalizing dyslipidemia and hepatic steatosis. In addition, rats fed a high-fat diet containing chia seeds had a decrease in thiol, plasma catalase, and glutathione peroxidase levels, and an increase in liver glutathione reductase levels (Rossi et al., 2013; Marineli et al., 2015b). Dandelion has been reported to significantly inhibit lipid accumulation in the liver, reduce insulin resistance, and have positive effects in the prevention and treatment of non-alcoholic fatty liver disease (Davaatseren et al., 2013). In our study, sinusoidal dilation and hyperemia, as well as necrosis of hepatocytes in some areas, were observed in liver tissue of DM group rats. DC and DD group hepatocytes indicated lower levels of necrotic changes compared to DM group hepatocytes. Considering the changes observed in our study and the information in the literature, our results suggest that the use of chia and dandelion may be protective against damage to the liver caused by diabetes.

As a result of oxidative stress, the amount of ROS (reactive oxygen species) increases, and insulin resistance develops with the secretion of cytokines in high amounts from activated macrophages and monocytes. In Type 2 diabetes mellitus, insulin resistance increases along with inflammation associated with oxidative stress and activation of monocytes, and insulin secretion decreases due to the destruction of pancreatic island cells (Navarro-Gonzales and Mora-Fernandez, 2008; Elmarakby and Sullivan, 2012). Tumor necrosis factor-α is a proinflammatory cytokine34 released from myeloid cells as a result of activation of the MAPK (mitogen-activated protein kinase) and NFkB (nuclear factor-kB) signaling pathways. TNF-α is found mainly in human fat (adipose) tissue. TNF-α and TNF-α mRNA levels in adipose tissue increase in direct proportion to the level of obesity and hyperinsulinemia, and TNF-α levels decrease as a result of a decrease in adipose tissue due to weight loss (Hotamisligil et al., 1995; Kern et al., 1995). In addition, elevated serum TNF-α levels have been reported in patients with type 1 and type 2 diabetes. Because of this effect, it has been noted that TNF-α can be used to control diabetes and evaluate the pro-inflammatory immune response that develops in diabetes (Foss-Freitas et al., 2006). In a study that examined TNF-α expression and immunohistochemical distribution in chronic liver damage, TNF-α positive cells were rarely observed along sinusoids in the control group's hepatic

tissue, while immunoreactivity was not observed in hepatocytes (Orfila et al., 1999). A study conducted in diabetic rats indicated moderate immunoreactivity of TNF-α around the central veins in the liver tissue of control group rats, while strong immunoreactivity was reported in the diabetes mellitus group (Satin et al., 2016). In the study, moderate immunoreactivity of TNF-α was detected around the central veins in the control, sham, chia, dandelion groups, and strong immunoreactivity of TNF-α was detected in the DM group. It was also noted that chia and dandelion administration reduced the increased the immunoreactivity of TNF-α in diabetes mellitus.

Interleukin-6 is a proinflammatory cytokine that allows the differentiation of monocytes into macrophages (Chomarat et al., 2000). In addition, IL-6 increases insulin resistance and glucose transport in fat cells. Thanks to this effect, it has been reported that IL-6 may play a role in insulin-stimulated glucose transport (Stouthard et al., 1996; Rotter et al., 2003). When both cytokine levels were evaluated together in diabetic rats, hepatic steatosis and degree of inflammation, serum TNF-α and IL-6 levels, hepatic TNF-α and IL-6 mRNA expression, immunoreactivity of TNF-α in liver tissue were significantly higher than in the control group (Zhang et al., 2009; Li et al., 2018). The determination that immunoreactivity of TNF-α and IL-6 was strong in the DM group and moderate in DC and DD groups suggests that chia and dandelion use may have positive effects on increased proinflammatory cytokine levels in diabetes.

CONCLUSION

The frequency of diabetes-related complications and its high death rates around the world also increases the importance of treating this disease. Natural-origin treatment methods for chronic diseases such as diabetes in developed and developing countries are gaining popularity due to fewer side effects. Many traditional medicines are made of medicinal plants, minerals, and organic substances. Therefore, chia and dandelion plants are also considered to be effective in the treatment of many diseases today. It is emphasized that some cytokines, such as TNF-α and IL-6, are effective in the proinflammatory response and may have positive effects on certain diseases that occur in the liver. In our study, we determined that Chia use was especially effective in lowering high blood glucose, while chia and dandelion administration reduced TNF-α and IL-6 immunoreactivity in liver tissue in rats with diabetes. When our histopathological, immunohistochemical, and statistical results are evaluated together, we concluded that the use of chia and dandelion can have a positive effect on TNF-α and IL-6 levels and that these plants, which stimulate the pro-inflammatory response, can be used as a natural source of treatment for diabetes.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests

Financial Disclosure: This study was supported by Kafkas University Scientific Research Projects Coordination Unit (2019-TS-63).

Author's Contributions: ŞYA designed, collected, analyzed and interpreted experiments prepared the data and the article. ŞYA, PAK, SEY and EU performed the experiment. EKS designed the experiments, oversaw the project, and critically read the paper. SD performed histopathological examinations. All authors approved the final version of the published article.

ŞYA:Şükran Yediel Aras, PAK:Pınar Aksu Kiliçle, SEY:Sevda Eliş Yildiz, EU:Erhan Uluman, BB:Buket Bakir, EKS:Ebru Karadağ Sari, SD:Serpil Dağ

REFERENCES

Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, *et al.* Study of the anti-hyperglycemic effect of plants used as antidiabetics. J Ethnopharmacol. 1998; 61(2):101-110.

Ayerza R, Coates JW. Effect of dietary α-linolenic fatty acid derived from chia when fed as ground seed, whole seed and oil on lipid content and fatty acid composition of rat plasma. Ann Nutr Metab. 2007; 51(1):27-34.

Ayerza R, Mealla AM. El Cultivo de la Chia en Mexico. Buenos Aires: Agropecuaria El Valle S.A Buenos Aires, (unpublished), 1993.

Baş H, Pandır D, Kalender S. Furan-induced hepatotoxic and hematologic changes in diabetic rats: the protective role of lycopene. Arh Hig Rada Toksikol. 2016; 67:194-203.

Chani EMM, Pacheco SOS, Martinez GA, *et al.* Long-term dietary intake of chia seed is associated with increased bone mineral content and improved hepatic and intestinal morphology in sprague dawley rats. Nutrients. 2018; 10(7):922.

Cho SY, Park JY, Park EM, *et al.* Alternation of hepatic antioxidant enzyme activities and lipid profile in streptozotocin-induced diabetic rats by supplementation of dandelion water extract. Clinica Chimica Acta. 2002; 317:109-117.

Chomarat P, Banchereau J, Davoust J, Palucka AK. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. Nat Immunol. 2000; 1(6): 510–514.

Davaatseren M, Hur HJ, Yang HJ, *et al.* Taraxacum official (*Dandelion*) leaf extract alleviates high-fat diet-induced nonalcoholic fatty liver. Food Chem Toxicol. 2013; 58:30-36.

Djeu JY, Serbousek D, Blanchard DK. Release of tumor necrosis factor by human polymorphonuclear leukocytes. Blood. 1990; 76:1405-1409.

Elmarakby AA, Sullivan JC. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. Cardiovasc Ther. 2012; 30(1):49-59.

Fernandez- Martinez E, Lira- Islas IG, Carino- Cortes R, *et al.* Dietary chia seeds (*Salvia hispanica*) improve acute dyslipidemia and steatohepatitis in rats. J Food Biochem. 2019; 43(9):e12986.

Foss-Freitas MC, Foss NT, Donadi EA, Foss MC. In vitro TNF-alpha and IL-6 production by adherent peripheral blood mononuclear cells obtained from type 1 and type 2 diabetic patients evaluated according to the metabolic control. Ann N Y Acad Sci. 2006; 1079:177-180.

Guevara-Cruz M, Tovar AR, Aguilar-Salinas CA, *et al.* A dietary pattern including nopal, chia seed, soy protein, and oat reduces serum triglycerides and glucose intolerance in patients with metabolic syndrome. J Nutr. 2012; 142(1):64-69.

Honek A, Martinkova Z, Saska P. Effect of size, taxonomic affiliation and geographic origin of dandelion (*Taraxacum agg.*) seeds on predation by ground beetles (Carabidae, Coleoptera). Basic Appl Ecol. 2011; 12(1):89-96.

Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor alpha in human obesity and insulin resistance. J Clin Invest. 1995; 95:2409-2415.

Kern PA, Saghizadeh M, Ong JM, *et al.* The expression of tumor necrosis factor in human adipose tissue: regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest. 1995; 95:2111-2119.

Köklüdağ A. Sitokinler. In: Gümüşdiş G, Doğanavşargil E, eds. Klinik Romatoloji. İstanbul: Deniz Matbası; 1999. s.39-46.

Kotloff RM, Little J, Elias JA. Human alveolar macrophage and blood monocyte interleukin-6 production. Am J Respir Cell Mol Biol. 1990; 3:497-505.

Levinthal GN, Tavill AS. Liver disease and diabetes mellitus. Clinical Diabetes. 1999; 17(2):73-81.

Li XW, Chen HP, He YY, *et al.* Effects of Rich-Polyphenols Extract of dendrobium loddigesii on anti-diabetic, anti-inflammatory, anti-oxidant, and gut microbiota modulation in db/db mice. Molecules. 2018; 23:3245.

Manna P, Das J, Ghosh J, Sil PC. Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, IkappaBalpha/NF-kappaB, MAPKs, and mitochondria-dependent pathways: Prophylactic role of arjunolic acid. Free Radic Biol Med. 2010; 48(11):1465-1484.

Manuel SR, Bienvenu J, Whiche J. Cytokines. In: Burtis CA, Aschwood ER, eds. Tietz Textbook of Clinical Chemistry. 5th ed. Philadelphia: WB Sounders Company; 1999. p.541-616.

Marineli RS, Lenquiste SA, Moraes EA, Maróstica MR. Antioxidant potential of dietary chia seed and oil (*Salvia hispanica L.*) in diet-induced obese rats. Food Res Int. 2015a; 76:666-674.

Marineli RS, Moura CS, Moraes EA, Lenquiste SA, Lollo PCB, Morato PN, *et al.* Chia (*Salvia hispanica L.*) enhances HSP, PGC-1a expressions and improves glucose tolerance in diet-induced obese rats. Nutrition. 2015b; 31(5):740-748.

Martinet Y, Yamauchi K, Crystal RG. Differential expression of the tumor necrosis factor/cachectin gene by blood and lung mononuclear phagocytes. Am Rev Respir Dis. 1988; 138:659-665.

Navarro-Gonzales JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol. 2008; 19(3):433-442.

Orfila C, Lepert LC, Alric L, *et al.* Expression of TNF-alpha and immunohistochemical distribution of hepatic macrophage surface markers in carbon tetrachloride-induced chronic liver injury in rats. Histochem J. 1999; 31(10):677–685.

Rossi AS, Oliva MA, Ferreira MR, Chicco A, Lombardo YB. Dietary chia seed induced changes in hepatic transcription factors and their target lipogenic and oxidative enzyme activities in dyslipidaemic insulin-resistant rats. Br J Nutr. 2013; 109:1617-1627.

Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin resistant subjects. J Biol Chem. 2003; 278(46):45777-45784.

Sanchez SS, Abregu AV, Aybar MJ, Sánchez Riera AR. Changes in liver gangliosides in streptozotocin induced diabetic rats. Cell Biol Int. (2000; 24(12):897-904.

Satin K, Petpiboolthai H, Anupunpisit V. Effect of Curcumin on characterization and localization of interleukin-13 and tumor necrosis factor-alpha in liver of diabetic rats. J Med Assoc Thai. 2016; 99 (8):187-195.

Shanmugam N, Reddy MA, Guha M, Natarajan R. High glucose-induced expression of pro-inflammatory cytokine and chemokine genes in monocytic cells. Diabetes. 2003; 52:1256-1264.

Stouthard JML, Oude Elferink RPJ, Sauerwein HP. Interleukin-6 enhances glucose transport in 3T3-L1 adipocytes. Biochem Biophys Res Commun. 1996; 220(2):241-245.

Sung SS, Bjorndahl JM, Wang CY, Kao HT, Fu SM. Production of tumor necrosis factor/cachectin by human T cell lines and peripheral blood T lymphocytes stimulated by phorbol myristate acetate and anti-CD3 antibody. J Exp Med. 1988; 167:937-953.

Tierney LM, McPhee SJ, Papadakis MA. Current Medical Diagnosis & Treatment. New York: Lange Medical Books/McGraw-Hill; 2002. p.1203-1215.

Tolman KG, Fonseca V, Dalpiaz A, Tan MH. Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. Diabetes Care. 2007; 30(3):734-743.

Vuksan V, Jenkins AL, Brissette C, *et al.* Salba-chia (*Salvia hispanica L.*) in the treatment of overweight and obese patients with type 2 diabetes: A double-blind randomized controlled trial. Nutr Metab Cardiovasc Dis. 2017; 27(2):138-146.

World Health Organization (WHO). Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus. World Health Organization. 1999. Available at: https://apps.who.int/iris/handle/10665/66040 Accessed April 25, 2021.

World Health Organization (WHO). Diabetes (10 November 2021). Available at: https://www.who.int/news-room/fact-sheets/detail/diabetes. Accessed June 06, 2022.

World Health Organization (WHO). World health statistics 2020: monitoring health for the SDGs, sustainable development goals. World Health Organization. Available at: https://apps.who.int/iris/handle/10665/332070 Accessed April 25, 2021.

Yaman T, Doğan A. Streptozotosin ile diyabet oluşturulan sıçanlarda meşe palamudu (*Quercus branti Lindl.)* ekstraktların karaciğer ve pankreası koruyucu etkileri. Dicle Üniv Vet Fak Derg. 2016; 1(2):7-15.

Zhang X, Li Z, Liu D, *et al.* Effects of probucol on hepatic tumor necrosis factor-a, interleukin-6 and adiponectin receptor-2 expression in diabetic rats. J Gastroenterol Hepatol. 2009; 24:1058-1063.

Zitnik RJ, Zheng T, Elias JA. cAMP inhibition of interleukin-1 induced interleukin-6 production by human lung fibroblasts. Am J Physiol. 1993; 264:253-260.

Original Article https://doi.org/10.47748/tjvr.1091743

TJVR 2022; 6 (2): 53-60

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

The prevalence of ear diseases in cat and dogs in Kocaeli provinces

Ibrahim Canpolat Murat Tanrısever Selçuk Başer

[1] *Department of Surgery, Faculty of Veterinary Medicine, University of Firat, Türkiye*

Correspondence: *Ibrahim Canpolat (icanpolat@firat.edu.tr)*

Received: 24.03.2022 Accepted: 26.06.2022

ABSTRACT

Objective: In this study, it was aimed to determine the prevalence of ear diseases in cats and dogs brought to clinics in Kocaeli provinces.

Materials and methods: For this purpose, a general ear examination of 66 cats and 46 dogs brought to the clinic in Kocaeli were performed and the ear canal and eardrum were examined with an otoscope. In addition, a radiographic imaging method (x-ray), as well as microbiological and histopathological examinations were performed. After diagnosing the diseases, they were recorded and evaluations about ear diseases were analyzed statistically.

Results: As a result, the prevalence of ear disease in cats and dogs is high in Kocaeli province; It has been determined that improving the cleaning, feeding and sheltering conditions of animals will be effective in reducing these cases, and periodic clinical examinations will enable a fast and effective treatment process by early diagnosis of these diseases.

Keywords: *Cat, dog, Ear diseases, Parasites, Diagnosis*

INTRODUCTION

The ear, which has the function of providing balance in addition to the task of enabling living things to hear the sounds around them, is one of the important sense organs that directly affect the quality of life. In this context, identifying and treating ear diseases is very important in terms of animal health and quality of life.

Anatomically, the parts of the ear, which consist of three parts: the outer ear, the middle ear and the inner ear, are related to each other, and a negativity occurring in any part can affect other parts (Sasikala et al., 2011). Ear diseases generally have a progressive nature (Cabañes, 2020). For this reason, a late diagnosis of ear disease may cause the treatment process of the disease to be very difficult or treatment cannot be possible (Marignac, 2005). In recent years, an increase has been observed in the number of cats and dogs brought to clinics with complaints of head shaking and ear scratching (Fatjó and Bowen, 2020). Head shaking and scratching the ears, which are the most obvious symptoms of ear diseases, can also occur in cats and dogs without ear diseases in some cases. In addition, in some cats and dogs, ear diseases may not cause any symptoms until they reach an advanced level, or animal owners may notice these symptoms quite late. This makes it difficult to diagnose and treat ear diseases before they reach the chronic level. Diagnosis and treatment of ear diseases, which is one of the diseases that are frequently encountered with the increase in the pet animals population, reveals the importance of studies in this field. (Morris, 2004)

Aural hematoma, which is one of the most common auricular diseases in cats and dogs, is a trauma condition that occurs due to constant head

shaking or constant scratching of the auricle. Otitis externa is a disease that occurs in the structures in the external ear canal. Otitis media is an inflammation of the middle ear cavity and tympanic membrane. Otitis interna, which occurs in hereditary or advanced infections, causes cases such as difficulty in walking in animals, instability in behavior due to the effect of the balance part, severe dizziness called vertigo, and coordination disorder in movements. External ear canal foreign bodies, which are more common in dogs than cats, include splinters, grass spiders (flounder grass) and sand grains. Inflammatory polyps, which are common tumor formations in the nasopharynx and eustachian tube, can be seen more frequently in cats aged 1-1,5 years. One of the common diseases in cats and dogs is tumors. Tumors that can occur in the auricle skin of old cats and dogs can be seen more frequently in cats (Janssens et al., 2016). It is of great importance to make the correct diagnosis in order to achieve successful results regarding the course, results and treatment process of ear diseases. An effective treatment process can be planned and carried out by making an accurate diagnosis as a result of taking anamnesis and effective otoscopic, dermatological, radiological and laboratory examinations (Pamuk et al., 2009). Controlling the active inflammation in the ear is the first goal of the ear disease treatment process. For this reason, after the continuous and predisposing factors that are effective in the emergence of the disease are taken under control, the antecedents should be determined and eliminated (Janssens et al., 2016). After the ear is cleaned and local factors are determined and eliminated, the appropriate treatment method should be determined by evaluating the test results and physical examination findings obtained within the framework of the anamnesis (Girao et al., 2006). The methods commonly used in the treatment of chronic ear diseases in cats and dogs are vertical and horizontal ear canal drainage, total ear ablation, lateral and ventral bulla osteotomy. In this study, it was aimed to reveal the prevalence of ear diseases in cats and dogs in Kocaeli, the seasonal distribution of ear diseases and the race, age and gender distribution.

MATERIALS and METHODS

This study was approved by the ethics committee of Fırat University on 22.05.2019 and with the decision no. 2019/81.

In the study, 526 cats and 342 dogs brought to two clinics in Kocaeli between 01/06/2019-01/06/2020 were examined. 112 animals of different breeds, ages and sexes, 66 cats and 46 dogs, were brought to two clinics (Güneş and Selçuk Veterinary Clinic) in Kocaeli province with complaints such as scratching their ears, shaking their heads, ear discharge, keeping their heads tilted, and bad ear odor between these dates. After the clinical examination, otoscopic examinations were performed. Radiological, microbiological and histopathological examinations were performed for animals deemed necessary. "Otology Patient Registration Form" has been prepared in order to ensure that patient registration and follow-up can be carried out in a regular and systematic way, and all information about the patients' definitions, clinical findings, otoscopic findings, and radiographic findings are written on these forms. In the clinical examination that started with inspection, the animal's gait, posture, ear posture, head movements, balance and itching were observed and recorded on the form. For direct and indirect ear examination, the animals were taken to the examination table and the external ear canal and auricle were examined and conditions such as bad odor, redness, discharge, swelling, redness of the ear skin, shedding of the ear hair and external ear canal opening were evaluated. After the palpation examination, the animal was placed in the lateral position for otoscopic examination and the external auditory canal was cleaned with cotton-wrapped alligator forceps before the examination. Ear discharge and earwax were swapped from the animals encountered with ear discharge and bad odor before the cleaning process, and then the cleaning process was started. Scraping samples were taken from animals with crusting, skin rash and redness for native parasitological examination. After cleaning, the pinna was placed in a suitable position for otoscopic examination and the presence of a foreign body, wound, hyperemia, tumor and hyperplasia in the meatus acusticus externa was investigated and the membrane tympani was examined.

In the study, the data obtained from the otology patient registration forms, in which the examination data were recorded, were transferred to the SPSS package program and analyzed. While determining the frequency and percentage values in the evaluation of the demographic data of animals such as province, species, age, race,

gender, and qualitative data on disease diagnoses; Arithmetic mean, standard deviation, independent groups t-test and one-way analysis of variance (ANOVA) tests were used to determine the distribution of diseases according to characteristics such as province, season, age, race and gender.

RESULTS

Within the scope of the research, a total of 868 animals, 526 cats and 342 dogs were examined in Kocaeli. The incidence of ear diseases was determined to be 12.9% in Kocaeli province. Ear diseases were found to be 12.5% in cats and 13.5% in dogs (Table 1). Considering the distribution of the animals brought to the clinic according to the season in which they got sick, it was determined that 28% got sick in the spring, 35% in the summer, 20% in the autumn and 17% in the winter.

Table 1. Findings regarding the incidence of ear disease.

Province	Kind	Number of animals examined	Number of animals with ear disease	%
Kocaeli	Cat	526	66	12.5
	Dog	342	46	13.5
	Total	868	112	12.9

Table 2. Distribution of animals by species.

Kind	f	%
Cat	66	58.9
Dog	46	41.1
Total	112	100

Table 3. Distribution of the cats examined in the study according to their breeds.

Breed (cat)	f	%
Angora cat	1	1.5
Bombay	2	3.0
British shorthair	7	10.6
Persian cat	4	6.0
Crossbred	9	13.6
Sarman	5	7.5
Scottish fold	18	27.2
Siamese	1	1.5
Tekir	17	25.7
Tri color	2	3.0
Total	66	100

It is seen that 58.9% of the animals participating in the research are cats and 41.1% are dogs (Table 2).

In this study, it was seen that there was no statistically significant relationship between the breeds of cats and the diagnosis of the disease ($F=2.004$; $p>0.05$). When Table 3 is examined, the most diagnosed cat breeds are Scottish fold and Tabby breeds; otitis externa (34.9%) and ear mites (30.4%) were diagnosed most often in Scottish fold cats, while otitis externa (47.9%) and wound (17.5%) diagnoses were seen in tabby cats.

In the study, it was observed that there was no statistically significant relationship between the breeds of dogs and the diagnosis of the disease ($F=1.785$; $p>0.05$).

Table 4. Distribution of the dogs examined in the study according to their breeds.

Breed (Dog)	f	%
German shepherd	1	2.1
Cocker spaniel	3	6.5
Golden retriever	5	10.8
Kangal	6	13.0
King Charles spaniel	1	2.1
Greek hound	3	6.5
Labrador retriever	1	2.1
Labrador crossbred	1	2.1
Crossbred	12	26.0
Pekingese	1	2.1
Pomeranian	1	2,1
Pug	3	6.5
Rotweiller	1	2.1
Setter	4	8.6
Terrier	2	4.3
Yorkshire terrier	1	2.1
Total	46	100

Table 5. Distribution of cats examined in the study by age.

Age (Cat)	f	%
3 months	7	9.3
4 months	2	3.5
5 months	2	2.3
6 months	4	7.0
8 months	2	2.3
9 months	2	2.3
1 years	21	25.6
1,5 years	3	1.1
2 years	12	24.9
3 years	4	7.0
4 years	5	12.5
5 years	1	1.1
6 years	1	1.1
Total	66	100

Table 6. Distribution of the dogs examined in the study by age.

Age (Dog)	f	%
3 Months	1	1.2
4 Months	2	2.4
6 Months	2	2.4
7 Months	1	1.2
8 Months	2	2.4
1 years	10	21.5
2 years	9	21.5
3 years	6	19.4
4 years	4	9.4
5 years	5	11.4
6 years	1	2.4
7 years	1	2.4
9 years	1	1.2
10 years	1	1.2
Total	46	100

Table 7. Distribution of the cats examined in the study by gender

Gender (Cat)	f	%
Male	36	53.5
Female	30	46.5
Total	66	100

Table 8. Distribution of the dogs examined in the study by gender.

Gender (Dog)	f	%
Male	25	58.6
Female	21	41.4
Total	46	100

When the table is examined, it is seen that the most diagnosed dog breeds are the cross breeds and Kangal breeds; While it is seen that otitis externa (38.7%) and foreign body (32.2%) diagnoses are made most in cross breed dogs, it is seen that otitis externa (69.2%) and foreign body (15.4%) diagnoses are made in Kangal breed dogs. (Table 4).

It shows that the cats examined within the scope of the findings obtained according to the age of the cats in the study are concentrated in the young age group (Table 5).

Table 9. Distribution of diagnoses made to animals within the scope of the study.

Diagnosis	f	%
Wound	11	9.8
Otitis externa	40	35.7
Foreign body	17	15.2
Fungal disease	11	9.8
Otitis media	11	9.8
Ear scabies	12	10.7
Inflammation	2	1.8
Hematoma	4	3.6
Otitis interna	4	3.6
Total	112	100

It was seen that there was no statistically significant correlation between the ages of the cats examined and the diagnosis of the disease ($F=2.289$; $p>0.05$). When the table is examined, it is seen that the most diagnosed age group is cats in the 0-1 age group and 2-4 age group; while otitis externa (33.3%) and ear scabies (20.4%) diagnoses were the most common in cats in the 0-1 age group, otitis externa (57.6%) and fungal infections were observed in cats in the 2-4 age group. (14.8%).

Table 10. Distribution of disease diagnoses by seasons.

Diagnosis	Spring		Summer		Autumn		Winter		f	p
	f	%	f	%	f	%	f	%		
Wound	9	18.0	3	5.0	3	8.6	6	21.4	1.039	0.377
Dermatitis	-	-	1	1.7	-	-	-	-		
Eczema	-	-	1	1.7	-	-	1	3.6		
Otitis externa	19	38.0	25	45.8	20	57.3	10	35.8		
Foreign body	10	20.0	11	18.3	3	8.6	-	-		
Fungal	6	12.0	4	2.6	3	8.6	2	7.1		
Otitis media	2	4.0	5	8.3	2	5.7	3	10.7		
Ear scabies	3	6.0	6	10.0	1	2.8	2	7.1		
Inflammation	-	-	2	3.3	-	-	-	-		
Hematoma	-	-	2	3.3	1	2.8	1	3.6		
Otitis interna	1	2.0	-	-	1	2.8	3	10.7		
Myiasis	-		-	-	1	2.8	-	-		
Total	50	100	60	100	35	100	28	100		

Table 11. Distribution of disease diagnoses by species.

Diagnosis	Cat		Dog		t	p
	f	%	f	%		
Wound	10	11.6	11	12.6	3.683	0.057
Dermatitis	1	1.2	-			
Eczema	1	1.2	1	1.1		
Otitis externa	38	44.2	36	41.7		
Foreign body	5	5.8	19	21.9		
Fungal disease	11	12.8	4	4.6		
Otitis media	3	3.4	9	10.4		
Ear scabies	10	11.6	2	2.2		
Inflammation	2	2.4	-			
Hematoma	2	2.4	2	2.2		
Otitis interna	3	3.4	2	2.2		
Myiasis	-	-	1	1.1		
Total	86	100	87	100		

It is seen that there is no statistically significant correlation between the ages of the dogs examined within the scope of the study and the diagnosis of the disease (F=0.360; p>0.05). When the table is evaluated, it is seen that the most diagnosed age group is dogs in the 0-1 age group and 2-4 age group; While otitis externa (38.1%) and foreign body (18.5%) diagnoses were the most common in dogs aged 0-1 (Table 6), otitis externa (43.2%) and foreign body diagnoses were observed in cats aged 2-4 years. object (25.0%) diagnoses were made.

It is seen that there is no statistically significant relationship between the genders of the cats (Table 7) examined and the diagnosis of the disease (t=0.554; p>0.05).

Again, it is seen that there is no statistically significant relationship between the genders of the dogs (Table 8) examined and the diagnosis of the disease (t=0.942; p>0.05).

Table 12. Microbiological examination findings.

Type	Species	Years	Gender	Season	Right ear	Left ear
CAT	Bombay	1	Male	Autumn	No reproduction	No reproduction
	Persian cat	2	Female	Summer	No reproduction	No reproduction
	Persian cat	2	Male	Winter	No reproduction	No reproduction
	Scottish fold	1.5	Male	Spring	No reproduction	No reproduction
	Scottish fold	2	Female	Summer	No reproduction	No reproduction
	Scottish fold	4	Male	Spring	*E. coli*	*E. coli*
	Scottish fold	1	Female	Winter	*Proteus* spp.	*Proteus* spp.
	Scottish fold	4	Female	Summer	*Pseudomonas* spp.	*Pseudomonas* spp.
	Scottish fold	6	Male	Summer	*E. coli*	*E. coli*
	Scottish fold	1	Male	Summer	No reproduction	No reproduction
	Tekir	1	Male	Spring	*E. coli*	No reproduction
	Tekir	3	Female	Summer	No reproduction	No reproduction
	Tekir	1	Male	Autumn	*Pseudomonas* spp.	*Pseudomonas* spp.
DOG	Golden	3	Male	Summer	*Staphylococcus* spp.	*Staphylococcus* spp.
	Golden	3	Female	Autumn	No reproduction	No reproduction
	Kangal	4 months	Male	Spring	*Proteus* spp.	*Proteus* spp.
	Labrador	6	Female	Spring	No reproduction	No reproduction
	Labrador crossbred	10	Female	Spring	*Streptococcus* spp.	No reproduction
	Pug	5	Female	Spring	*Staphylococcus* spp.	*Staphylococcus* spp.
	Pug	3	Male	Summer	No reproduction	*Staphylococcus* spp.
	Pug	2	Female	Autumn	*Pseudomonas* spp. + *Streptococcus* spp.	*Pseudomonas* spp.
	Setter	8 months	Male	Spring	*Staphylococcus* spp.	No reproduction
	Setter	3	Male	Winter	*Proteus* spp.	No reproduction
	Terrier	3	Female	Summer	No reproduction	No reproduction
	York shire	2	Male	Winter	*E. coli*	*E. coli*

It was determined that there was no statistically significant difference between the diagnoses made in the patients brought to the clinic and the seasonal variable (Table 9, 10) (F = 1.039; p>0.05).

It is observed that there is no statistically significant correlation between the types of patients examined and the diagnosis of the disease (Table 11) (F=3,683; p>0.05).

Swap samples taken from a total of 25 animals, 13 cats and 12 dogs, were examined in the study and the findings were interpreted in the table (Table 12).

DISCUSSION

In this study, which aimed to determine the prevalence of ear diseases in cats and dogs in Kocaeli, this rate was determined to be 12.9%. In Kocaeli province, it was determined that it was 12.5% in cats and 13.5% in dogs. This rate obtained in the study is lower than the rates reported in studies conducted in Antalya (Güler, 2014) and Istanbul (Demirutku, 2007). It has been determined that the incidence of ear diseases differs between provinces and the incidence of ear diseases is higher in Kocaeli province. It is thought that the high temperature and humidity values in Kocaeli province have an effect on this finding obtained in the research.

Demirutku (2007) determined that the diagnosis of diseases was observed at similar rates in the cases of cats and dogs examined in his study, and the diagnoses of ear diseases did not differ statistically significantly depending on the species variable.

Demirutku (2007) examined the prevalence of ear diseases in a patient group consisting of 279 dogs and 35 cats, and examined the animals brought to the Surgery Department of Istanbul University Faculty of Veterinary Medicine between 2002-2007. In the study, the changes in ear diseases in cats and dogs depending on various characteristics such as species, gender, age and race were investigated. While 48 auricle, 233 external ear canal and 11 both auricle and external ear canal diseases were found in dogs examined within the scope of the research, 4 auricle, 29 external ear canal and 2 both auricle and external ear canal diseases were found in cats. While only auricular diseases are 20 wounds, 9 auricular hematomas, 6 dermatitis, 5 tumors, 3 deformations, 2 vasculitis, 1 abscess, 1 rupture and 1 necrosis; only external ear canal diseases, 193 of them otitis externa, 22 of them foreign body, 10 of them tumor, 2 of them membrana tympani perforation, 2 of them foreign body and otitis externa, 1 of them flounder-derived membrane tympani perforation and 1 of them ceruminolite; it has been determined that 5 cases of auricular hematoma and otitis externa, 3 cases of auricular deformation and otitis externa, 2 cases of wound and otitis externa, and 1 of them tumor and otitis externa are the cases where auricle and external ear canal diseases are seen together. Becerman (2019), in his study investigating the prevalence of ear diseases in 250 dogs in Diyarbakır Metropolitan Municipality animal care and rehabilitation center, determined ear diseases by clinical, radiographic and otoscopic examinations. In the study, 17% of ear disease diagnoses were auricular hematoma, 32% wound, 9% foreign body, 4% auricular abscess, 11% purulent discharge, 28% scabies, and 3% dermatitis. It was determined that 44.8% of them were otitis externa due to different reasons. In this study, otitis externa and foreign body cases were common in cats and dogs; It has been determined that secondary factors such as external ear canal shape, trauma, increase in cerumen and moisture level, obstruction and primary factors such as foreign bodies, allergies, ectoparasites are the main factors that play a role in the emergence of otitis externa in animals. In this context, it is thought that the greater exposure of the ear canal to these effects is effective in this finding. This finding obtained in the study shows parallelism with the findings of the studies in the literature.

Many ear diseases, especially otitis externa, are directly affected by humidity, temperature and precipitation, and an increase in otitis externa cases may occur depending on the increase in these factors. In studies, it is seen that the season with the highest incidence of otitis externa is summer, when the temperature and humidity are the highest, and ear diseases are observed more frequently in this season compared to other seasons (Carlotti 1991; Harvey et al., 2001; Krahwinkel, 2003; Rosychuk, 2005). It is thought that the fact that animals are exposed to more environmental effects due to the increase in the time they spend outside during the summer season is effective on this finding. Becerman (2019), in his study conducted in Diyarbakır province, stated that ear diseases in animals are more common in the summer months and this increases depending on temperature, humidity and precipitation conditions. This finding obtained

in the study is similar to the findings of the studies in the literature.

Güler (2014) examined a total of 335 animals, 173 cats and 162 dogs, in his study to determine the prevalence of ear diseases in cats and dogs brought to a pet clinic operating in Antalya. He examined the seasonal distribution of ear diseases in cats and dogs and determined that 43% of ear diseases were seen in spring and 30% in winter. Demirutku (2007), in his study in Istanbul, stated that the seasons are effective in the emergence of ear diseases, but he did not perform a statistical analysis revealing this relationship in his research. This finding differs from the finding obtained in the study. It is thought that this situation is due to the special care conditions and geographical conditions of the animals.

It was determined that ear scabies and fungal diagnoses were more common in cats, while foreign body and otitis media diagnoses were more common in dogs. It is thought that this difference is due to special reasons such as the anatomical structures of the animals, their care conditions and social environments. Demirutku (2007) examined the prevalence of ear diseases in 279 dogs and 35 cats in his study and found that the diagnoses of ear diseases did not differ significantly according to the species, but showed a similar distribution. This finding obtained in the study is similar to the findings obtained in the studies in the literature.

In addition to the anatomical structure of the ear, the racial variant can also have a significant effect on ear diseases. In the study, the most common cat breeds with ear disease were Scottish fold and tabby; It was determined that the dog breed was Kangal and crossbreds. It has been determined that otitis externa is the most common diagnosis in all cat breeds, but ear scabies diagnoses are more common in the Scottish fold breed than in other breeds. It is thought that the inward-curving structure of the ears of Scottish fold cats is effective in this finding. It is thought that the fact that tabby cats have erect and medium-sized ears is more effective in the occurrence of ear injuries than other breeds. It has been determined that foreign body diagnosis is more common in crossbred dogs than in other dog breeds. It was determined that the diagnosis of otitis externa was most common in Kangals. Otitis externa, hematoma and auricle sores are frequently encountered in dog breeds with droopy ears such as Golden, Cocker spaniel and Labrador (Cole, 2004; Smeak, 2011; Swaim and Bradley, 1996; Plunkett, 2002). In his study, Fossum determined that ear diseases did not differ significantly according to the race variable, but diseases such as otitis externa, wounds and foreign bodies were more common in breeds with long ears. Becerman (2019) examined the relationship between ear diseases and dog breeds in his study. It was determined that 60% of the dog breeds examined in the study were Terrier, Kangal, crossbreed and German shepherd breeds, that the distribution of the disease did not differ significantly depending on the breeds, and that the rate of disease was high in crossbred and Kangal dogs, which are breeds that do not have long ears. Angus (2004) determined in their study that the incidence of many types of diseases, especially otitis externa, is higher in animals with long and drooping ears. Cole (2004) stated in his study that 20% of cases of otitis externa were encountered in dogs with drooping and long ears. Güler (2014) examined the relationship between the distribution of ear diseases and animal breeds and determined that there was no statistically significant difference between ear diseases and the breed variable, but the incidence of ear disease was higher in Cocker, Labrador and Golden retriever breeds than in other breeds. This finding obtained in the study is similar to the findings obtained in the studies in the literature.

It is stated that ear diseases are more common in dogs between the ages of 5-8 and cats between the ages of 1-4 (Rosychuk, 2005; Krahwinkel, 2003). Demirutku (2007), in his study, determined that the incidence of ear disease in dogs is mostly concentrated in the 5-8 age group. Again, Güler (2014) determined in his study that ear diseases are more common in animals aged 4-7 years. In this study, it was determined that ear diseases were seen at a higher rate in dogs aged 1-4 in cats and dogs in the 0-4 age group. This finding differs from the findings in the studies in the literature. It is thought that this difference is due to the regional and climatic conditions in which the animals live and the different care conditions.

In the study, it was determined that ear diseases in cats and dogs were at similar levels in both female and male animals. Apaydın and Hasandayıoğlu (2018) stated in their study that there was no statistically significant relationship between the gender variables of dogs and the diagnosis of ear diseases, and that gender was not an effective factor in ear diseases. This finding obtained in the

research shows parallelism with the findings obtained in the studies in the literature.

As clearly stated in Table 12, microbiological evaluations are presented. And this finding is in line with the findings of Becerman (2019), Demirutku (2007), Apaydın and Hasandayıoğlu (2018) and Güler (2014) in their studies.

CONCLUSION

As a result, the prevalence of ear disease in cats and dogs is high in Kocaeli province; It has been determined that improving the cleaning, feeding and sheltering conditions of animals will be effective in reducing these cases, and periodic clinical examinations will enable a fast and effective treatment process by early diagnosis of these diseases.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

Author's Contributions: SB is responsible for the examination and diagnosis of cases. IC and MT are responsible for the evaluation and writing of the cases.

SB: Selçuk Başer, IC: İbrahim Canpolat, MT: Murat Tanrısever.

REFERENCES

Angus JC. Otic cytology in health and disease. Vet Clin North Am Small Anim. Pract. 2004; 34:411-424.

Apaydın N, Hasandayıoğlu Ö. Köpeklerde dış kulak yolu hastalıklarının video-otoskopik, ultrasonografik ve radyografik yöntemlerle belirlenmesi. Kocatepe Vet J. 2018; 11(1):63-69.

Becerman V. Diyarbakır Büyükşehir Belediyesi hayvan bakım ve rehabilitasyon merkezindeki köpeklerde görülen kulak hastalıklarının insidansının belirlenmesi (Master Thesis). Kayseri: Erciyes University, Institute of Health Sciences, 2019.

Cabañes FJ. Diagnosis of Malassezia dermatitis and otitis in dogs and cats, is it just a matter of counting? Rev Iberoam Micol. 2020; 21:1130-1406.

Carlotti DN. Diagnosis and medical treatment of otitis externa in dogs and cats. JSAP. 1991; 32:394-400.

Cole LK. Anatomy and physiology of the canine ear. Vet Dermatol. 2004; 34:412-421.

Demirutku A. Kedi ve köpeklerde dış kulak hastalıkları üzerine klinik incelemeler (Master Thesis) İstanbul: İstanbul University, Institute of Health Sicences, 2007.

Fatjó J, Bowen J. Behavior and medical problems in pet animals. Adv In Small Anim Care. 2020; 1:25-33.

Fossum TW. Surgery of the ear. In: Small Animal Surgery. Missouri: Saunders Elsevier; 2002. p.229-253.

Girao MD, Prado MR, Brilhante RSN, *et al.* Malassezia pachydermatis isolated from normal and diseased external ear canals in dogs: A comparative analysis. Vet J. 2006; 172:544-548.

Güler ZE. Antalya bölgesinde pet kliniğine getirilen köpeklerdeki kulak hastalıklarının sınıflandırılması ve prevalansı (Master Thesis). Afyon: Afyon Kocatepe University, Institute of Health Sciences, 2014.

Harvey RG, Harari J, Delauche AJ. Ear diseases of the dog and cat. London: Manson Publishing; 2001.

Janssens SDS, Haagsman AN, Haar GT. Middle ear polyps: results of traction avulsion after a lateral approach to the ear canal in 62 cats (2004–2014). JFMS. 2016; 1:1-6.

Krahwinkel DJ. External ear canal. In: Textbook of small animal surgery. Slatter D, ed. Philadelphia: Saunders; 2003. p.1746-1756.

Marignac G. Diseases that affect the pinna; small animal ear diseases: An illustrated guide. Missouri: Elsevier Sunders; 2005.

Morris DO. Medical therapy of otitis externa and otitis media. Vet Clin North Am Small Anim. 2004; 34:541-555.

Pamuk K, Sarıtaş K, Demirkan İ, Korkmaz M. Afyon Kocatepe Üniversitesi Veteriner Fakültesi Cerrahi Kliniğine getirilen hastaların değerlendirilmesi: 1090 olgu (2001-2008). Kocatepe Vet J. 2009; 2(1):29-33.

Plunkett SJ. Emergency procedures for the small animal veterinarian. London: Saunders Ltd; 2002.

Sasikala V, Saravanan M, Ranjithkumar M, Sarma K, Vijayakaran K. Management of ear mites in cats. Indian Pet Journal. 2011; 11:5-53.

Smeak DD. Management of complications associated with total ear canal ablation and bulla osteotomy in dogs and cats. Vet Clin North Am Small Anim. Pract. 2011; 41:981-994.

Swaim SF, Bradley DM. Evaluation of closed-suction drainage for treating auricular hematomas. J Am Anim Hosp Assoc. 1996; 32:36-43.

Rosychuk W. Veterinary endoscopy for the small animal practitioner. USA: Elsevier; 2005.

Original Article https://doi.org/10.47748/tjvr.1120029

TJVR 2022; 6 (2): 61-66

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Comparison of fertility parameters in Romanov sheep synchronized with progesterone-based protocol plus PMSG or GnRH

Barış Güner[1] Ebru Karakaya Bilen[2]

[1] *Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Türkiye*
[2] *Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye.*

Correspondence: *Ebru Karakaya Bilen (ebilen@siirt.edu.tr)*

Received: 23.05.2022 Accepted: 08.07.2022

ABSTRACT

Objective: The aim of this study was to compare the fertility parameters in response to pregnant mare serum gonadotropin (conventional treatment) or gonadotrophin-releasing hormone (alternative treatment) in Romanov sheep subjected to a 7-d short-term protocol during non-breeding season.

Materials and Methods: All sheep (n:57) were subjected to short-term synchronization protocol. Intravaginal sponge impregnated with 20 mg fluorogestone acetate was inserted for 7 days and all sheep received 125 µg cloprostenol at sponge removal. Sheep were randomly assigned to receive no additional treatment (CON, n:16), 240 IU pregnant mare serum gonadotropin (PMSG, n:24) at sponge removal or 10 µg buserelin acetate (GnRH, n:17) at 30 h after sponge removal. Natural mating was performed following detection of estrous with fertile eight Romanov rams. Estrous response, pregnancy rate, lambing rate, and litter size were compared among groups.

Results: Estrous response and pregnancy rate were 86% and 75.4% in all sheep, respectively. Estrous response was numerically higher about 7% ($p>0.05$) in treatment groups (PMSG, 87.5%; GnRH, 88.2%) than CON (81.2%). However, pregnancy rate was numerically higher ($p>0.05$) in PMSG (83.3%) than GnRH (70.6%) and CON (68.7%). Similarly, lambing rate in the PMSG (79.1%) was approximately 15% numerically greater ($p>0.05$) than in GnRH (64.7%) and CON (62.5%). In addition, litter size in PMSG (2.1) was also numerically higher ($p>0.05$) than GnRH (1.9) and CON (1.9).

Conclusion: The use of GnRH provided similar estrous response compared to use of PMSG in Romanov sheep synchronized with short-term protocol. However, use of PMSG provided numerically higher pregnancy rate, lambing rate, and litter size than use of GnRH. Considering the serious ethical concerns and animal welfare for the production of PMSG, it is necessary to use alternative gonadotropins. Comprehensive studies are needed to compare the fertility parameters between application of PMSG and GnRH in Romanov sheep.

Keywords: *PMSG, GnRH, Fertility, Romanov, Sheep*

INTRODUCTION

Raising lambs for slaughter during winter allows the farmers to take advantage of the higher prices of lamb products (Abecia et al., 2012). The majority of sheep breeds perform different reproduction activities depending on season, feeding regime, latitude/longitude, the length of the photoperiod (Doğan et al., 2006). Reproduction of sheep is commonly controlled with hormone-based synchronization protocols including progesterone releasing devices/sponges (Guner et al., 2022).

Intravaginal device/sponge impregnated with progestagen is used for 12 to 14 days (long period) to mimic luteal phase of estrous cycle in sheep (Ungerfeld and Rubianes, 2002). However, similar or better fertility rates was reported after a short-term (5 to 7 days) progesterone-based synchronization protocol by reducing long-term progesterone exposure in previous studies (Vinoles et al., 2001; Martinez-Ros et al., 2019a; Guner and Saat, 2021).

It is well known that pregnant mare serum gonadotropin (PMSG) is frequently used to stimulate the estrous behavior and ovulation at progesterone sponge/device removal (Bruno-Galarraga et al., 2021). However, the production of PMSG from pregnant mares is considered to be a serious ethical concern (Vilanova et al., 2019). Current ethical concerns on animal welfare may lead to ban the production and using PMSG in future (Santos-Jimenez et al., 2020). Additionally, previous studies reported that repeated use of PMSG associated with PMSG neutralizing antibodies decreased fertility in sheep (Roy et al., 1999; Maurel et al., 2003; Guner et al., 2022). There have been inconsistency fertility rates after the use of gonadotropin releasing hormone (GnRH) as alternative gonadotropin instead of PMSG in progesterone-based synchronization protocols in different breeds (Reyna et al., 2007; Martemucci and D'Alessandro, 2011; Silva et al., 2015; Martinez-Ros and Gonzalez-Bulnes, 2019; Santos-Jimenez et al., 2020). There have been limited studies reporting fertility after progesterone-based synchronization protocol in Romanov sheep (Macías-Cruz et al., 2013; Martinez-Ros et al., 2019a). Besides, there has been no report related to efficacy of different gonadotropin (PMSG or GnRH) on fertility in Romanov sheep in literature. The objectives of the present study were to determine the efficacy of GnRH as alternative gonadotropin on estrous response and pregnancy rate in Romanov sheep.

MATERIALS and METHODS

The experimental procedures were approved by the Siirt University Animal Care Committee (Reference No. 2019-06).

Animals and management

This study was conducted on a total of 65 Romanov sheep, between the ages of 1-4, during non-breeding season (April-May) and housed under the same care conditions at Siirt University Goat Research and Application Center (37° 56' N, 41° 56' E). Sheep were fed with 3 kg of lentil hay and 400 g concentrate feed per head per day. Flushing was not applied to the sheep throughout the study.

Study design

Romanov sheep (n:65) were subjected to short-term synchronization protocol (Martinez-Ros et al., 2019a) and an intravaginal sponge containing 20 mg of fluorogestone acetate (Chronogest®, İntervet, Turkey) was inserted for 7 days. However, study was completed with 57 animals due to the absence of sponge before the sponge removal in 8 sheep. Sheep (n:57) were allocated to three groups including two treatments and one control group. All sheep received 125 μg cloprostenol (Estrumate®, İntervet, Turkey) at the time of sponge removal. No additional hormone was administered after application of 125 μg cloprostenol in the control group (CON, n:16). Sheep in PMSG group (n:24) received 240 IU of pregnant mare serum gonadotropin (PMSG; Chronogest PMSG®, İntervet, Turkey) at sponge removal. Sheep in GnRH group (n =17) received 10 μg buserelin acetate (GnRH, Receptal®, Intervet, Turkey) at 30 h after sponge removal. Estrous detection was made by teaser Romanov rams, starting 24 h after the sponge withdrawal for 3 days. Eight rams, known to be fertile, were used for natural breeding. A pregnancy diagnosis was performed via transrectal ultrasound (Easi-Scan equipped with a 4.5 MHz - 8.5 MHz; IMV, USA) at 30 days post natural mating. Number of single, twins or triplets was determined to calculate the litter size which is defined as number of lambs/total number of sheep that lambing.

Statistical analysis

The SPSS® 25.0 package program (SPSS Inc., Chicago, IL, USA) was used in the statistical analysis. Chi-square test was used to compare the estrous response, pregnancy rate, lambing rate, and litter size. The significance level was considered at $p<0.05$ for all analyzes.

RESULTS

The overall estrous response was 86.0% after short-term progesteron-based synchronization protocol in Romanov sheep in the present study. Considering the difference among groups, estrous response was numerically about 7% higher ($p>0.05$) in treatment groups (PMSG, 87.5%; GnRH, 88.2%) than control group (81.2%). Irrespective of

groups, overall pregnancy rate was 75.4% in all sheep. Although there was no statistical difference (p>0.05) in the pregnancy rate among groups, PMSG group (83.3%) was numerically higher than GnRH (70.6%) and CON (68.7%) groups (Table 1). The pregnancy loss interval from the first pregnancy examination to lambing was 7.0% (3/43) in this study. Similar to pregnancy rate, lambing rate in the PMSG group was approximately 15% numerically greater (p>0.05) (79.1%) than GnRH (64.7%) and CON (62.5%) groups (Table 1). There was no difference (p>0.05) in the percentage of single (20%, 70%, 10%), twin (36.4%, 36.4%, 27.2%), and triplets (21%, 47.4%, 31.6%) among CON, GnRH and PMSG groups, respectively. Irrespective of groups, the percentage of Romanov sheep that gave birth single (25%), twin (50%), and triple (25%) lamb. The number of lambs, in sheep that gave birth, ranged from 1 to 3 and the mean of litter size was 2.00±0.09. Litter size was numerically higher (p>0.05) in PMSG group (2.1) than GnRH (1.9) and CON (1.9) groups.

Table 1. Reproductive parameters in Romanov sheep received different gonadotropins with progesterone-based synchronization protocol

Reproductive Parameters	CON (n:16)	GnRH (n:17)	PMSG (n:24)	P value
Estrous detection rate (%)	81.2 (13)	88.2 (15)	87.5 (21)	NS
Pregnancy rate (%)	68.7 (11)	70.6 (12)	83.3 (20)	NS
Lambing rate (%)	62.5 (10)	64.7 (11)	79.1 (19)	NS
Litter size	1.9 (19/10)	1.9 (21/11)	2.1 (40/19)	NS

CON: sheep received no treatment after sponge removal, GnRH: sheep received 10 μg busereline acetate at 30 h after sponge removal, PMSG: sheep received 240 IU pregnant mare serum gonadotropin at the time of sponge removal, NS: not significant

DISCUSSION

Romanov is one of the most prolific breeds that provides higher reproductive efficiency in Turkey (Kutluca Korkmaz and Emsen, 2016). As in Turkey, farmers have practically implemented the crossbreeding the purebred Romanov sheep with other domestic sheep to maximize productivity in many countries (Đuričić et al., 2019; Murphy and Freking, 2021). However, Romanov is aseasonally polyestrous breed and distribution of lambing was not equal the throughout year (Đuričić et al., 2022). Therefore, progesterone-based synchronization protocol is widely used to gain higher income with more lamb production for slaughtering in Romanov sheep during winter (Murphy and Freking, 2021).

Pregnant mare serum gonadotropin is commonly applied in conjunction with progesterone-based synchronization protocols to increase estrous response, ovulation rate, pregnancy rate, litter size in sheep (Abecia et al., 2012). Unlike to common dose of PMSG (500 IU) in non-prolific breeds (Barrett et al., 2004), using a low dose of PMSG (250-300 IU) is sufficient to provoke estrous behavior and multiple lambing in prolific breeds such as Romanov (Macías-Cruz et al., 2013). Additionally, using low dose of PMSG decrease the cost of synchronization protocol (Macías-Cruz et al., 2013). Besides, the major concern for PMSG is collection of high blood from pregnant mares with unethical condition (Vilanova et al., 2019) and the development of PMSG neutralizing antibodies following repetition use (Guner et al., 2022). Therefore, recent studies focused on the determination of the efficacy of alternative gonadotropin such as gonadotropin-releasing hormone (GnRH) in conjunction with progesterone-based synchronization protocol in sheep (Martinez-Ros and Gonzalez-Bulnes, 2019; Santos-Jimenez et al., 2020.

In the present study, estrous response was 86% following short-term (7 d) progesterone-based synchronization protocol during non-breeding season in this study. Consistent with our results, higher estrous response ranging from 77.1 to 100% was reported after short or long-term progesteron-based synchronization protocol in different breeds (Ataman et al., 2006; Ustuner et al., 2007; Martinez-Ros et al., 2019a; Guner and Saat, 2021). Similar to our results, estrous response was higher (100%) following progesterone-based protocol in crossbreed Romanov sheep (Macías-Cruz et al., 2013). Additionally, estrous response was numerically about 7% higher in treatment groups (GnRH and PMSG) than in control group. Consistent with our results, Cavalcanti et al. (2012) reported that GnRH administration at 24 h after

short-term protocol did not change (95.2% vs. 100%) the estrous response compared to the control group (Cavalcanti et al., 2012). Martinez-Ros and Gonzalez-Bulnes (2019b) and Santos-Jimenez et al. (2020) reported that estrous response was equal (89.5%) or similar (88.9% vs. 94.5%, respectively) after either short-term CIDR-56h-GnRH or CIDR-PMSG protocol.

Contrary to previous reports, administration of GnRH after sponge removal statistically reduced the estrous response from 92.3 to 66.6% (Martemucci and D'Alessandro, 2011) and from 90 to 30% (Silva et al., 2015) in different breed sheep synchronized short-term protocol. Administration of GnRH is recommended at least 24–36 h after progestagen removal or luteolysis. However, acceleration of LH surge within 1–4 h post-administration may induce premature ovulation of pre-ovulatory follicles and may not allow secrete adequate estradiol for estrous behavior (Silva et al., 2015; Martinez-Ros and Gonzalez-Bulnes, 2019b). Similar estrous response demonstrated that administration of GnRH at 30 h after sponge removal did not suppress estrous behavior compared to PMSG in Romanov sheep in this study. Unlike Romanov (aseasonally polyestrous breed), application of GnRH is not recommended due to inadequate follicle development during non-breeding season in non-prolific sheep. Considering the importance of breed (Romanov) differences, Ben Saïd et al. (2007) reported that prolific breeds (Romanov) require a very small estradiol signal to induce estrous behavior and ovulation rates compared to non-prolific breeds. However, small difference in estrous response in treatment groups compared to control group could have resulted from less estradiol response needed to stimulate estrous behavior n Romanov sheep.

In previous studies reported that long-term progesterone-based synchronization protocols lead to extension of the lifespan of the ovulatory follicle, sub-luteal progesterone concentration at sponge removal, higher risk of vaginitis, and low fertility rates (Vinoles et al., 1999; Ungerfeld and Rubianes, 2002). In a comprehensive study conducted on 1750 sheep (Menchaca et al., 2018), a higher pregnancy rate in 6 days protocol (43.5%) compared to 14 days protocol (37.8%) confirmed the reports of previous studies. Although short-term synchronization protocols offer similar or higher fertility rates, short-term protocols are less widely used by practitioners due to unaware of these detrimental effects compared to long-term protocols (Menchaca et al., 2018; Martinez-Ros et al., 2019a; Uriol et al., 2019). Several retrospective studies revealed that Romanov breed had primarily superior fertility rate (from 92 to 95.9%) during breeding season (Casas et al., 2005; Đuričić et al., 2019; Đuričić et al., 2022). The pregnancy rate was 75.4% after short-term synchronization protocol regardless of groups in this study. Similar to our results, Martinez-Ros et al. (2019a) determined the pregnancy rate as 78.9% following short-term (7-day) progesterone-based synchronization protocol in Segureña×Romanov breed. In the present study, the pregnancy rate was higher than previous report (65%) that was carried out 12-day progesterone-based synchronization protocol in Romanov × Pelibuey breed (Macías-Cruz et al., 2013). Our result was within the range of pregnancy rates (66.7 to 85.7%) after short-term progesterone-based synchronization protocol in non-prolific different breeds such as Merino-Akkaraman, Kıvırcık, and Awassi in Turkey (Ataman et al., 2006; Özyurtlu et al., 2011; Guner and Saat, 2021).

Considering the pregnancy rate among groups, numerically higher pregnancy rate was obtained in PMSG group (83.3%) than those in GnRH (70.6%) and CON (68.7%) in this study. Similar to higher pregnancy rate, lambing rate and litter size was also numerically higher in PMSG group compared to other groups in this study. It was reported that Romanov breed had three times more granulosa cells in their preovulatory follicles than other prolific breeds (Ricordeau et al., 1990; Macías-Cruz et al., 2013). Low PMSG doses could be sufficient to not only increase estradiol levels by improving growth of antral and non-ovulatory follicles but also postovulatory luteal function (Macías-Cruz et al., 2013; Gonzalez-Bulnes et al., 2020). However, GnRH has no effect on growth or regression of subordinate or ovulatory follicles (Reyna et al., 2007). Additionally, there has been limited information related to fertility results by comparing the GnRH and PMSG in progesterone-based synchronization protocol. Similar to our results, Martinez-Ros and Gonzalez-Bulnes (2019b) reported that pregnancy rate was approximately 10% (68.4% vs. 57.9%) lower with administration of GnRH at 56 h after sponge removal compared to PMSG. Although the time of GnRH application was equal in our study, the use of GnRH drastically decrease the pregnancy rate from 92.3% to 33.3% compared to use of PMSG in crossbreed Altamurana sheep (Martemucci and D'Alessandro,

2011). Most reports indicated that use of GnRH (at ~48 h after sponge removal) was co-administered with PMSG instead of single administration of GnRH. However, the pregnancy rate decreased by approximately 10% (Zonturlu et al., 2018) or 20% (Cavalcanti et al., 2012) in sheep received GnRH and PMSG compared to single administration of PMSG in synchronization protocol.

CONCLUSION

In conclusion, alternative gonadotropins are required instead of using PMSG due to ethical concerns in PMSG production and welfare of pregnant mares in progesterone-based synchronization protocol in sheep. The use of GnRH at 30 h after sponge removal was sufficient to show similar estrous behavior compared to low dose PMSG in Romanov sheep. However, numerically higher pregnancy rate, lambing rate, and litter size were obtained with the use of PMSG in sponge removal compared to use of GnRH. Further comprehensive studies are needed to compare the fertility parameters between PMSG and GnRH in Romanov sheep.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

Author's Contributions: All authors (EKB and BG) contributed to the study design. EKB performed synchronization protocols. EKB and BG drafting and revising the manuscript.

EKB: Ebru Karakaya Bilen, BG: Barış Güner

REFERENCES

Abecia J, Forcada F, González-bulnes A. Hormonal control of reproduction in small ruminants. Anim Reprod Sci. 2012; 130:173-179.

Ataman MB, Aköz M, Akman O. Induction of synchronized oestrus in akkaraman cross-bred ewes during breeding and anestrus seasons: The use of short-term and long-term progesterone treatments. Rev Med Vet. 2006; 157:257-260.

Barrett DMW, Bartlewski PM, Batista-Arteaga M, Symington A, Rawlings NC. Ultrasound and endocrine evaluation of the ovarian response to a single dose of 500 IU of eCG following a 12-day treatment with progestogen-releasing intravaginal sponges in the breeding and nonbreeding seasons in ewes. Theriogenology. 2004; 61:311-327.

Ben Saïd S, Lomet D, Chesneau D, *et al.* Differential estradiol requirement for the induction of estrus behavior and the luteinizing hormone surge in two breeds of sheep. Biol Reprod. 2007; 76:673-680.

Bruno-Galarraga M, Cano-Moreno V, Lago-Cruz B, Encinas T, Gonzalez-Bulnes A, Martinez-Ros P. The use of hcg for inducing ovulation in sheep estrus synchronization impairs ovulatory follicle growth and fertility. Animals. 2021; 11:10-17.

Casas E, Freking BA, Leymaster KA. Evaluation of Dorset, Finnsheep, Romanov, Texel, and Montadale breeds of sheep: III. wool characteristics of F1 ewes. J Anim Sci. 2005; 83:2743-2751.

Cavalcanti AS, Brandão FZ, Nogueira LAG, da Fonseca JF. Effects of GnRH administration on ovulation and fertility in ewes subjected to estrous synchronization. Rev Bras Zootec. 2012; 41:1412-1418.

Dogan I, Nur Z. Different estrous induction methods during the non-breeding season in Kivircik ewes. Vet Med. 2006; 51:133-138.

Đuričić D, Benić M, Žaja IŽ, Valpotić H, Samardžija M. Influence of season, rainfall and air temperature on the reproductive efficiency in Romanov sheep in Croatia. Int J Biometeorol. 2019; 63:817-824.

Đuričić D, Dobos A, Grbavac J, *et al.* Climate impacts on reproductive performance of Romanov sheep in the moderate climate. J Anim Behav Biometeorol. 2022; 10(1):1-6.

Gonzalez-Bulnes A, Menchaca A, Martin GB, Martinez-Ros P. Seventy years of progestagen treatments for management of the sheep oestrous cycle: Where we are and where we should go. Reprod Fertil Dev. 2020; 32:441-452.

Guner B, Kulaksiz R, Saat N, *et al.* Effect of pre-synchronisation with progestogen and eCG on reproductive activity in synchronised ewes during anoestrous season. Vet Med. 2022; 67(5):231-239.

Guner B, Saat N. Comparison of pregnancy rates after short-term and long-term synchronization protocol in ewes- Pilot study. Erciyes Üniv Vet Fak Derg. 2021; 13(3):69-74.

Kutluca Korkmaz M, Emsen E. Growth and reproductive traits of purebred and crossbred Romanov lambs in Eastern Anatolia. Anim Reprod. 2016; 13:3-6.

Macías-Cruz U, Ponce-Covarrubias JL, Álvarez-Valenzuela FD, Correa-Calderón A, Meza-Herrera CA, Avendaño-Reyes L. Reproductive efficiency of Pelibuey and Romanov × Pelibuey ewes synchronized with synthetic progesterone and low doses of PMSG under a hot environment. Czech J Anim Sci. 2013; 58(12):546-553.

Martemucci G, D'Alessandro AG. Synchronization of oestrus and ovulation by short time combined FGA, PGF2α, GnRH, eCG treatments for natural service or AI fixed-time. Anim Reprod Sci. 2011; 123:32-39.

Martinez-Ros P, Gonzalez-Bulnes A, Garcia-Rosello E, Rios-Abellan A, Astiz S. Effects of short-term intravaginal progestagen treatment on fertility and prolificacy after natural breeding in sheep at different reproductive seasons. J Appl Anim Res. 2019a; 47(1):201-205.

Martinez-Ros P, Gonzalez-Bulnes A. Efficiency of CIDR-based protocols including GnRH instead of eCG for estrus synchronization in sheep. Animals. 2019b; 9(4):1-11.

Maurel MC, Roy F, Hervé V, *et al.* Réponse immunitaire à la eCG utilisée dans le traitement de l'induction d'ovulation chez la chèvre et la brebis. Gynecol Obstet Fertil. 2003; 31(9):766-769.

Menchaca A, dos Santos-Neto PC, Cuadro F, Souza-Neves M, Crispo M. From reproductive technologies to genome editing in small ruminants: An embryo's journey. Anim Reprod. 2018; 15:984-995.

Murphy TW, Freking BA. Comparison of performance of F1 Romanov crossbred ewes with wool and hair breeds during fall lambing and body weight and longevity through six production years. J Anim Sci. 2021; 99(1):1-7.

Özyurtlu N, Ay S, Küçükaslan İ, Güngör Ö, Aslan S. Effect of subsequent two short-term, short-term, and long-term progestagen treatments on fertility of Awassi ewes out of the breeding season. Ankara Üniv Vet Fak Derg. 2011; 58(2):105-109.

Reyna J, Thomson PC, Evans G, Maxwell WMC. Synchrony of ovulation and follicular dynamics in Merino ewes treated with GnRH in the breeding and non-breeding seasons. Reprod Domest Anim. 2007; 42:410-417.

Ricordeau G, Thimonier J, Poivey JP, Driancourt MA, Hochereau De Reviers MT, Tchamitchian L. I.N.R.A. Research on the Romanov sheep breed in France: a Review. Livest Prod Sci. 1990; 24:305-332.

Roy F, Maurel MC, Combes B, *et al.* The negative effect of repeated equine chorionic gonadotropin treatment on subsequent fertility in Alpine goats is due to a humoral immune response involving the major histocompatibility complex. Biol Reprod. 1999; 60(4):805-813.

Santos-Jimenez Z, Martinez-Herrero C, Encinas T, Martinez-Ros P, Gonzalez-Bulnes A. Comparative efficiency of oestrus synchronization in sheep with progesterone/eCG and progesterone/GnRH during breeding and non-breeding season. Reprod Domest Anim. 2020; 55:882-884.

Silva BDM, Silva TASN, Moreira NH, *et al.* Ovulation induction in ewes using GnRH in long and short-term synchronization protocols. Anim Reprod. 2015; 12:312-315.

Ungerfeld R, Rubianes E. Short term primings with different progestogen intravaginal devices (MAP, FGA and CIDR) for eCG-estrous induction in anestrus ewes. Small Rumin Res. 2002; 46(1):63-66.

Uriol M, Martinez-Ros P, Rios A, Encinas T, Gonzalez-Bulnes A. Onset of oestrus and periovulatory events in sheep exposed to 5 and 14 days of CIDR treatment with and without eCG. Reprod Domest Anim. 2019; 54:1489-1492.

Ustuner B, Gunay U, Nur Z, Ustuner H. Effects of long and short-term progestagen treatments combined with PMSG on oestrus synchronization and fertility in Awassi ewes during the breeding season. Acta Vet Brno. 2007; 76:391-397.

Vilanova XM, De Briyne N, Beaver B, Turner PV. Horse welfare during equine chorionic gonadotropin (eCG) production. Animals. 2019; 9(12):1-10.

Vinoles C, Forsberg M, Banchero G, Rubianes E. Effect of long-term and short-term progestagen treatment on follicular development and pregnancy rate in cyclic ewes. Theriogenology. 2001; 55:993-1004.

Vinoles C, Meikle A, Forsberg M, Rubianes E. The effect of subluteal levels of exogenous progesterone on follicular dynamics and endocrine patterns during the early luteal phase of the ewe. Theriogenology. 1999; 51:1351-1361.

Zonturlu AK, Kaçar C, Kaya S, Emre B, Korkmaz Ö, Arı UÇ. Effect of double GnRH injections on reproductive parameters in Awassi ewes receiving long-term progesterone. J Appl Anim Res. 2018; 46:1103-1107.

Original Article https://doi.org/10.47748/tjvr.1117331

TJVR 2022; 6 (2): 67-71

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Perceptions of students about the use of plastination in anatomy lessons

Barış Can Güzel[1] Saime Betül Baygeldi[1] Zait Ender Özkan[1]

[1] *Department of Anatomy, Faculty of Veterinary Medicine, Fırat University, Elazığ, Türkiye*

Correspondence: *Saime Betül Baygeldi (sbaygeldi@firat.edu.tr)*

Received: 16.05.2022 Accepted: 17.07.2022

ABSTRACT

Objective: This study was to examine the opinions of first-year veterinary faculty students about the use of plastinated anatomic prosections in addition to wet cadavers in anatomy practical lessons.

Materials and Methods: The students were shown plastinated organs and organs fixed in formaldehyde. Then a questionnaire comprising 7 questions was administered to the 100 student participants. The questionnaire responses were analyzed statistically using SPSS vn. 22.0 Frequencies software.

Results: In response to question 1, 58.4% of the students thought that there was no anatomic difference between the plastinated organs and the organs fixed in formaldehyde. In contrast to the strong smell of formaldehyde, 88% of the students stated that the plastinates were odourless. The use of plastinated organs was thought to improve the quality of education by 89.1% of the students, compared to the negative aspects of formaldehyde. It was stated by 84.2% of the students that plastinated organs should be included in anatomy lessons due to the thoughts of the majority of the students that plastinated tissues could make a greater contribution to anatomy lessons. In response to the final question, 92.1% of the students stated that they felt no abhorrence of the plastinated organs.

Conclusion: Plastination may be especially useful for educational institutions without access, space, or the financial resources for dissection, and can emphasize unique or pathological samples. The results of this study demonstrated that plastinated samples were perceived as a useful addition to traditional resources in the teaching of anatomy.

Keywords: *Anatomy, Plastination, Questionnaire*

INTRODUCTION

The rapid tempo of technological and economic developments has brought about greater demands from education systems. The most important requirements for students are to focus on the importance of lifelong learning to be able to adapt to global changes, to continuously develop knowledge and skills, to think critically, and to inspire creativity and innovation (Integrated ICT into education, 2009).

In the education and training of medical, dental and veterinary practitioners, anatomy is one of the most important and clinically relevant syllabus requirements. In recent years there have been significant changes in the teaching of anatomy to meet the demands and developments of syllabus design (Drake, 1998; Drake et al., 2009; Pyle, 2012; Souza and Devi, 2014). Although many anatomists prefer the use of dissection rather than other teaching tools, the debate is still ongoing as to whether cadaver dissections are appropriate or not in anatomy education (Patel and Moxham, 2006; Korf et al., 2008; Drake et al., 2009; Pyle, 2012; Estai and Bunt, 2016).

Formaldehyde (FA), which is said to be a carcinogenic agent is widely used in anatomy laboratories as a solution of formaldehyde and water. At room temperature, formaldehyde is a colourless gas, and can be determined by the smell in concentrations of 0.5-1.0 parts formaldehyde in a million parts of air (parts per million–ppm) (Costa and Amdur, 1996). Formaldehyde constitutes an occupational risk in both classroom and laboratory classes for anatomy instructors who are counseling students and conducting administrative activities and research. In addition, students who are working with tissues preserved in formaldehyde and instructors who are demonstrating or supervising students conducting certain dissection or prosection activities are at risk of exposure to formaldehyde during anatomy laboratory sessions (Mirabelli et al., 2011).

In laboratories where tissues are preserved in formaldehyde-based solutions, the routes to potential formaldehyde exposure are (a) absorption through the respiratory tract with inhalation through the nose or mouth, (b) absorption through the skin after dermal contact, (c) splashes into the mouth which is swallowed or from eating or smoking using the hands, (d) absorption into the digestive system through injection. As mummified tissues in particular are close to the respiratory regions of the students and instructors, the risk of inhalation exposure is high (Costa and Amdur, 1996). Acute formaldehyde exposure is associated with the destruction of the eyes, nose, throat, and respiratory pathways. Long-term exposure has been associated with mild neurological symptoms such as headache, dizziness, and genetic damage. The classification of the carcinogenity of formaldehyde is based to a large extent on carcinogenity in human nasal pathways and genotoxicity in human lungs and nasal epithelial cells, and in rodent lung epithelial cells (IARC, 2006; ATSDR, 2010).

Instructors and students want to be able to use a truly ideal educational material which is odorless, dry, robust and resistant, also protecting their health, without the need to use protective equipment such as gloves and masks (Bilge et al., 2014).

The desire to preserve human organic tissue is as old as humanity. In the past, different mummification methods have been developed but none have been defined as excellent. Plastination is one of the newest and most ideal preservation methods.

Plastination is an organic tissue preservation method which is widely used in anatomy to produce durable anatomic samples of the whole body or body parts. Plastination has been developed as much for research as for teaching purposes (Sora et al., 2019). It is an alternative tissue preservation technique that was developed by Dr von Hagens at the end of the 1970s (von Hagens, 1979).

It is defined by the International Plastination Association as a tissue preservation technique in which "the fluids and lipids in biological tissues are replaced with curable polymers resulting in hardened, dry, odorless, and durable samples" (International Society for Plastination, 2016). The general public has become familiar with plastination through exhibitions at the Body Worlds and Body Works museums (BODIES, 2016; Klaus et al., 2018).

The aim of this study was to examine the opinions of first-year veterinary faculty students about the use of plastinated anatomic prosections in addition to wet cadavers in anatomy practical lessons.

MATERIALS and METHODS

The study was conducted in the Anatomy Department of Firat University Veterinary Faculty. First, the students were shown plastinated organs and organs fixed in formaldehyde. Then a questionnaire comprising 7 questions was administered to the 100 student participants. The questionnaire responses were analyzed statistically using SPSS vn. 22.0 Frequencies software.

The questions on the questionnaire were:

Is there an anatomic structural difference between plastinated samples and samples fixed in formaldehyde?

Is there an odour to plastinated samples?

Can you touch plastinated samples without gloves?

Do you think that plastinated samples are healthier than samples fixed in formaldehyde?

Do you think that the use of plastinated samples improves the quality of your education?

Would you like to use plastinates in all anatomy lessons?

Do you feel any abhorrence when touching plastinated samples?

RESULTS

The questionnaire responses given by the students are shown in Figure 1.

In response to question 1, 58.4% of the students thought that there was no anatomic difference between the plastinated organs and the organs fixed in formaldehyde.

In contrast to the strong smell of formaldehyde, 88% of the students stated that the plastinates were odourless.

Although cadavers were touched using gloves in the majority of laboratory lessons, it was stated by 93.1% of the students that plastinated organs could be touched without gloves.

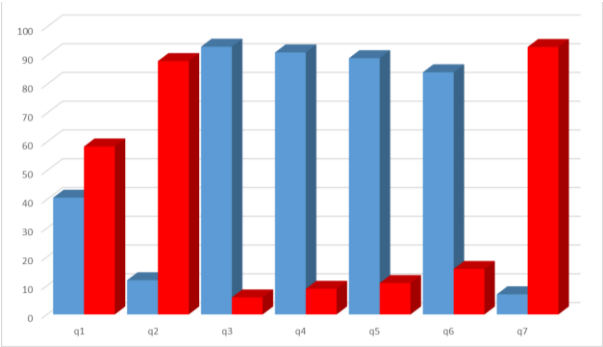

Figure 1. In the graphic columns of the answers received from the students, the blue "Yes" represents the red "No" answers.

It was reported by 91.1% of the students that plastinated tissues were healthier, whereas chemical remnants were present on organs fixed with formaldehyde.

The use of plastinated organs was thought to improve the quality of education by 89.1% of the students, compared to the negative aspects of formaldehyde. It was stated by 84.2% of the students that plastinated organs should be included in anatomy lessons due to the thoughts of the majority of the students that plastinated tissues could make a greater contribution to anatomy lessons.

In response to the final question, 92.1% of the students stated that they felt no abhorrence for the plastinated organs.

DISCUSSION

The role of dissection in the teaching of anatomy increased in the second half of the 20th century, resulting in new preservation techniques and anatomic models based on diagnostic observation (Elizondo-Oman˜a et al., 2005).

Plastination, as a biological tissue preservation method, was developed by Gunther von Hagens in the anatomy laboratory of Heidelberg University in 1978 and was used at that time for both teaching and research. The use of traditional cadaver dissection and more modern teaching resources allows different approaches in the teaching of anatomy, and is important in the development of practical and theoretical skills in both traditional and modern methods (Weiglein, 1997). However, there is an ongoing debate about which method or tools support the most productive learning experience (Patel and Moxham, 2008).

This study is the first to have reported the general knowledge, and perceptions of students about plastination and its use as an anatomy teaching resource in the Veterinary Faculty of Firat University. The results showed that the students considered plastination to be a valuable, new tool for learning anatomy.

Plastinates have become an ideal educational tool as they can be used outside the dissection room without the need for any special conditions. The students consider their use to be of benefit in the education system. It is generally accepted that plastinates are of great value as a learning and teaching resources (von Hagens et al., 1987; Fasel, 1988; Weiglein, 1997; Jones, 2002; Latorre et al., 2007; Frushstorfer et al., 2011), and therefore, many institutions now use plastinates to teach anatomy at degree level.

There are very few studies related to the opinions of veterinary faculty students about the use of plastinated samples. Latorre et al. (2007) investigated how the knowledge of veterinary and medical students developed using plastinated samples.

Fruhstorfer et al. (2011) investigated the opinions of first-year medical faculty students when wet cadaver samples were replaced with plastinated samples.

As stated in a previous study (Riederer, 2014), the use of plastinated samples is not limited to the dissection room, and it is an important factor that they can be used in practice for teaching purposes, can be exhibited in museums, and can be used at conferences for any educational purpose.

It is important to have high-quality resources during education on morphological subjects such as anatomy. The context in which anatomy is taught creates a difference in the learning perceptions of the students. Local curriculum

factors are important in creating an environment that facilitates learning (Smith et al., 2014).

The use of plastinates is helpful for students in identifying and understanding the necessary structures in anatomy. Several students have recommended that plastinated samples could be important in identifying structures and in understanding the 3-dimensional aspects of these structures. In a study by Reeves et al. (2004), it was reported that student dissection skills developed when additional computer learning support was used in gross anatomy.

For students to acquire anatomic knowledge and skills, it is necessary to use both traditional and modern techniques (Elizondo- Oman˜**a**, 2005). Fruhstorfer et al. (2011) expressed concerns that the majority of students who used only plastinates were completely removed from wet cadaver dissection.

During this study, the instructors reported that the use of plastinated samples facilitated teaching during the lessons and they wanted more samples. The same opinions were reflected in the student questionnaire.

CONCLUSION

The results of this study showed that the students felt that the use of plastinated samples both in the dissection room and in the anatomy museum was helpful for both learning and understanding anatomy. They recommended working with plastinated samples rather than touching wet cadavers. In practical anatomy applications, plastinates were greatly liked by the students as a teaching tool.

Plastination is accepted as a useful addition to cadaver dissection by most anatomy instructors and provides an important teaching resource in the curriculum of medical and veterinary faculties.

Plastination may be especially useful for educational institutions without access, space, or the financial resources for dissection, and can emphasize unique or pathological samples. The results of this study demonstrated that plastinated samples were perceived as a useful addition to traditional resources in the teaching of anatomy.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

Author's Contributions: BCG, SBB and ZEÖ designed the study. BCG performed statistical analysis. SBB participated in drafting and revising the manuscript.

BCG: Barış Can Güzel, SBB: Saime Betül Baygeldi, ZEÖ: Zait Ender Özkan

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) Addendum to Toxicological Profile for Formaldehyde, Health Effects. Avaliable at: "http://www.atsdr.cdc.gov/ToxProfiles/formaldehyde_addendum.pdf. Accessed, 2010.

International Society for Plastination. About Plastination. Avaliable at: http://isp.plastination.org/about.html. Accessed October 13, 2016.

Bilge O, Çelik S, Boduç E. Uzun yıllar önce tespiti yapılmış lokomotor sistem örneklerinin plastinasyonu ve eğitimde kullanımı. Ege Tıp Dergisi. 2014; 53:84-87.

Bodies the Exhibition Disclamer (BODIES). The exhibition. Avaliable at: http:// www.premierexhibitions.com/exhibitions/4/4/bodies-exhibition/bodies-exhibition-disclaimer. Accessed June 15, 2016.

Costa DL, Amdur MO. Air pollution. In: Klaassen CD, eds. Casarett and Doull's Toxicology: The Basic Science of Poisons. New York: McGraw-Hill; 1996. p.857-82.

Drake RL. Anatomy education in a changing medical curriculum: Anat Rec. 1998; 253:28-31.

Drake RL, McBride JM, Lachman N, Pawlina W. Medical education in the anatomical sciences: the winds of change continue to blow. Anat Sci Educ. 2009; 2:253-259.

Elizondo-Oman˜ a RE, Guzma´n-Lo´pez S, Garcı´a-Rodrı´guez MDLA. Dissection as a teaching tool: past, present, and future. Anat Rec B New Anat. 2005; 285B(1):11-15.

Estai M, Bunt S. Best teaching practices in anatomy education: A critical review. Ann Anat. 2016; 208:151-157.

Fasel JHD. Use of plastinated specimens in surgical education and clinical practice. Clin Anat. 1988;1(3):197-203.

Fruhstorfer BH, Palmer J, Brydges S, *et al.* The use of plastinated prosections for teaching anatomy the view of medical students on the value of this learning resource. Clin Anat. 2011; 24(2):246-252.

IARC Workıng Group on the Evaluation of Carcinogenic Risks to Humans, et al. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. IARC monographs on the evaluation of carcinogenic risks to humans, 2006; 88:478.

Integrated ICT into education: Lesson learned. UNESCO Asia and Pacific Regional Bureau for Education, Bangkok, Thailand. Available at: http://www.gesci.org/ old/files/docman/ICT_integrating_education.pdf. Accessed August 4, 2009.

Jones DG. Re-inventing anatomy: the impact of plastination on how we see the human body. Clin Anat. 2002; 15(6):436-440.

Klaus RM, Royer DF, Stabio ME. Use and perceptions of plastination among medical anatomy educators in the United States. Clin Anat. 2018; 31(2):282-292.

Korf HW, Wicht H, Snipes RL, *et al.* The dissection course - necessary and indispensable for teaching anatomy to medical students. Ann Anat. 2008; 190:16-22.

Latorre RM, Garcı´a-Sanz MP, Moreno M, *et al.* How useful is plastination in learning anatomy? J Vet Med Educ. 2007; 34(2):172-176.

Mirabelli MC, Holt SM, Cope JM. Anatomy laboratory instruction and occupational exposure to formaldehyde. Occup Environ Med. 2011; 68:375-378.

Patel KM, Moxham BJ. Attitudes of Professional anatomists to curricular change. Clin Anat. 2006; 19:132-141.

Patel KM, Moxham BJ. The relationships between learning outcomes and methods of teaching anatomy as perceived by professional anatomists. Clin Anat. 2008; 21(2):182-189.

Pyle MA. New models of dental education and curricular change: their potential impact on dental education. J Dent Educ. 2012; 76:89-97.

Reeves RE, Aschenbrenner JE, Wordinger RJ, *et al.* Improved dissection efficiency in the human gross anatomy laboratory by the integration of computers and modern technology. Clin Anat. 2004; 17(4):337-344.

Riederer BM. Plastination and its importance in teaching anatomy. Critical points for long-term preservation of human tissue. J Anat. 2014; 224(3):309-315.

Smith CF, Martinez-A´ lvarez C, McHanwell S. The context of learning anatomy: does it make a difference? J Anat. 2014; 224(3):270-278.

Sora MC, Latorre R, Baptista C, López-Albors O. Plastination A scientific method for teaching and research. Anat Histol Embryol. 2019; 48(6):526-531.

Souza AD, Devi R. Cytokinesis blocked micronucleus assay of peripheral lymphocytes revealing the genotoxic effect of formaldehyde exposure. Clin Anat. 2014; 27:308-312.

von Hagens G. Impregnation of soft biological specimens with thermosetting resins and elastomers: Anat Rec. 1979; 194:247-255.

von Hagens G, Tiedemann K, Kriz W. The current potential of plastination. Anat Embryol (Berl). 1987; 175(4):411-21.

Weiglein AH. Plastination in the neurosciences. Acta Anat (Basel). 1997; 158(1):6-9.

Case Report https://doi.org/10.47748/tjvr.1110108

TJVR 2022; 6 (2): 73-76

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Unusual localization of squamous cell carcinoma clinically mimicking mammary carcinoma in an Akkaraman sheep

Taha Burak Elifoğlu[1] Merve Bişkin Türkmen[2] Hasan Ceyhun Macun[1] Oğuz Kul[2]

[1] *Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, Türkiye*
[2] *Department of Pathology, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, Türkiye.*

Correspondence: *Taha Burak Elifoğlu (tbelifoglu@kku.edu.tr)*

Received: 27.04.2022 Accepted: 02.06.2022

ABSTRACT

Squamous cell carcinoma (SCC) is a malignant epithelial tumor of skin. All species of animals are vulnerable to SCC and, sheep are very rarely developing this type of skin carcinoma. The purpose of this report was to describe unusual mammary lobe localization of SCC showing a great resemblance to the mammary tumor according to its gross and clinical examinations. The sheep was brought to department of obstetrics and gynecology and, tumoral tissue was totally extirpated with a suspicion of mammary tumor. The diagnosis made as differentiated squamous cell carcinoma originated from the skin surface and invading through the dermis and subcutis but mammary gland parenchyma was remained intact. There are only few reports of SCC cases belonging to the skin of the mammary area in sheep. Therefore, it is thought that this case will make a scientific contribution with its originality and rarity.

Keywords: Epithelial tumours, Sheep, Squamous cell carcinoma, Udder

INTRODUCTION

There are various local sheep breeds in different regions of Turkey and Akkaraman sheeps have the largest population among other breeds. Akkaraman is a fat-tailed sheep which has high survival and production rates even in poor feeding conditions (Ozmen et al., 2020).

Squamous cell carcinoma (SCC) is a malignant epithelial tumor of skin that consists of anaplastic epithelia exhibiting morphologic differentiation attempts to the keratinocytes. There are several factors that are associated with tumor development such as prolonged exposure to ultraviolet light, lack of hair or sparse hair coat, and lack of skin pigmentation. Anatomical predilection sites are the ears, eyes, nose, perineum, and any depigmented skin regions of the body (Namjoo et al., 2012). Angora goats have breed predisposition for the development of SCC especially in their head region because of their depigmented hair and direct UV exposure during grazing. Though the SCC is a common skin tumor of all species of domestic animals, it is occasionally seen in sheep (Goldschmidt and Goldschmidt, 2016). SCC tumors occurs most frequently on the muzzle, lips, eyelids, pinnae, perineum and, vulva in sheep (Macedo et al., 2008; Mauldin and Peters-Kennedy, 2016). The purpose of this case report is to draw attention to a rare case of squamous cell carcinoma mimicking mammary carcinoma and to do scientific contribution by rarity of the case.

MATERIALS and METHODS

In this case, a 5-year-old Akkaraman sheep was brought to Department of Obstetrics and Gynecology Clinics with complaints of stiffness and swelling in its mammary. The owner of the animal reported that he had bought new animals 1-2

months ago and this animal came together in that flock. For this reason, although the exact information about the anamnesis is not known, it has been told by previous owners that the animal gave birth 2-3 months ago and there has been no change in the mass since new owners bought the animal. However, they brought it to our clinic because of the complaints of loss of appetite and increased weight loss for last two weeks.

In the first examination, it was determined that the mass hanging from the abdomen, which is thought to originate from the mammary gland, enveloped the teat (Figure 1), and at the same time, firm consistency was detected in the palpation of the right mammary lobe. The tumor was observed on right mammary lobe region, and it had an irregular shape and 15x11.5x9 cm dimensions with a 3 cm ulcerative area on the skin surface (Figure 2).

As a result of increased weight loss, loss of appetite, loss of interest to surroundings and irregular shape of mass, it was decided that sheep had poor prognosis and because of loss of milk yield and future economic concerns and treatment expenses, animal was culled, and slaughtered. Immediately after slaughtering, tumoral tissue was totally extirpated with a suspicion of mammary tumor, then sent for pathological examination. Also, at post-mortem systemic necropsy examination revealed no other mass foci or metastasis. The tissue was firstly trimmed and fixated in buffered 4% paraformaldehyde solution. After routine tissue follow-up procedures, paraffin embedded and thin sections of 5 µm were cut, deparaffinized and stained with hematoxylin-eosin (HE) and Masson's Trichrome stains (Munro, 1971). It was histopathologicaly examined and photographed under trinocular light microscope (Olympus BX51, DP25 Digital camera).

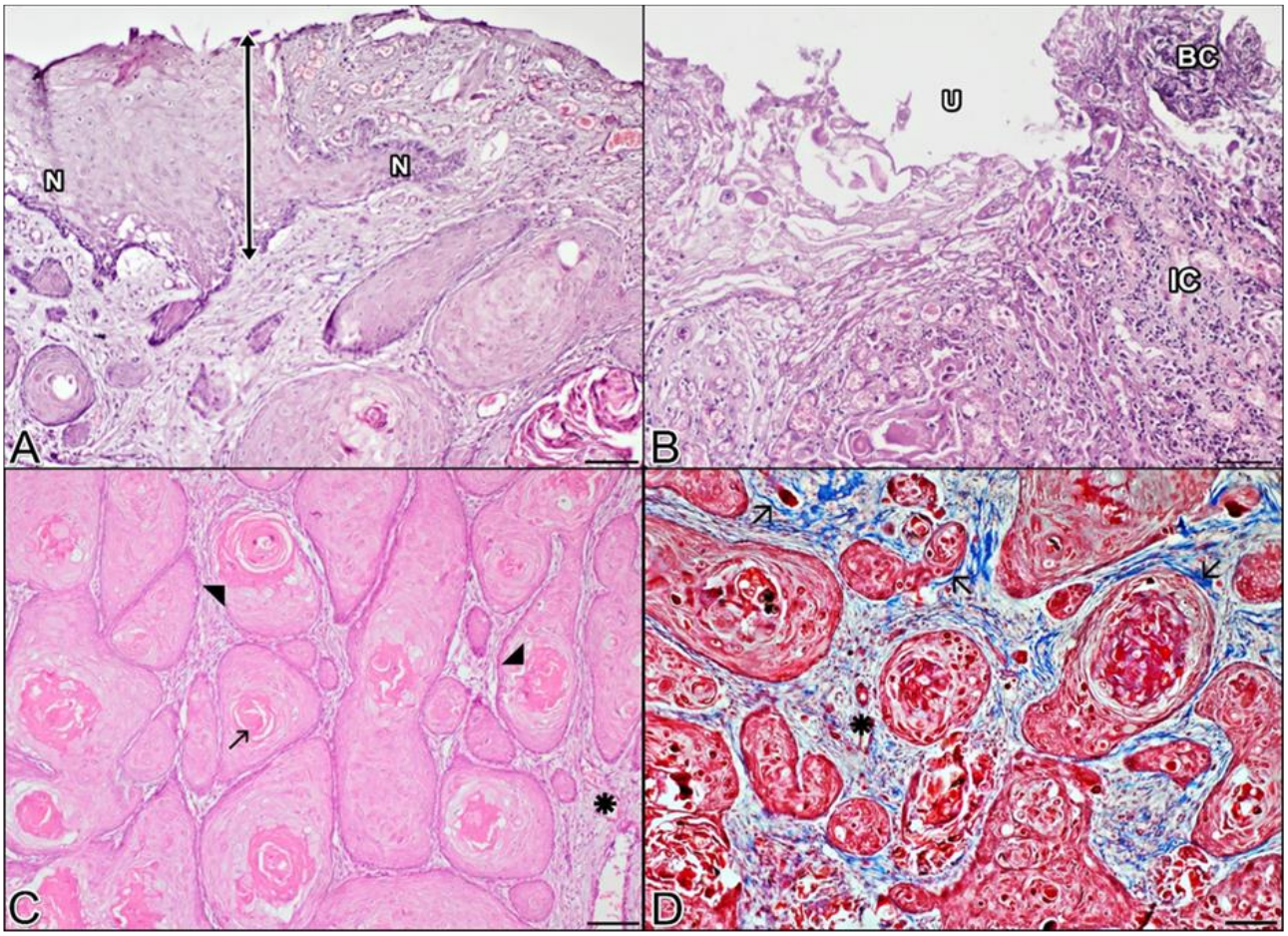

Figure 3. Sheep, well differentiated squamous cell carcinoma, mammary skin. (A) Epidermal hyperplasia (double headed arrow) and tumoral cells invading the dermis. Hyperplasia of basal layer cells (N). Haematoxylin and eosin staining HE. (x100) Bar=100µm. (B) Bacterial colonies (BC) and inflammatory cells (IC) infiltrations in ulcerated area (U). HE. (x100) Bar=80µm. (C) Parakeratotic (arrowhead) and hyperkeratotic keratin pearls (arrow) and fibrovascular stroma (star). HE. (x200) Bar=100µm. (D) Collagen fibrils (arrow) and fibro vascular stroma (star). Masson's trichrome staining. (x200) Bar=100µm.

Figure 1. Appearance of mass that hanging from the abdomen on the teat

Figure 2. Measurements of mass before removal

RESULTS

Microscopic examination showed hyperplasia and acanthosis of the epidermis and the origin of the tumor was keratinocytes in the epithelium. The basal layer keratinocytes underlying the epidermis had hyperchromatic and pleomorphic nucleuses, some were also necrotic in appearance. Neoplastic cell proliferation and infiltration to the dermis, with islands of invasive neoplastic epithelial cells were prominent as shown in Figure 3. Tumor cells with irregular eosinophilic cytoplasm surrounding the keratinous material at the center, making neoplastic island or cords, also called keratin pearls or cancer pearls were observed. Some of these cancer pearls 'were not containing any chromatin material (hyperkeratotic), while others had chromatin residues but were not form a complete concentric structure (parakeratotic). Abundant neutrophil leukocytes infiltrations and mononuclear cells were observed inside the tumor parenchyma due to erosion, ulceration and bacterial contamination on the skin (Figure 3). The epithelial origin of the tumor was confirmed by Masson's trichrome staining and there was observed thick collagen fibrils surrounding neoplastic cell islands. As a result of these findings, though appearance and palpation of mass looked like mammary tumor, the case was evaluated as well differentiated squamous cell carcinoma which is located and involved in mammary area but not originated from mammary gland.

DISCUSSION

This tumor type has been reported in different breeds of sheep, such as Merino, Polwarth, Ile de France, and Corriedale (Tustin, 1982; Ramos et al., 2007; Abo-Aziza et al., 2017). Though there are only few cases of SCC in udder in different breeds of sheep (Ahmed, 2018), no SCC case report in Akkaraman sheeps was found in researches and in literature reviews. In that manner it will be a unique and rare case of SCC in Akkaraman sheep. Lesions can be reported in any part of the body but usually appear on areas of the skin with a lack of pigment or wool (Mauldin and Peters-Kennedy, 2016). In this case, sheep was first shown to have well-differentiated SCC in the right mammary lobe skin region near to udder. Although this area is hairless, it is one of the regions likely to be exposed to sunlight due to its anatomical nature. For example, in photosensitization cases, photodynamic agents in the skin of the mammary and teat are easily activated by encountering UV rays and severe erosion and edema occur. However, in Akkaraman sheeps, SCC cases reported to date do not have a mammary area. Even more interesting, any mass shaped in this region tends to be clinically referred to as a mammary tumor. In this case, the diagnosis of SCC, which has no connection with the mammary tissue, was made in the histopathological examination of the mass, which was clinically suspicious of mammary tumor. As a result, rarely there are reports of SCC cases belonging to the skin of the mammary area. Thus, this case is unique and have scientific contribution by its rarity.

ACKNOWLEDGMENTS

This report was presented in International Scientific Research Congress Dedicated to the 30 th Anniversary of Baku Eurasia University, 2022 as oral presentation.

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

Author's Contributions: TBE and HCM performed clinical examination. MBT and OK performed histopathologic examination. TBE, MBT, HCM and OK participated in drafting and revising the manuscript.

TBE: Taha Burak Elifoğlu, MBT: Merve Bişkin Türkmen, HCM: Hasan Ceyhun Macun, OK: Oğuz Kul

REFERENCES

Abo-Aziza FAM, Zaki AA, El-Shemy A, Abd Elhalem SS, Amer AS. Immunological and histopathological changes in sheep affected with cutaneous squamous cell carcinoma and treated immunotherapeutically. Vet World. 2017; 10(9):1094.

Ahmed YF. Aggressive form of mammary squamous cell carcinoma in sheep. Vet Sci Technol. 2018; 09:8.

Goldschmidt MH, Goldschmidt KH. Epithelial and melanocytic tumors of the skin. In: Donald JM, eds. Tumors in Domestic Animal. 5th ed. Iowa: John Wiley and Sons Ames; 2016. p.129-131.

Macedo J, Riet-Correa F, Medeiros Dantas AF, Dantas Simoes SV. Diseases of the skin in sheep and goats from the Brazilian semiarid. Pesqui Veterinária Bras. 2008; 28(12):633-642.

Mauldin EA, Peters-Kennedy J. Integumentary system. In: Maxie MG, eds. Jubb, Kennedy & Palmer's Pathology of Domestic Animals. 2nd ed. St. Louis, Missouri: Elsevier; 2016. p.509-736.

Munro BH. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3th ed. New York: Elsevier; 1971. p.249.

Namjoo AR, Soroori S, Farid M, Nourani H. An unusual squamous cell carcinoma in a sheep: a case report. Comp Clin Path. 2012; 21(6):1383-1386.

Ozmen O, Kul S, Gok T. Determination of genetic diversity of the Akkaraman sheep breed from Turkey. Small Ruminant Research. 2020; 182:37-45

Ramos AT, Norte DM, Elias F, Fernandes CG. Squamous cell carcinoma in cattle, sheep and horse: study of 50 cases in south of Rio Grande do Sul. Brazilian J Vet Res Anim Sci. 2007; 44:5-13.

Tustin H. High incidence of squamous cell carcinoma of the vulva in Merino ewes on a South African farm. J S Afr Vet Assoc. 1982; 53(3):141-143.

Review https://doi.org/10.47748/tjvr.1083287

TJVR 2022; 6 (2): 77-84

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Some reproductive characteristics in common donkey male (*Equus asinus*)- A mini review

Sofiane Aissanou[1] Omar Besseboua[2] Abdelhanine Ayad[1]

[1] *Department of Physical Biology and Chemistry, Faculty of Nature and Life Sciences, University of Bejaia, Algeria*

[2] *Department of Agronomic and Biotechnological Sciences, Faculty of Nature and Life Sciences, University Hassiba Benbouali, Chlef, Algeria.*

Correspondence: *Abdelhanine Ayad (abdelhanine.ayad@univ-bejaia.dz)*

Received: 05.03.2022 Accepted: 18.07.2022

ABSTRACT

In contrary to most domestic livestock species, the common donkey (*Equus asinus*) is widely known as an animal with marked seasonality in reproductive activity. The annual cycle of daily photoperiod has been identified as the determining factor in sexual activity. A synthesis of the particularities of donkey reproduction is important and constitutes a basis of scientific reflection for managing asine livestock farms and establishing a well conservation plan for the different breeds around the world. It is necessary to have a perfect knowledge of the seasonal physiological changes in order to optimize the reproductive characteristics of donkeys. The objective of this paper is to review the current state of knowledge on the reproduction seasonality of common donkeys. We start with a remainder of some anatomical of the genital apparatus and sexual behavior aspects. Moreover, the investigations undertaken by many authors reveal the influence of the season on testicular biometry, histology, seminal and hormonal parameters in male donkeys populations. In conclusion, despite scientific controversy on the reproductive seasonal character in donkeys, it could likely be influenced by several factors mainly the photoperiod.

Keywords: *Sexual Behavior, Sperm, Hormone, Seasonality, Male, Donkeys*

INTRODUCTION

For as long as donkeys were known, the female was always more scientifically interested than the male. Many of the earliest accounts of donkeys were only interested in female. However, the male donkey (ass, jackass) has many interesting characteristics and there is abundant literature dating back to the past century. In recent years, donkeys have become more widely used in Central European countries by equine breeders for leisure activities in ecotourism and hippotherapy. Field reports revealed a continuing decline in world populations of the asinine species (Kugler et al., 2008) with the advent of motorization in transport and work (Figure1), this can be a risk of extinction of the species and cause a negative impact on animal biodiversity (Vlaeva et al., 2017). A synthesis of the particularities of donkey reproduction is important and constitutes a basis of scientific reflection for managing asine livestock farms and establishing a well conservation plan for the different breeds around the world (Rodrigues et al., 2021). The aim of the article is to give information about the anatomical and physiological aspects of male donkey reproduction.

Histoanatomy of the genital apparatus

The male reproductive tract consists of a pair of testes, epididymis, vas deferens, ejaculatory duct, and accessory sex glands (prostate… *etc*.).

Figure 1. Use of the common donkey (*Equus asinus*) in transport (a) and agriculture (b) before the advent of motorization

Genital tract

The donkey penis and prepuce are anatomically like stallions (Hagstrom, 2004). In donkeys, the penis in resting measures 50 cm long, with a diameter of 2.5-5 cm, and the presence of two nipples on each side of the sheath (Canisso et al., 2019). The penis body is clearly protruding along the entire length of the copulatory organ and is enveloped almost up to its tip by the bulbous spongy muscle (Chabchoub et al., 2007). The dilatation of the glans is more important in donkeys (Tibary et al., 2006) and its length increase clearly in erection, its size could range from 35.5 to 45.5 cm in diameter for a small donkey weighing 100 kg (Purdy, 2010).

The prostate forms a large glandular structure which consisted of right and left lobes, extended over the dorsal and lateral faces of the urethra (Morel, 2003). It shows an isthmus almost a centimeter thick and 2 to 3 cm long in the cranio-caudal direction, of a slate color and becoming yellowish after castration in donkeys. The prostate of the donkey is enveloped by a thick capsule which has outer fibrous and inner fibro-muscular layers. It seems that the prostate gland of a donkey is more active during the spring season compared to the other seasons of the year; this activity could be strongly related to the breeding season (Abou-Elhamd et al., 2013).

Testis and epididymis

Donkeys have larger, more globular testicles compared to stallions with smaller, laterally constricted, ovoid-shaped testes (Figure 2).

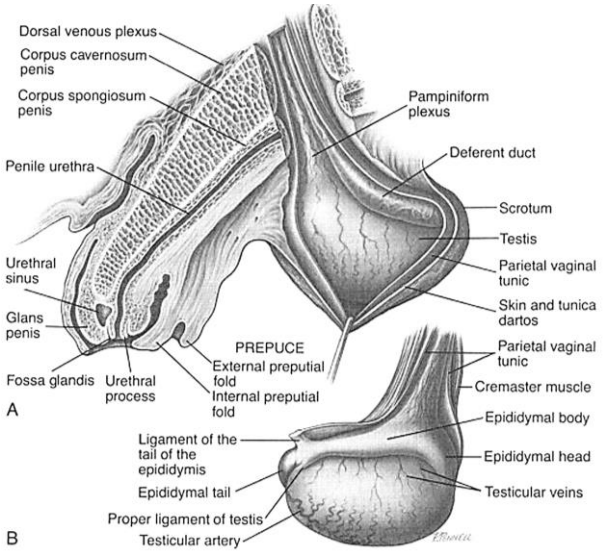

Figure 2. Chart illustrating a longitudinal section of the distal penis (A) and lateral aspect of the testis (B) in the common donkey (*Equus asinus*) (Nickel et al., 1979).

It measures on average 6 to 7 cm in wide, 9 to 10.5 cm in length and 3.7 to 5.2 cm in height (Table 1). Testicular size increases with age, nevertheless, the degeneration can be noted from the age of 15 years (Chabchoub et al., 2007). Donkeys' testicle is almost horizontal, with their long axis lightly oblique in the ventro-caudal direction without a difference between the right and left gonad (Barone, 1990). The testis of the donkey was completely descended into the scrotum around birth. It was covered by a thick tunica albuginea consisting of outer and inner fibrous layers in addition to the middle vascular one (Banks, 1993). Discrete bundles of smooth muscle cells were demonstrated in the outer fibrous layer of the tunica albuginea. These cells give the tunica albuginea a contractile function, which may aid in sperm transport (Abd-Elhafeez et al., 2017).

The epididymis is 12 to 13 cm long in the donkey and the duct can reach 70 to 85 cm. It weighs 17 to 25 g and its head receives from 12 to 23 efferent cones (Barone, 1990; Aissanou and Ayad, 2020). The corpus is relatively thick. The cauda epididymis is bulging, medially deviated and more prominent in donkeys with an average diameter of 2.45±0.08 cm (Aissanou and Ayad, 2020). The ligament of the tail of the epididymis and the testis are thick and

distinct (Chabchoub et al., 2007). The histomorphological study of the testicular compartments showed that the seminiferous tubule diameter was 222±6 µm (Neves et al., 2002); 221.51±2.32 µm (Aissanou and Ayad, 2022) and 205.6±6.65 µm (Moustafa et al., 2015). Similarly, germ cell epithelial height values in mature individuals were recorded; 68.71±1.1 µm in the Algerian donkeys (Aissanou and Ayad, 2022); 72.7±1.98 µm in Egypt (Moustafa et al., 2015) and 70.0±5.3 µm in the wild donkey (*Equus asinus africanus*) (Nipken and Wrobel, 1997).

Sexual behavior

The pre-copulatory behavior manifested by some signs such as naso-nasal contact; flehmen; nibbling and/or sniffing of the head, neck, back of the knee, flank, perineum and tail; and olfactory examination of urine or excreta without an erection or immediate copulation (Henry et al., 1998; McDonnell, 1998).

The role of vocalization appears to be an important factor in successful communication between the two partners in mating, where 78% of female vocalizations under pasture breeding management were in estrus (Henry et al., 1991). The male donkey is territorial compared to horses which keep a group of mares and it mates with jennies that approach him or cross his territory (Figure 3).

Henry et al. (1991) reported that the first mating of males was recorded during the day on pasture after 39.9±30.4 and 25.9±17.8 min of intermittent teasing periods. While the inter-copulatory interval was 88.4±71.5 and 93.3±54.5 min, when erection is achieved, the jacks return to the jennies to mate and proceed straight to the mounts once the males smelled the perineal area. For the jacks, the time from the approach in erection to ejaculation ranged 32±20 and 51±50 s. On the other hand, the time of mount to ejaculation was included 19±5.5 and 19±5.0 s. The ejaculation usually takes place after 4–8 pelvic thrusts lasting 19-30 s (Henry et al., 1991; Gastal et al., 1996; Quartuccio et al., 2011). It is also noted that from the first contact with the female (appetitive sexual behavior) to the ejaculation (consummatory sexual behaviour) ranges from 6 to 32 min (Clayton et al., 1981; Henry et al., 1991; Gastal et al., 1996). Other observations were recorded that jacks would relax on the jenny's back for five to ten seconds following ejaculation before assuming the quadrupedal station, with pelvic flares ranging from 5 to 6 before ejaculation (Henry et al., 1998). In the post-copulatory phase which takes about 15–30 min, the jack shows a total disinterest in the female, stays in the resting area, and eventually refuses the female approach (Henry et al., 1991; Quartuccio et al., 2011). The variation of erection latency observed by Costa (1991) and Veronesi et al. (2008) (1 to 45 min and 14 to 39.3 min, respectively) may well in part be due to the particularly sensitive nature of jacks to environmental disturbances such as noise, weather, and general management (Henry et al., 1991)

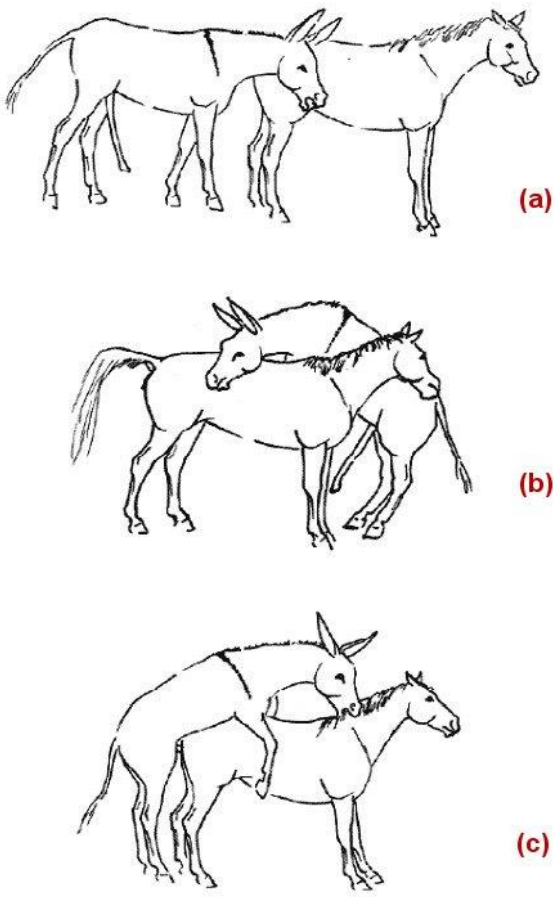

Figure 3. Donkey jack sexual behavior in presence of the female in deep estrus. (a) The donkey, with a full erection, approaches the female that is presenting typical passive behavior (top); (b) the donkey male may mount laterally the female; (c) successful copulation.

Seasonality

There are general factors that can influence the seasonal reproduction of animals, such as related to genotype and individual; and other are related to the animal's environment (Bronson, 1989). It is known that mammals generally use the duration of daylight to regulate not only their reproduction but also many other seasonal processes such as hibernation, growth, moulting, *etc*. In the literature, the scientific opinion is controversial on the seasonality character of the asine species, it likely

could be influenced by photoperiod and other factors such as breed and environmental conditions (Tibary et al., 2006). The investigations were undertaken to study the influence of the season on testicular biometry, histology, seminal and hormonal parameters are very limited in male donkey populations (Aissanou and Ayad 2020; 2022).

Photoperiod is the main environmental factor that influences seasonal patterns. Long or short days inhibit or stimulate sexual activity in animals. The light detected by the retina is translated into a neuroendocrine message by the epiphysis through the secretion of melatonin which influences seasonal reproductive activity (Reiteret al., 2018). Low or high concentrations of melatonin during daylight and nighttime respectively, are molecular indicators of photoperiod (Carcangiu, 2013).

Also, the seasonal changes in reproduction including especially hormonal activity, gametogenesis, and testicular size may show fluctuations depending on the period of the year. It is well known FSH acts specifically on Sertoli cells, playing an important role in the maintenance of qualitatively and quantitatively normal spermatogenesis. LH acts on Leydig cells to stimulate testosterone secretion (Dutta et al., 2019). Moreover, testosterone secretion, which is the major testicular androgen, is a necessary prerequisite for the maintenance of established spermatogenesis in the adult testes (Roberts and Chauvin, 2019).

The gel free volume and mass motility score in autumn were more ($P<0.05$) than in summer and winter. The pH of semen in winter and summer differed from the autumn season. The sperm concentration during summer was more than in autumn and winter ($P<0.05$). However, the live sperm percentage did not differ significantly due to season (Roy et al., 2003). Also, other observations have been reported on the biochemical characteristics of semen, which the means concentrations of glucose, cholesterol, acid phosphatase and aspartate aminotransferase activities in the seminal plasma were significantly higher in autumn and winter compared to summer (Roy et al., 2004).

In Brazilian donkeys, the seasonal effect was observed only on seminal pH among all the sperm parameters (Gastal et al., 1997). Whereas Contri et al. (2010) noted a significant difference in the volume and viability in fresh Martina Franca donkey sperm characteristics, which values were higher during the short-day season, *i.e.* in November and December. On the other hand, no significant differences in testicular morphometric traits or in seminal parameters were observed in the Italian Martina Franca donkey during the year (Carllucio et al., 2013). It was also demonstrated that the seasonal change of plasma testosterone level in the breeding season (Mar-Sep, 2.18±0.27 ng/mL) were significantly higher than in the non-breeding season (Oct-Feb, 1.50±0.18 ng/mL) in Chinese donkeys and correlated with photoperiod and temperature (Jiaha, 1983). Moreover, the seasonal variation of 17β-Estradiol differs by season because the average level in April-September was higher than in October-March.

Shuler et al. (2019) evaluated the testicular endocrine function of male donkeys (*Equus asinus*) under field conditions in Germany. A highly significant influence of season on the evolution of steroids concentrations namely estrone, estrone sulfate and testosterone through the year was recorded (Figure 4).

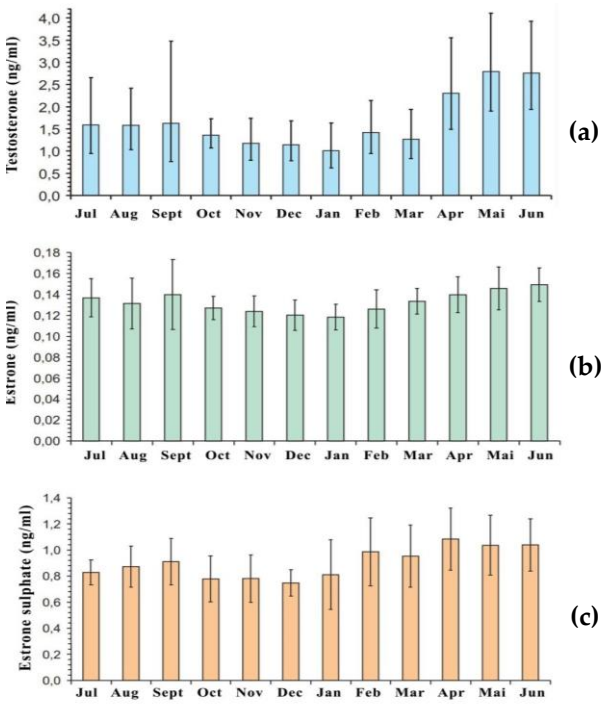

Figure 4. Testosterone (a), estrone (b) and estrone sulfate (c) secretory profile (mean ± SE) in donkeys (*Equus asinus*) in blood serum during different months of the year (Schuler et al., 2020).

The concentration values were low from November to January and high in April, May and June. Thus, the results showed that breed also had an effect on the expression of seasonality between dwarf and standard donkeys. In addition, seasonal interactions and geographical location seem to have

a considerable influence on reproductive seasonality in donkey populations around the world (Tibary et al., 2006).

Recently, it has been reported in testis histomorphological investigation that sexual activity usually occurs in winter and autumn in local donkeys from northern Algeria (Aissanou and ayad, 2022). Likewise, the highest gonado-somatic index and scrotal circumference values were recorded in the autumn and winter season. As well as the values of the testicular and epididymal biometrics were significantly higher in the autumn and winter season than in the spring and summer seasons (Aissanou and Ayad, 2020).

Table 1. Mean values (± SD) of the donkey (*Equus asinus*) breeds testis biometry.

Measurement	Algerian donkey[1]	Ethiopian donkey[2]	Martina Franca donkeys[3]	Miranda donkey[4]
Testicular length (cm)	6.8±0.2	13.4±3.78	9.6	8.04±1.2
Testicular Width (cm)	4.92±0.13	6.96 ± 1.65	6.8	6.06±1.19
Testicular Height (cm)	3.79±0.12	ND	5.2	4.63±0.86
Testicular Weight (g)	80.91±5.8	277±32.7	ND	ND
Testicular Volume (cm^3)	73.42±5.53	ND	ND	126.28±56.52

[1]Aissanou and Ayad, 2020; [2]Lemma and Deressa, 2009; [3]Carluccio et al., 2004; [4]Martins-Bessa et al., 2021. ND: not determined

Table 2. Mean (± SD) of the donkey (*Equus asinus*) breeds seminal parameters.

Parameters	Pêga donkey[1]	Catalonian donkey[2]	Andalusian donkey[3]	Martina Franca donkey[4]
Gel free semen volume (mL)	47.2±28.6	56.61±23.18	80.6±11.1	90±43.4
Gel volume (mL)	71.7±54.8	ND	ND	107.2±41.6
Total motility (%)	84.2±6	68.40±16.59	90.2±2.7	81.9±3.7
Progressive motility (%)	74.4±7	ND	70.1±4.1	76.6±4.8
Sperm concentration (10^6 per mL)	253±91.2	280.88±228.94	259.4±37.6	350.4±139.7
Total abnormalities	7.9±3.0	18.99±8.62	12.2±2.1	ND
pH	ND	7.77±0.35	7.2±00	7.6±0.2
VCL (m/s)	ND	80.20±51.72	106.6±0.2	115.9±14.2
VSL (m/s	ND	49.80±43.18	78.5±0.3	195.9±27.7
VAP (m/s)	ND	59.39±43.04	94.4±0.2	136.8±12.5
LIN (%)	ND	60.50±28.79	73.3±0.2	60.8±5.4
STR (%)	ND	79.21±24.50	82.2±0.2	89±40

[1] Canisso et al., 2010; [2] Miro et al., 2005; [3] Dorado et al., 2013; [4]: Contri et al., 2010. ND: not determined

Table 3. Effect of different extenders in post thawing donkey semen cryopreservation

Author	Extender	Breed	Concentration	Hours post-thaw	Progressive motility (%)	Total motility (%)	VAP (μm/s)
Álvarez et al. (2019)	Lactose–jenny colostrum extender	Zaragoza, Spain	50% of lactose, 20% of jenny colostrum, 25% of Glucose–EDTA and 5% DMF.	0	ND	58.3	50
Oliviera et al. (2014)	CLC	Pega	3mg	0	49.2 ± 2.7	81.1 ± 2.9	89.6 ± 2.7
Rota et al. (2012)	Eg-INRA96	Amiata	2.8 mL	0	33	53	107
				1	31	43	100
				2	21	31	85
Rota et al. (2012)	Gly-INRA96	Amiata	4.4 mL	0	32	49.5	110
				1	28	42	95
				2	21	32	83

CLC: Cholesterol-loaded cyclodextrin; Eg:Ethylene glycol; Gly: Either glycerol, ND: not determined

Sperm quality and cryopreservation

Puberty is the first release of fertile sperm. This is well correlated with the development of gonads, which the donkeys are under breeding conditions and environmental factors. It has been demonstrated that puberty, by the presence of spermatozoa in the epididymis, is observed more early in the low density of donkeys having a high population density than the other having low population density of animals (Choquenot, 1991). The success of the cryopreservation process is conditioned by the survival of the plasma membrane of the sperm, the acrosome, and the mitochondria (Sieme et al., 2015). Indeed, the optimal conditions of cryoconservation are aimed to provide the physicochemical properties during freezing, as well as the optimal cooling rates, maintaining temperatures, and warming rates for the sperm (Parks and Graham, 1992).

Many studies have aimed to describe the reproductive capacities of the different breeds in different localities (Table1). Carluccio et al. (2013) reported that the percentage of viable spermatozoa in the donkey's ejaculates is much higher compared with values reported for the horse, (80% *vs.* 55 to 65%). Other published results suggest that vitamin C and E could increase the quality of frozen sperm acting on sperm viability, acrosome cell membrane integrity, and mitochondrial activity. Also, the fusion of both vitamins provides a better effect than the single addition (Yu et al., 2019). According to Álvarez et al. (2019), the use of an extender containing lactose-jenny colostrum can be successfully used for donkey semen cryopreservation and could effectively improve donkey sperm qualities after freezing-thawing (Table 3).

Rota et al. (2012) reported that the use of either glycerol or ethylene glycol as a cryoprotectant after thawing and dilution with seminal plasma appears to improve fertility and pregnancy rates of inseminated jennies. While Oliviera et al. (2014) demonstrated that the use of cholesterol-loaded methyl-b cyclodextrin on donkey semen before cryopreservation increased the viability of thawed spermatozoa (Table 3).

The vitrification technic by direct exposure of sperm to liquid nitrogen is a method of biotechnology related to reproduction and seems increasing in popularity as an alternative to conventional freezing. Although, Hidalgo et al. (2020) showed that donkey sperm could not be vitrified using only glycerol as permeable cryoprotectant agents. Vitrification using non-permeable cryoprotectant agents (sucrose 0.1 M and BSA 5%) enhanced sperm motility and viability after warming.

In another study, donkey semen diluted in skimmed milk glucose showed superior sperm motility than lactose - egg yolk. The pregnancy rate for mares inseminated with semen diluted in skimmed milk glucose was higher than that obtained using lactose - egg yolk (56.52% *vs*. 4.76%, respectively) (Carvalho, et al., 2017**)**. Moreover, total cholesterol (mg/dl), total protein (g/dl) and triglycerides (mg/dl) were significantly higher in donkey's seminal plasma (74.65±9.70, 3.63±0.50 and 61.72±7.3, respectively) than in horse (64.72±10.23, 1.25±0.19 and 39.57±8.35, respectively) (Pal et al., 2009). These results could be explained by the fact that donkeys tend to be more fertile than horses, presenting a conception rate of 78% compared to a mare's average of 65% (Hagstrom, 2004).

CONCLUSION

Equine professionals recognize that donkey is an abandoned animal source, and their value is often underestimated in the equine world. Although the present mini-review has focused on the reproduction in male donkeys, it would be also needed to mention other important physiologic aspects in order to ensure genetic diversity. Reproductive physiology is a key essential to undertake research in safeguarding the genetic inheritance of endangered animal species. Therefore, it is very important to review some aspects of reproduction to understand asine breeding management. The donkey belongs to the family of Equidae, and exhibits some similar features of reproduction to horse. It appeared that the seasonality is well marked in donkeys, with other factors besides photoperiod and ambient temperature influencing the asine reproduction. Previous studies revealed that there is variability in sperm quality and production in donkeys around the world.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

Author's Contributions: Conceptualisation: A.A., S.A; methodology: A.A and S.A; validation: O.B and A.A; investigation: S.A; resources: A.A., O.B. and

S.A; writing—original draft preparation: S.A., A.A.; supervision: A.A. All authors read and approved the final manuscript.

REFERENCES

Abd-Elhafeez HH, Moustafa MNK, Zayed AE, Sayed R. The development of the intratesticular excurrent duct system of donkey (*Equus asinus*) from birth to maturity. Histol Cytol Embryol. 2017; 1:1-8.

Abou-Elhamd AS, Salem AO, Selim AA. Histomorphological studies on the prostate gland of donkey Equusasinus during different seasons. J Histol. 2013; 2013:19.

Aissanou S, Ayad A. Influence of age, body weight and season on testicular and epididymis biometrics in donkeys (*Equus asinus*). Int J Morphol. 2020; 38:1434-1443.

Aissanou S, Ayad A. Histomorphometric changes of testicular tissues by season and age of Algerian local donkeys (*Equus asinus*). Acta Scient Anim Scien. 2022; 44:55775-55775.

Álvarez C, Luño V, González N, Gil L. A preliminary study on the use of jenny colostrum to improve quality in extenders for freezing donkey semen. Cryobiology. 2019; 87:110-114.

Banks WJ. Applied veterinary histology. 3rd ed. USA: Mosby Year Book; 1993.

Bronson FH. Mammalian reproductive biology. University of Chicago Press; 1989

BaroneR. Anatomie comparée des mammifères domestiques, tome 4. Paris: Vigot; 1990. p.951.

Canisso IF, Panzani D, Miro J, Ellerbrock RE. Key aspects of donkey and mule reproduction. Vet Clin Equine Pract. 2019; 35:607-642.

Carcangiu V, Mura MC, Parmeggiani A, Piccione G, Bini PP, Cosso G, Luridiana S. Daily rhythm of blood melatonin concentrations in sheep of different ages. Biol Rhythm Res. 2013; 44:908-915.

Carluccio A, Villani M, Contri A, Tosi U, Battocchio M. Preliminary study on some seminal and testicular morphometric characteristics in Martina Franca jackass. Ippologia. 2004; 15:23-26.

Carluccio A, Panzani S, Contri A, Bronzo V, Robbe D, Veronesi MC. Influence of season on testicular morphometry and semen characteristics in Martina Franca jackasses. Theriogenology. 2013; 79:502-507.

Carvalho LE, Silva JM, Palhares MS, *et al*. Physical characteristics and fertility of fractionated donkey semen cooled at 5° C. Arq Bras Med Vet Zootec. 2017; 69:29-38.

Chabchoub A, Tibary A, Trimeche A. Particularités et spécificités de la reproduction du baudet. Nouv Prat Vet Equine. 2007; 100:36-40

Choquenot D. Density-dependent growth, body condition, and demography in feral donkeys: testing the food hypothesis. Ecology. 1991; 72(3):805-813.

Clayton HM, Lindsay FEF, Forbes AC, Hay LA. Some studies of comparative aspects of sexual behaviour in ponies and donkeys. Appl Anim Ethol. 1981; 7:169-174.

Contri A, Amicis IDE, Veronesi MC, Faustini M, Robbe D, Carluccio A. Efficiency of different extenders on cooled semen collected during long and short day length seasons in Martina Franca donkey. Anim Reprod Sci.2010; 120:136-141.

Costa AJSA. Andrologic evaluation of jacks Pêga breed. (Thesis). Belo Horizonte, Minas Gerais: Federal University of Minas Gerais, 1991.

Dorado J, Acha D, Gálvez MJ, Ortiz I, Carrasco JJ, Díaz B, Hidalgo M. Sperm motility patterns in Andalusian donkey (*Equusasinus*) semen: Effects of body weight, age, and semen quality. Theriogenology. 2013; 79:1100-1109.

Dutta S, Biswas A, Sengupta P, Nwagha U. Ghrelin and male reproduction. Asian Pac J Reprod. 2019; 8:227.

Gastal MO, Henry M, Beker AR, Gastal EL, Gonçalves A. Sexual behavior of donkey jacks: influence of ejaculatory frequency and season. Theriogenology. 1996; 46:593-603.

Gastal MO, Henry M, Beker AR, Gastal EL. Effect of ejaculation frequency and season on donkey jack semen. Theriogenology.1997; 47:627-638.

Hagstrom DJ. Donkeys are different: an overview of reproductive variations from horses. University of Illinois, USA, Department of Agriculture, Local Extension Councils Cooperating, 2004; 1-5.

Henry M, Mcdonnell SM, Lodi LD, Gastal EL. Pasture mating behavior of donkeys (*Equusasinus*) at natural and induced oestrus. J Reprod Fert.1991; 44:77–86.

Henry M, Lodi LD, Gastal MMFO. Sexual behaviour of domesticated donkeys (*Equusasinus*) breeding under controlled or free range management systems. Appl Anim Behav Sci. 1998; 60:263-276.

Hidalgo M, Diaz-Jimenez M, Consuegra C, Pereira B, Dorado J. Vitrification of Donkey Sperm: Is It Better Using Permeable Cryoprotectants. Animals. 2020; 10:1462.

Jiaha Z. Seasonal variations of plasma testosterone, 17 β-estradiol and cortisol levels in Guan Zhong male donkeys. Chinese J Anim Vet Sci. 1983; 4.

Kugler W, Grunenfelder HP, Broxham E. Donkey breeds in Europe. Switzerland: St. Gallen; 2008.

Lemma A, Deressa B. Study on reproductive activity and evaluation of breeding soundness of jacks (*Equusasinus*) in and around DebreZeit, Ethiopia. Livest Res Rural Dev. 2009; 21:126.

Martins-Bessa A, Quaresma M, Leiva B, Calado A, Navas González FJ. Bayesian linear regression modelling for sperm quality parameters using age, body weight, testicular morphometry, and combined biometric indices in donkeys. Animals. 2021; 11:176.

Mcdonnell SM. Reproductive behavior of donkeys (*Equusasinus*). Appl Anim Behav Sci. 1998; 60:277-282.

Miró J, Lobo V, Quintero-Moreno A, Medrano A, Peña A, Rigau T. Sperm motility patterns and metabolism in Catalonian donkey semen. Theriogenology. 2005; 63:1706-1716.

Morel MCGD. Equine reproductive physiology, breeding and stud management. 2nd ed. CABI Publishing; 2003.

Moustafa MNK, Sayed R, Zayed A, El-Hafeez AHE. Morphological and morphometric study of the development of seminiferous epithelium of donkey (*Equusasinus*) from birth to maturity. J Cytol Histol. 2005; 6(6):1.

Neves ES, Chiarini-Garcia H, França LR. Comparative testis morphometry and seminiferous epitheliumcycle length in donkeys and mules. Biol Reprod. 2002; 67:247-255.

Nickel R, Schummer A, Seiferle E, Sack WO. The viscera of the domestic mammals. 2nd rev. Berlin. 1979; 2:261-71.

Nipken C, Wrobel KH. A quantitative morphological study of age-related changes in the donkey testis in the period between puberty and senium. Andrologia. 1997; 29(3):149-161.

Oliveira RR, Rates DM, Pugliesi G, *et al*. Use of cholesterol-loaded cyclodextrin in donkey semen cryopreservation improves sperm viability but results in low fertility in mares. Reprod Domest Anim. 2014; 49(5):845-850.

Pal Y, Legha RA, Tandon SN. Comparative assessment of seminal characteristics of horse and donkey stallions. IJAS 2009; 79(10):1028-1029.

Parks, JE, Graham JK. Effects of cryopreservation procedures on sperm membranes. Theriogenology. 1992; 38:209-222.

Purdy SR. Reproduction in Donkeys. In Large animal. Proceedings of the North American Veterinary Conference, Orlando, Florida, USA, 16-20 January 2010:p.249-252.

Quartuccio M, Marino G, Taormina A, Zanghì A, Cristarella S. Seminal characteristics and sexual behaviour in Ragusano donkeys (*Equusasinus*) during semen collection on the ground. Large Anim Rev. 2011; 17:151-155.

Reiter RJ, Tan DX, Rosales-Corral S, Galano A, Zhou XG,Xu B. Mitochondria: central organelles for melatonin' s antioxidant and anti-aging actions. Molecules. 2018; 23:509.

Roberts KP, Chauvin TR. Molecular mechanisms of testosterone action on the testis. Curr Opin Endocr Metab Res. 2019; 6:29-33.

Rodrigues JB, Raw Z, Santurtun E, Cooke F, Clancy C. Donkeys in transition: Changing use in a changing world. BJVRAS. 2021; 58:e174325-e174325.

Rota A, Panzani D, Sabatini C, Camillo F. Donkey jack (*Equusasinus*) semen cryopreservation: Studies of seminal parameters, post breeding inflammatory response, and fertility in donkey jennies. Theriogenology. 2012; 78(8):1846-1854.

Roy AK, Yadav MP, Sengar OPS. Effect of season on the physical characteristics of donkey (*Equusasinus)* semen. Indian J Anim Sci. 2003; 73:620-622

Roy AK, Yadav MP, Sengar OPS. Effect of season on the biochemical characteristics of donkey (*Equusasinus*) semen. Indian J Anim Sci. 2004; 74:345-347

Schuler G, Bernhardt-welte AW, Failing K, Hoffmann B. Concentrations of testosterone, estrone and estrone sulfate in peripheral blood of donkey stallions in relation to season. Tierarztl PraxAusg G. Grosstiere/Nutztiere. 2019; 47(5):294-297.

Sieme H, Oldenhof H, Wolkers WF. Sperm membrane behaviour during cooling and cryopreservation. Reprod Dom Anim. 2015; 50:20-26.

Tibary A, Sghiri A, Bakkoury M, Fite C. Reproductive patterns in donkeys. In Proceedings of the 9th International Congress of the World Equine Veterinary Association,. 2006: p.311-319.

Vlaeva R, Barzev G, Georgieva S, Ivanova I. Dynamics in the development of donkey population in Bulgaria. Trakia J Sci. 2017; 15(1):57.

Veronesi MC, Probo M, Govoni N, Tosi U, Kindahl H, Faustini M, Carluccio A. Testosterone, 15-ketodihydro-PGF2α, cortisol, estronesulphate and LH plasma concentrations in jackass around the time of semen collection Proceedings of 6th Biannual Meeting of the Association for Applied Animal Andrology. 2008; C9, 35.

Yu X, He S, Wang L, Kang M, Zhu Y, Wang S, Sun X. Effects of Vitamin C and Vitamin E on cryopreservation of Guanzhong donkey semen. Pak J Zool.2019; 51:1777-1781.

Review https://doi.org/10.47748/tjvr.1121211

TJVR 2022; 6 (2): 85-95

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Apoptosis in cancer

Melisa Beyhan Yılmaz[1] Dilek Muz[2]

[1] *Department of Tumor Biology and Immunology, Health Science Institute, Tekirdağ Namık Kemal University, Tekirdağ, Türkiye*
[2] *Department of Virology, Faculty of Veterinary Medicine, Tekirdağ Namık Kemal University, Tekirdağ, Türkiye*

Correspondence: *Dilek Muz (dilekmuz@nku.edu.tr)*

Received: 25.05.2022 Accepted: 25.08.2022

ABSTRACT

Apoptosis, also known as programmed cell death, has become a target for treating many diseases, especially cancer. Many factors are influential in the cell's pathway to apoptosis. The defects in these pathways may transform the cell to become malignant, and the organism may face a lethal outcome such as cancer. Understanding apoptosis will provide clues in guiding the pathogenesis of diseases. Two main pathways leading to apoptosis, intrinsic and extrinsic, take an active role. The granzyme B pathway is also considered an apoptotic pathway, and this pathway is activated by enzymes secreted by immune cells such as T and NK. Many caspase molecules have initiator and enforcer roles and are active at critical points in the cell's apoptosis process. In cancer treatments, activating molecules in these pathways and repairing disrupted pathways are among the target approaches. This review discusses target strategies for inhibiting apoptotic pathways and molecules in cancer cells and activating these apoptotic pathways.

Keywords: *Cell death, Apoptosis, Caspases, Bcl-2 family, Cancer*

INTRODUCTION

Cell deaths occur in the organism under physiological and pathological conditions by various stimuli throughout life (Danial et al., 2018). Unexpected increases or decreases in cellular deaths can cause different disorders such as cancer, autoimmune diseases, and immunodeficiency (Tait et al., 2014). Apoptosis, called "programmed cell death," has critical importance essential to organism balance. This balance system is described with various morphological and biochemical mechanisms within the cell (Elmore, 2007), activated by multiple factors. Many pathological conditions such as cancer, metabolic disorder and viral infections can disrupt this balance and activate apoptotic pathways (Kyansakul et al., 2017; Özkaraca et al., 2021). This review, it is aimed to discuss the programmed cell death pathway and the status of molecules, proteins and enzymes involved in this death pathway in cancer cells.

1. APOPTOSIS MECHANISMS

Apoptosis is controlled by extrinsic and intrinsic pathways driven by various cellular signals (Jan and Chaudhry, 2019). The apoptosis mechanism generally occurs through two main processes. These are the mitochondrial pathway and death receptor pathway (Green and Llambi, 2015).

1.1. Extrinsic Pathway

Death receptors (e.g., FAS, TRAIL, TNF) on the apoptotic cell surface have essential roles in forming extrinsic pathways. An exogenous signal that leads to the extrinsic pathway triggers the primary death ligand-receptor proteins interaction. Death receptors (DR) have 80 amino acids motif death domain (DD) on the cell cytoplasmic surface to be later formed in the death-inducing signaling

complex (DISC). DR proteins are classified as tumor necrosis factor (TNF) receptor gene family (Fulda and Debatin, 2006). The TNF receptor 1 (TNFRI), Fas ligand (Fas-L), TNF-related apoptosis-induced ligand-receptor 1 (TRAIL-R1), and TRAIL-R2 are among the best-described death receptors (Fulda and Debatin, 2006). TNF, Fas-L, or TRAIL stimuli are expressed as triple protein structures on the membranes of cytotoxic T or NK cells and bind to their receptors on the membrane of the pathogen-infected cell, leading to the death of the infected cell. Binding death ligands reveal an attachment area to related receptors for the adaptor protein; thus, the DD interaction sites at the cytoplasmic end of the receptors bring together the adapter protein FADD (Fas-Associated Death Domain) procaspase-8 proteins to establish DISC. Procaspase-8 turns into caspase-8 after DISC formation; that is, caspase-8 is activated after several cuts, and then the apoptosis cascade begins with the activation of caspase -3, -6, -7 (Figure 1) (Enari et al., 1998; Stroh and Schulze-Osthoff, 1998; Yamada et al., 1999).

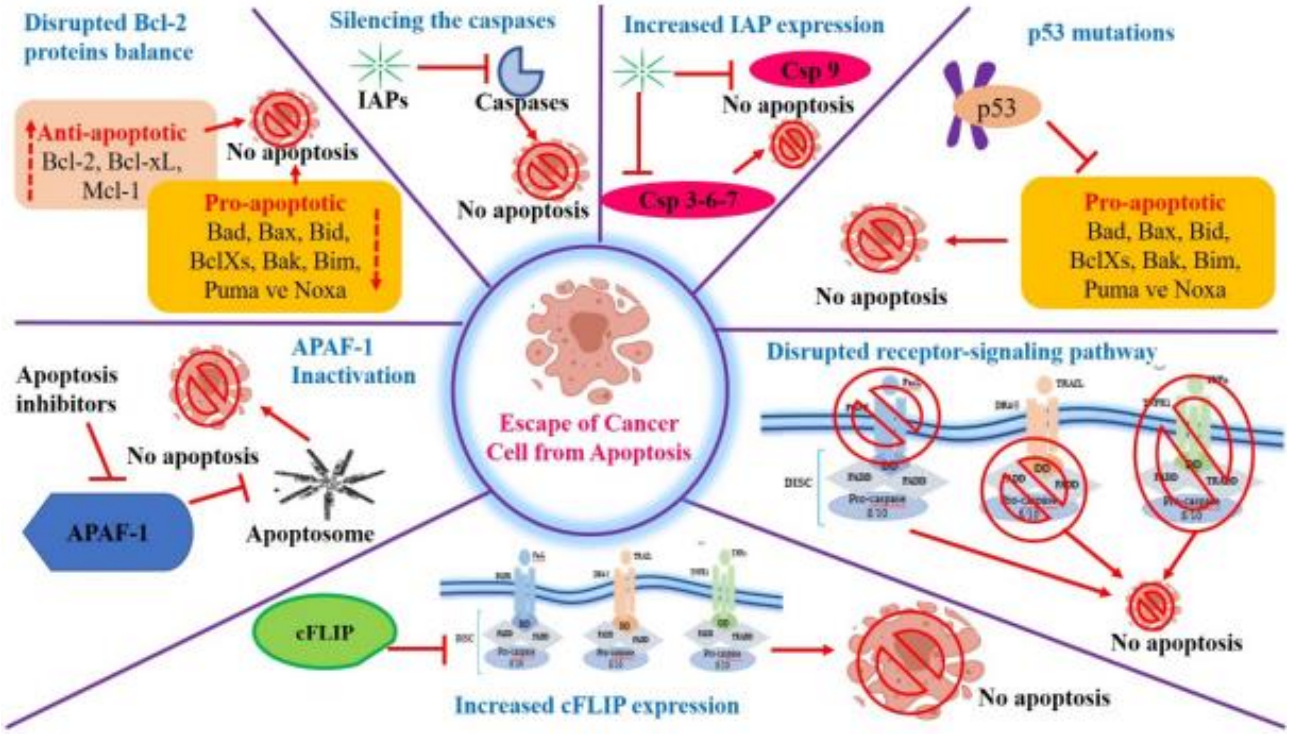

Figure 1. Mechanisms of escape of cancer cells from apoptosis and carcinogenesis.

1.2 Intrinsic Pathway

"Internal stresses" such as an oncoprotein, DNA damage, hypoxia, or infection can prompt the intrinsic pathway. This pathway is promoted by intracellular stimuli such as oxidants, high concentration of intracellular Ca2+, and pH changes resulting in increased mitochondrial permeability and releasing of proapoptotic proteins into the cytoplasm (Radogna et al., 2007). Bcl-2 family proteins have leading regulator roles in apoptotic mechanisms, including proapoptotic (e.g., Bax, Bcl-Xs, Bak, PUMA) and anti-apoptotic proteins (e.g., Bcl-2, Bcl-W, Bcl-XL) (Kannan and Jain, 2000). The p53 protein functions in the intrinsic pathway to increase the level of the proapoptotic Bcl-2 family of proteins. It decreases the release of anti-apoptotic Bcl-2 proteins and causes apoptosis-related death of damaged cells. Moreover, p53 can increase the expression of CD95 and TRAIL receptor 2 (TRAIL-R2/DR5), directing cells to the internal pathway and the external apoptotic pathway (Johnston et al., 2002). Overexpression of proapoptotic proteins on the mitochondrial membrane's outer surface increases the permeability of the inner mitochondrial membrane to ions and solute molecules by opening the pores on the outer membrane. Following the increase in the inner membrane permeability of mitochondria, the flow towards the mitochondrial matrix, swelling of the organelle, and the physical deterioration of the outer membrane result in the expression of Cyt-c within the cytosol (Giorgio et al., 2005). Cyt-c is highly important in mitochondrial ATP production

and carries electrons between the ETS (Electron Transport System) III and ETS IV complexes. However, after being released from the mitochondria, activating factor 1 protein (APAF-1) enters the cytosol and binds to the adapter Cyt-c molecule, leading to the formation of the apoptosome complex required for Cyt-c caspase activation. For the apoptosome to affect effector caspases, it must bind to pro-caspase-9 and activate this caspase. Pro-caspase-9 activates caspase-9, and as a result, it activates effector caspase-3, -6, and -7, causing cell death (Figure 1) (Lopez and Tait, 2015).

1.3 Morphological Changes in Apoptosis

The cell is seen to shrinkage and pycnosis in the early stage of apoptosis (Elmore, 2007). While shrinkage forms, the nucleus shrinks, condensation occurs, and nucleus fragmentation is observed (karyorrhexis). The fragmentation separates the apoptotic cell from the neighboring cells, protrusions form (Saraste and Pulkki, 2000), and cell organelles divide into membrane-covered apoptotic bodies without fragmentation (Kerr et al., 1994). Membranes and mitochondria protect the unity in the body (Saraste and Pulkki, 2000). The phagocytic cells, such as macrophages and dendritic cells, recognize cell membrane changes and engulf apoptotic cells. These changes arise from the migration of the phosphatidylserine molecule inside the cell membrane to the outer wall of the cell membrane, which occurs by the activity of the aminophospholipid transferase enzyme (Saraste and Pulkki, 2000). Signals on cell surfaces signal phagocytic cells for phagocytosis, which eliminates the apoptotic cell. The DNase-II enzyme in phagocytic cells insists on DNA degradation of apoptotic cells resulting in broken into pieces into 50kb pieces and then disrupting the nuclear units (Nagata et al., 2003).

2. APOPTOSIS AND CARCINOGENESIS

The idea that apoptosis may affect the malignant phenotype was raised in the 1970s. It has been shown that apoptosis contributes to decreasing malignant cells, tumor progress and inhibiting hyperplasia. Conversely, cancer cells also avoid and reduce apoptosis (Kerr et al., 1972; Wyllie et al., 1980). Apoptosis is formerly accepted as a physiological death mechanism, nonimmunogenic, or even telogenic. However, late apoptotic cells enter the immunological process by being specifically identified with phagocytic cells by the "eat me" signals they display on their surface. Immunogenic apoptosis termed immunogenic cell death (ICD), is described by its capability to alert the immune system and respond. In tumor cells, apoptosis is induced by multiple signal stimuli, serving tumor-specific neoantigens on the cell surface creating dangerous signals to immune system cells. Although carcinogenesis events such as the conversion of proto-oncogenes to oncogenes, taking continuous growth signal to cell, invasion, and metastasis activates apoptotic pathways, inversely malignant cells suppress apoptotic processes in various ways. These pathways called the apoptosis escape mechanisms of cancer cells, contain the imbalance of pro-apoptotic and anti-apoptotic proteins, decreased caspase function, and deterioration of the death signal mechanism (Figure 1) (Wong, 2011).

2.1. BCL-2 Family Proteins

The Bcl-2 protein family consists of proapoptotic proteins (e.g., Bak, Bax, Bcl-Xs, Bim, Bid, Hrk/DP5, Bim/Bod, Bmf, Noxa, Puma/Bbc-3, etc.) and antiapoptotic proteins (Bcl- 2, Bcl-W, Bcl-XL, Mcl-1, Bfl-1, etc.). Anti-apoptotic Bcl-2 members enable cancer formation and development by promoting mutated or transformed cells (Akl et al., 2014). They preserve mitochondrial functions and prevent mitochondrial degradation. By protecting the mitochondria membrane integrity, cyt-c, which enables initiation of the intrinsic apoptosis pathway, is prevented from being released into the cytoplasm. Bcl-XL, one of the anti-apoptotic proteins, can inhibit cancer treatment by activating oxidative phosphorylation inhibitors (OXPHOS) when apoptotic stimuli reach the cell (Neil et al., 2004). Together with this inhibition, they inhibit the apoptotic pathway. Levels of Mcl-1 and Bfl-1 anti-apoptotic proteins occur in reply to cell death alerts (Campbell and Tait, 2018). Bcl-2 and Bcl-XL are stable proteins in the cell, but other anti-apoptotic proteins are formed by polyubiquitination and proteasomal degradation. The increase of miRNAs such as micl-195, miR-24-2, and miR-365-2 along with Bcl-2 has been mentioned in cancer cells (Pandey et al., 2016), and also Bcl-2 caused an increase in AKT and IKK by acting NF-Kβ pathway (Mortenson et al., 2007; Kumar et al., 2008; Tucker et al., 2008).

Pro-apoptotic Bcl-2 proteins possess 9-16 amino acid homology domains (BH3). These BH3 domains are necessary to bind to anti-apoptotic proteins and induce apoptosis. These proteins become active precursors of apoptosis during

cellular stress (Bouillet and Strasser, 2002). The X protein associated with Bak and Bcl-2, namely the BAX protein, can inhibit Bcl-2 with these two pro-apoptotic proteins and promote mitochondrial outer membrane permeabilization (MOMP) (Elkholi et al., 2011). Decreased expression in pro-apoptotic genes containing only the encoded BH3 region promotes apoptosis deficiency and tumor formation (Lomonosova and Chinnadurai, 2008). The Bim pro-apoptotic protein is involved in Myc-induced apoptosis, and the observation of Bim deficiency suggests prolonging the survival process in Myc tumors. Cytokine deficiency is also reported in the tumors with Bim deficiency. The overexpression of Bid pro-apoptotic protein works as a tumor suppressor in cells of myeloid origin cancer types such as prostate, ovarian, colon, and brain cancer. Puma pro-apoptotic protein does not cause tumors by itself, but it triggers survival in many cancer cells and increases apoptotic resistance in some cancer types (Vo and Letai, 2013).

2.2. Increasing Flip Expression and Expression of Apoptosis Inhibitors

FLIP is an anti-apoptotic protein called FLICE-like inhibitory protein, overexpressed in cancer cells and associated with poor prognosis (Humphreys et al., 2018). FLIP is located in the exogenous apoptotic pathway in the cell. Celular-FLIP (c-FLIP) is expressed in two forms, long-FLIP (l-FLIP) and short-FLIP (s-FLIP) in cells, it binds to FADD, caspase-8, caspase-10, or death receptor TRAIL-5. FLIP provides immortality in a cancer cell by inhibiting caspases that would be activated after apoptotic signals (Hyer et al., 2006). Both s-FLIP and l-FLIP of FLIP bind to FADD in the DISC domain and prevent caspase-8 and caspase-10 from activating. Some cancer types have been associated with c-FLIP expression, and resistance to chemotherapy and TRAIL-induced apoptosis was reported (Piras et al., 2011). It has been shown to repair apoptosis pathways by cytokine and chemotherapeutic agents after downregulation or silence of c-FLIP. Thus c-FLIP is a crucial target protein in cancer therapy**.**

2.3.Expression of Apoptosis Inhibitor Proteins

Apoptosis inhibitory proteins (IAP) mainly inhibit apoptosis, and XIAP is the most functionally important member of this gene family. XIAP is generally found in the apoptosome structure and regulates the apoptosome structure. The expression of apoptosis inhibitor proteins in the cell prevents the binding of caspase-9 to apoptosome after the apoptotic signals are received and inhibit the apoptotic pathway. XIAP can also directly link and restrict the effector caspases. Inhibition of caspase-9 inhibits effector caspases, and cell death does not occur (Berthelet and Dubrez, 2013). Survivin, one of the apoptosis inhibitor proteins, is a member of the IAP family, and can directly stop the death pathway by inhibiting caspase-3. Survivin expressed during fetal development and carcinogenesis rarely existent in normal adult cells. It acts as an oncogene, and its overexpression promotes tumor growth in many ways in cancer cells. İmpairment of the apoptosis mechanism, caspase inhibition, resistance to antitumor drugs, and survival of cancer stem cells may be promoted by survivin. The expression level of the survivin may be used as a biomarker in apoptotic resistance in cancer treatments (Garg et al., 2016). The increasing survivin in the cell results in cancer development and drug resistance, whereas reduced expression results in apoptosis in susceptibility to chemotherapy. It has been reported that a survivin inhibitor YM155 triggers cell death in some cancer types (Xie et al., 2016). Apoptosis inhibitor proteins, IAPs, function as negative regulators of caspases and cell death mechanisms. In addition to their apoptotic functions, IAPs regulate the release of genes essential for inflammation, immunity, cell migration through ubiquitin. Signaling pathways regulated by IAPs are degraded in cancer cells. SMAC mimetics target inhibitors of apoptosis proteins (Silke and Meier, 2013).

2.4. Silencing the P53

The p53 protein encoded by the TP53 gene functions as a tumor suppressor protein in the cell. DNA damage is critical in protecting cells from stresses, including oncogene activation. Cellular stress may lead to the accumulation of p53. Mutations in the TP53 gene encode the p53 protein are seen in about 50% of cancers. These mutations increase cells' survival and reduce cancer prognosis (Zhu et al., 2013). The TP53 gene mutations result in approximately 10-15% of the p53 protein's inability to function. Silencing of p53 modifications usually occurs in a small gene region between exons of the TP53 gene encoding the p53-DNA binding domain (Perri, Pisconti, and Scarpati, 2016). A point mutation (R337H) was defined in a study on the COOH-terminal region of p53. This mutant p53 forms a tetramer similar to wild-type p53, but its activity has been shown to

be much lower than wild-type p53. A new p53 mutation was detected in human neuroblastomas (p53ΔC). The results have shown that mutant p53 may have low pro-apoptotic impacts (Ozaki and Nakagawara, 2011).

2.5. APAF-1 Inactivation

Apaf-1 protein is a pro-apoptotic protein that enables caspase-9 activation by interacting with cyt-C released from mitochondria. Inactivation of Apaf-1 in cancer cells can inhibit p53 mediated apoptotic pathways. The Apaf-1 absence caused by p53 mutation has been demonstrated by much research and is associated with tumor development. The increase of Apaf-1 expression is no obligation for the apoptotic pathway, but its' presence is essential for the death signaling in the intrinsic apoptotic pathway (Soengas et al., 2001). Inhibition of Apaf-1 has been described in advanced-stage melanoma cells and the allelic losses of Apaf-1 in melanoma cells with loss of expression. Using 5-aza-2p-dioxide in treatment, Apaf-1 may have a function, and Apaf-1 related apoptotic defects can be eliminated (Soengas et al., 2001).

2.6. Silencing the Caspases

Caspases have significant effects on apoptosis pathways. Caspase activity maintains the balance between aggressiveness and death in cancer cells. It is a biomarker in apoptosis research (Kurmyshkina et al., 2015). Lacking expression of caspase-8, an initiating caspase in apoptotic pathways, increases malignancy in NCI-H82 (lung cancer model) cells. The functional loss of caspase-8 resulted in resistance against the exogenous way initiated by death ligands such as TNF, TRAIL, and CD95 after receiving the signal (Hensley et al., 2013). Loss of function mutations in the CASP10 gene encoding the caspase-10 protein cause impairment of the function of FAS (CD95), one of the death receptors that activate the exogenous pathway. As a result, autoimmune diseases may occur (Clemente et al., 2015). Palmerini et al. (2001), in a study on colon cancer, showed that caspase-7 fell below its average level and had less apoptotic activity. The apoptotic proteins in treatments are very promising. The caspase function losses in human cancers affected cancer development and had a poor prognosis (Soung et al., 2003). Like caspase-3, -7, caspase-6 is also one of the effector caspases. Studies have suggested that both low and high expression of caspase-6 may promote tumor development. However, it has been demonstrated that the mutant form of caspase-6 reduces intrinsic pathway activity in cancer cells. Although the number of studies on caspase-6 is insufficient, its effects are a matter of debate (Dagbay et al., 2017).

2.7. Loss of Death Receptors

Resistance to apoptosis has been demonstrated to be related to death receptors in many cancer types (Ivanov et al., 2003). Allele losses in loci of chromosomes (10q24 and 8p21-22) gene encoding FAS and TRAIL-R2 have been identified in breast cancer. These mutations in death receptors also result in metastatic effects in cancer cells. For example, mutations in the FAS gene have been defined in metastatic cells in a patient with T-cell leukemia (Shin et al., 2001). It has been shown that TRAIL-mediated apoptosis inhibits pancreatic cancer, melanoma, and neuroblastomas (Piras et al., 2011). DR4 polymorphisms were defined in human ovarian cancer (SKOV3) and bladder cancer. In this polymorphism, A1322G nucleotide change of the DR4 gene was. This polymorphism resulted in the replacement of lysine with arginine from amino acids in the DD region of the DR4 protein. Polymorphic DR4 develops resistance to apoptosis in cancer types such as lung and head and neck cancer. Different DR4 polymorphisms have also been identified in recent studies (Zhang and Fang, 2005). The C626G nucleotide change was T209R, while the G422A nucleotide change caused the R141H shift. The R141H polymorphism in the ligand-binding region of DR4 results in suppressing apoptosis (Zhang and Fang, 2005). Mutations in the DR5 gene, one of the death receptors, were described in head and neck, lung, breast, and non-Hodgkin lymphomas. These mutations are in the DD region and block TRAIL-induced apoptosis. At the same time, due to mutations in the DR5 gene, FADD and caspase-8 could not interact with DD regions, and apoptotic cell death pathways were also inhibited (Zhang and Fang, 2005). Mutations in the gene of another death receptor, the FAS ligand, have been reported in lymphatic and epithelial cancer types (Takakuwa et al., 2002). The mice's CD8 + cells were detected to abolish their cells. Another study reported FAS ligand deletions in patients with ALPS (congenital immune lymphoproliferative syndrome). At the same time, these mutations in the FAS gene lead to the inhibition of FAS-mediated apoptosis (Maeda et al., 1999).

3. APOPTOSIS IN CURRENT CANCER TREATMENT STRATEGIES

The many therapies are promising to destroy cancer cells with ICD by stimulating apoptotic mechanisms in cells without damaging the healthy cells.

Chemotherapy has been used for many years to treat cancer. Chemotherapeutic agents function by inhibiting proliferation in cancer cells and disrupting the genetic content of these cells (Johnstone et al., 2002). The chemotherapy targets inhibition of DNA synthesis, cell cycle arrest, and activation of multiple cell death pathways (Seitz et al., 2010; Pan et al., 2016;). Generally, chemotherapeutic agents activate apoptotic cell death pathways destroyed by anti-inflammatory phagocytes with tolerogenic signals (Elliott et al., 2009; Yoon et al., 2015). As an example of this event, it is seen that apoptotic pathways are activated after the application of paclitaxel in lymphoma cells. The chemotherapeutic agent cisplatin, which causes DNA damage, and doxycycline, which inhibits enzymes involved in protein synthesis, activate both intrinsic and extrinsic apoptotic pathways (Wang et al., 2004; Onoda et al., 2006; Kim et al., 2008;). Mutations in the receptors that recognize damage-associated molecular patterns (DAMPs) on the dendritic cell have been proven to cause breast cancer and it has been seen that these patients cause early relapse after chemotherapy (Kim et al., 2016).

In the treatment with radiotherapy, also known as ionizing radiation, the structure of DNA is disrupted by high-energy radio waves, which causes the activation of apoptotic cell death pathways. At the same time, the stress created by the radiation that the cell is exposed to can activate death pathways other than apoptotic death pathways (Ogura et al., 2009; Sia et al., 2020). At the same time, it was observed that as the height of the radio waves given to the patient increased, it became active in necrosis, except for apoptotic death pathways. After this necrosis, a high level of HMGB1 release occurs. In addition, a study showed that the RIP-kinase pathway was inactivated after radiotherapy-induced necrosis, resulting in raised survival in non-small cell lung cancer patients (Wang et al., 2016; Wang et al., 2018).

The gene therapy applied to cancer patients is to prevent anti-apoptotic and pro-apoptotic genes, disruptions in cell death, and escape of cancerous cells from the immune system. Genes related to apoptosis (e.g., Caspases and BCL-2 family, etc.) can also act on cancer cells independent of apoptotic cell death (Lebedeva et al., 2003; Jia et al., 2012). One of the critical initiatives of gene therapy is to be administered to cancerous cells to restore cell cycle regulating and tumor suppressor proteins such as p53, Rb (retinoblastoma), p16INK/CDKN2, PTEN (Vogelstein et al., 2000; Shanker et al., 2011). Gene therapy has long been studied for use in cancer cells. One of the oldest studies was the study conducted in 1996 to control non-small cell lung cancer with a viral vector expressing the p53 gene linked to the actin promoter (Roth et al., 1996). In addition, in a study to stop the growth of melanoma cancer cells, antisense oligonucleotides were used to target the c-Myc gene, and cancer growth in melanoma cells was slowed down with this gene therapy (Putney et al., 1999). In addition, tumor growth was suppressed by using antisense RNAs for mutations in the RAS gene family that are ordinary in colon cancers (Fleming et al., 2005; Krens et al., 2010;).

Immunotherapy stimulates a host's natural immune response mechanisms to attack cancer. Monoclonal or recombinant antibodies are the most commonly used ligand in immunotherapy. They bind to the specific and overexpressed antigen specified to the cancer cell surface, thereby enhancing the recognition of the cancer cell to the cells of the immune system and preventing proliferation and metastasis. The most commonly used antibodies in this way for therapeutic purposes are those that bind to HER2 (human epidermal growth factor receptor 2), EGFR (epidermal growth factor receptor), TfR (transferrin receptor), and PSMA (prostate-specific membrane antigen) (Sharkey and Goldenberg 2009). In another immunotherapeutic method called adaptive cellular therapy, T cells isolated from patients are cured ex vivo and reintroduced to the patient (Rosenberg et al., 2008). Chimeric antigen receptor (CAR)-T cell therapy specifically recognizes the antigens of cancer cells by modifying target T cells to express the CAR receptor. CRISPR/Cas9 technology, another popular method, and techniques such as plasmid DNA and mRNA transfer are used in designing CAR receptors. (McCune, 2018; Miliotou and Papadopoulou, 2018).

Oncolytic virotherapy is tumor immunotherapy mimicking viral infection using an oncolytic virus that targets cancer cells and kills them. Oncolytic

viruses can enter cancer and healthy cells, induce cell signaling pathways and activate stress in cancer cells. It stimulates the ICD in tumor cells and occurs as an effective immune reaction instead of cancer cells' aim as the primary mechanism. Concurrently, apoptotic pathways are activated by ER stress aggravation in the cancer cell, leading to its death. Human herpes simplex virus-1 (HSV-1) has been proven its' recombinant forms oncolytic capable. HSV-1 RH2 can impel squamous cell carcinoma cells to apoptosis by HMGB1 and ATP releasing and the exposure of CRT (Donnelly et al., 2013). Cell death of glioma cells has developed following NDV infection with CRT translocation, HMGB1 releasing, and rising antigen expression (Takasu et al., 2016). It has been shown that HSV-2 causes DAMPs in murine mammary gland cancer cells (Workenhe et al., 2014). Coxsackievirus B3-infected human non-small cell lung cancer cells also similarly leaded apoptosis (Donnelly et al., 2013).

3.1. Breaking Apoptosis Resistance in Cancer Treatment

The one of most critical difficulty in cancer treatments is resistance to treatment. Today, the combined applications of targeted therapies aim to break the resistance mechanisms (Mohammad et al., 2015).

3.1.1. The Activation of P53

The p53 gene is the most studied in cancer research due to mutations in approximately 50% of cancer types. Some studies have focused on drug-like small molecules targeting the p53 system (Kogan and Carpizo, 2019). Small molecules that activate both mutant and wild-type p53 were studied in cell-based treatments to induce apoptosis. MDM2 is an oncogene that can interact with p53, resulting in the inactivation of p53 in many cancer types. Small molecules are discovered to target blocking the MDM2-p53 protein-protein interaction. A molecule called MI-219 inhibits the MDM2-p53 interaction by phosphating, and thus p53 pathways can be activated in normal cells (Suzuki and Matsubara, 2011). Nutlin 3a is another small molecule that inhibits p53 and E3 ligase MDM2 interaction.

Many cancer types have been associated with mutant p53 and metastatic phenotype. Mutated p53 aggravates proliferation and metastasis of cancer cells by connecting to transcription factors such as NF-Y, E2Fs, NFkBp65, NfkBp50, SREBP, YAP, and VDR, or NRF2. As a result, raised proliferation causes autophagic cell death and inhibition of DNA repair mechanisms, ROS accumulation, and cell survival (Blandino and Agostino, 2018). Small molecules explicitly targeting the mutant p53 were investigated and triggered apoptosis in cancer cells by using them. The PRIMA-1 and MIRA-1 molecules have been researched in p53-targeted therapies. MIRA-1, one of these molecules, revealed to its toxicity rate was high. Conversely, it is shown that PRIMA-1 and PRIMA-1MET can reactivate mutated p53 proteins and their transcriptional activity and transform to wild-type p53, resulting in increased expression of Puma, Bax, and Noxa in cells (Blandino and Agostino, 2018). Tenovin is another small molecule that can act as an activator of p53 and increase the level of p53 protein. It targets SIRT-1 and SIRT-2 proteins influent to cell proliferation, repressing the cell growth and inducing apoptosis in the cell. It has been displayed that it inhibits tumor cell survival in mice. Another molecule, RITA, binds and reactivates the p53 molecule, leading cancer cells to apoptosis (Suzuki and Matsubara, 2011).

3.1.2. The Activation of Caspases

The death pathway is targeted by anti-cancer drugs that trigger caspase activation and is frequently preferred in cancer treatments (Fulda and Debatin, 2000-2013). The absence of caspases in cancer cells is influential to the apoptosis pathway. The deletion and mutation in caspase molecules such as caspase-3, -7, -8, -9 have been reported in some cancer types (Jia et al., 2012). The anti-cancer effects of therapy approaches based on the activation of caspase molecules have been proven. Justicin A, a derivative of Justicin, a herbal medicine, increases Cyt-c release by inactivation of anti-apoptotic proteins concurrently with caspase-8 activation and activating intrinsic and extrinsic pathways (Hensley et al., 2013). It is known that the hypermethylation in the promoter regions in the caspase-8 gene in some types of cancer. The gene transfer or dimethylation treatments to caspase-8 were examined in these cancer types (MacKenzie and Clark, 2008). Xie. et al. (2001) created an adenoviral vector (ADV ARR-PB-iCasp9) prostate cell-specific promoter (ARR-PB) to increase the expression of caspase-9. This vector-activated caspase-9 in prostate cancer cells caused apoptosis in mice. Another study showed that LY2181308 oligonucleotide activates caspase-3 and inhibits survivin mRNA. It also has been determined that this oligonucleotide activates the caspase-dependent apoptosis death pathway in

tumor cells and suppresses survival (Carrasco et al., 2011; Tanioka et al., 2011).

3.1.3. The Activation of the Extrinsic Pathway Using TRAIL Agonists

Mutations in the TRAIL gene or down expression of TRAIL protein receptors contribute to developing apoptosis resistance in tumor cells (Dai et al., 2015). TRAIL agonists designed for treatment include recombinant TRAIL proteins and DR4 /DR5 agonist antibodies. The dulanermine is a recombinant TRAIL agonist as rhApo2L.0 / TRAIL, binding to DR4 and DR5 to eliminate cancer cells and lead to apoptosis. Dulanermine acts apoptosis activity selectively only in cancer cells. The clinically tested TRAIL agonists are mapatumumab for DR4, drozitumab, conatumumab, lexatumumab, tigatuzumab, LBY-135 antibody for DR5. The use of agonistic antibodies combination increases the quality of life of cancer patients and is promising (Ralff and El-Deiry, 2019). Karstedt et al. (2017) demonstrated that the TRAIL agonists induced apoptosis in metastatic breast cancer and kidney cancer. Moreover, NK-T cells stimulated by the α-galaxylceramide molecule enhance TRAIL-mediated antitumor function (Falschlehner et al., 2009). A new TRAIL agonist (Karstedt et al., 2017; Yagolovich et al., 2019) mimicking TRAILR1 and TRAILR2, a fusion protein called APG350, contains the TRAIL receptor domains that can bind to the Fc portion of the IG1 antibody. It has been reported it induced apoptosis in cancer types such as breast, colon, and lung (Legler et al., 2018).

3.1.4. XIAP / IAP Suppression Using SMAC / DIABLO Mimetic

IAPs, antiapoptotic proteins, are inhibitor proteins that inhibit caspase, negative regulators of apoptosis, and play a role in controlling many cellular pathways. Expressions of XIAP (X-linked IAP) and IAPs have tried to inhibit treatment in several cancer types (Cossu et al., 2019). cIAP-1 and cIAP-2 exert weaker effects on caspases than XIAP. However, they interact with two TNFRs (TRAF-1 and TRAF-2) in the TNFα-initiated NF-Kβ pathway (Wu et al., 2007). Smac/Diablo mimetics (second mitochondria-derived activator of caspase) lead to the induction of caspase release and activation of apoptosis by interacting with IAP proteins. Thus, immortalized cells become more sensitive to chemotherapy and radiotherapy (Zhao et al., 2020). The molecule receives ONE; for Smac / Diablo mimetics, XIAP agonists were used. Studies on Smac/Diablo mimetics have observed that besides targeting XIAP, it interacts with proteins such as IAP-1 (cIAP-1) and cIAP-2, providing proteasomal degradation-related degradation (Cossu et al. 2019). Smac/Diablo mimetics are designed as monovalent and divalent. The univalent protein attaches to a single AVPI binding motif by mimicking IAPs. The other type is bivalent, which binds to two AVPI motifs by imitating IAP and prevents caspase inhibition. Smac/Diablo mimetics in the melanoma cell line lead to XIAP degradation, thereby sensitizing the cells to TRAIL treatment (Lecis et al., 2010) and supporting the degradation of procaspase 8 to caspase 8. The antitumor activity of APG-1378 (bivalent), one of the newly identified Smac/Diablo mimetics, was observed in hepatocellular carcinoma (HCC), APG-1378 reduced IAP protein levels (Chen et al., 2018). In addition, it has been observed that it is insufficient for apoptosis. It has been shown to induce apoptosis in combination with IAP-1378 and TRAIL; APG-1378 increases the killing capacity of NK (Natural Killer) cells.

3.1.5. BCL-2 Suppression with BH3 Mimetic

Many cancer types cancer carry mutations preventing the activation of BH3-specific proteins. Mutations in BH3 pro-apoptotic proteins make cancer cells resistant to radiation, chemotherapy, and cytotoxic agents. Therefore, it attempted to design molecules that mimic only BH3 pro-apoptotic proteins named therapeutic BH3 mimetics (Bouillet and Strasser, 2002; Adams and Cory, 2018). A BH3 mimetic drug approved is venetoclax / ABT-199, a BCL-2 inhibitor that links to the BH3 domains (Merino et al., 2018). Combining BH3 mimetics with treatments such as oncogenic kinase inhibitors is suggested in new studies. Kinase inhibition increases pro-apoptotic expression of BH3 only, like BIM and PUMA. Thus, BCL-2 proteins that BH3 mimetics cannot target are also inhibited (Adams and Cory, 2018).

CONCLUSION

One of the critical ways cancer cells achieve immortality is resistance to apoptosis. Under normal conditions, for cancer cells to survive in the organism and gain the ability to reproduce continuously, they need to get rid of many obstacles that will enable the activation of apoptotic pathways. In future studies, the tools used by cancer cells for apoptotic resistance and the mechanisms of apoptosis should also be clarified. Many ways can activate preventing

cancer cell proliferation and expanding cancer cells. With the clarification of these pathways, therapeutic agents will play a more active role in cancer treatments. This review reviewed the target strategies for cancer treatment by giving information about apoptotic pathways and their status in cancer cells.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

Author's Contributions: MBY and DM collaborated on the entire article.

MBY: Melisa Beyhan Yılmaz, DM: Dilek Muz

REFERENCES

Adams JM, Cory S. The Bcl-2 arbiters of apoptosis and their growing role as cancer targets. Cell Death Differ. 2018; 25(1):27-36.

Akl H, Vervloessem T, Kiviluoto S, *et al.* A dual role for the anti-apoptotic Bcl-2 protein in cancer: Mitochondria versus endoplasmic reticulum. Biochimica Et Biophysica Acta(Bba). 2014; 10(1843):2240-2252.

Berthelet J, Dubrez L. Regulation of apoptosis by ınhibitors of apoptosis (Iaps). Cells. 2013; 2(1):163-187.

Blandino G, Agostino SD. New therapeutic strategies to treat human cancers expressing mutant P53 proteins. J Exp Clin Cancer Res. 2018; 37(1):30.

Bouillet P, Strasser A. BH3 -only proteins - evolutionarily conserved proapoptotic Bcl-2 family members essential for initiating programmed cell death. Journal of Cell Science. 2002; (115): 1567-1574.

Campbell K. J, Tait SWG. Targeting Bcl-2 regulated apoptosis in cancer. Open Biol. 2018; 8(5):180002.

Carrasco R. A, Stamm N. B, Marcusson E, *et al.* Antisense inhibition of survivin expression as a cancer therapeutic. Mol Cancer Ther. 2011; 10(2):221-232.

Chen Z, Chen J, Liu H, *et al.* The SMAC Mimetic APG-1387 Sensitizes Immune-Mediated Cell Apoptosis in Hepatocellular Carcinoma. Frontiers in pharmacology. 2018; 9:1298.

Clemente N, Boggio E, Gigliotti CL, et al. A Mutation İn Caspase-9 Decreases The Expression Of Baffr And Icos İn Patients With İmmunodeficiency And Lymphoproliferation. Genes & Immunity. 2015; (16): 151–161.

Cossu F, Milani M, Mastrangelo E, Lecis D. Targeting The BIR Domains Of Inhibitor Of Apoptosis (IAP) Proteins İn Cancer Treatment. Comput Struct Biotechnol J. 2019; (17):142-150.

Dagbay K. B, Hill, M. E, Barrett E, Hardy J. A. Tumor-Associated Mutations in Caspase-6 Negatively Impact Catalytic Efficiency. Biochemistry. 2017; 29; 56(34): 4568–4577.

Dai X, Zhang J, Arfuso F, et al. Targeting TNF-related apoptosis-inducing ligand (TRAIL) receptor by natural products as a potential therapeutic approach for cancer therapy. Exp Biol Med (Maywood). 2015; 240(6):760-773.

Danial N. N, Hockenbery DM. Chapter 18-Cell death. In Hematology, 7th ed.; Hoffman, R., Benz, E.J., Silberstein, L.E., Heslop, H.E., Weitz, J.I., Anastasi, J., Salama, M.E., Abutalib, S.A., Eds.; Elsevier: Amsterdam, The Netherlands, 2018; 186-196.

Donnelly O. G, Errington-Mais F, Steele L, et al. Measles virus causes immunogenic cell death in human melanoma. Gene Ther. 2013; 20(1):7-15.

Elkholi R, Floros KV, Chipuk JE. The role of bh3-only proteins in tumor cell development, signaling and treatment. Genes Cancer. 2011; 2(5):523–537.

Elliott MR, Chekeni FB, Trampont PC, et al. Nucleotides Released by Apoptotic Cells Act as a Find-Me Signal to Promote Phagocytic Clearance. Nature. 2009; 461(7261): 282-286. https://dx.doi.org/10.1038/nature08296.

Elmore S. Apoptosis: A Review Of Programmed Cell Death. Toxicol Pathol. 2007; 35(4): 495-516.

Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature. 1998; 391(6662): 43-50.

Falschlehner C, Schaefer U, Walczak H. Following Traıl's Path in The Immune System. Immunology. 2009; 127 (2): 145-154.

Fleming JB, Shen GL, Holloway SE, Davis M, Brekken RA. Molecular consequences of silencing mutant K-ras in pancreatic cancer cells: Justification for K-ras-directed therapy. Mol Cancer Res. 2005; (3): 413-423.

FuldaS, Debatin KM. Caspase Activation in Cancer Therapy. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience. 2000-2013.

Fulda S, Debatin KM. Extrinsic Versus İntrinsic Apoptosis Pathways İn Anticancer Chemotherapy. Oncogene. 2006; (25): 4798-4811.

Garg H, Suri P, Gupta JC, Talwar GP, Dubey S. Survivin: a unique target for tumor therapy. Cancer Cell Int. 2016; 23:16:49.

Giorgio M, Migliaccio E, Orsini F, et al. Electron Transfer Between Cytochrome C And P66shc Generates Reactive Oxygen Species That Trigger Mitochondrial Apoptosis. Cell. 2005; 122(2): 221-33.

Green DR, Llambi F. Cell Death Signaling. Cold Spring Harb Perspect Biol. 2015; 12(3): A006080.

Hensley P, Mishra M, Kyprianou N. Targeting Caspases İn Cancer Therapeutics. Biol Chem. 2013; 394(7): 831-843. https://dx.doi.org/10.1515/hsz-2013-0128.

Humphreys L, Espona-Fiedler M, Longley DB. Flıp As A Therapeutic Target İn Cancer. Febs J. 2018; 285(22): 4104-4123.

Hyer ML, Samuel T, Reed JC. The Flıp-Side Of Fas Signaling Clinical Cancer Research. 2006; 15(12):5929-31. https://dx.doi.org/10.1158/1078-0432.CCR-06-2098.

IvanovVN, Bhoumik A, Ronai Z. Death Receptors And Melanoma Resistance To Apoptosis. Oncogene. 2003; (22): 3152-3161.

Jan R, Chaudhry G. Understanding Apoptosis And Apoptotic Pathways Targeted Cancer Therapeutics. Adv Pharma Bull. 2019; 9(2): 205-218.

Jia LT, Chen SY, Yang AG. Cancer gene therapy targeting cellular apoptosis machinery. Cancer Treat Rev. 2012; (38): 868-876.

Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: A Link Between Cancer Genetics And Chemotherapy. Article History. 2002; 2(108): 153-164.

Kannan K, Jain S. Oxidative Stress And Apoptosis. Pathophysiology. 2000; 7(3): 153-163.

Karstedt SV, Montinare A, Walczak H. Exploring the TRAILs less travelled: TRAIL in cancer biology and therapy. Nature Reviews Cancer. 2017; (17): 352-366.

Kerr JF, Winterford C, Harmon B. Apoptosis. its Significance in Cancer and Cancer Therapy. Cancer. 1994; 15;73(8): 2013-2026.

Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer. 1972; (26): 239-257.

Kim JS, Lee JH, Jeong WW. et al. Reactive oxygen species-dependent EndoG release mediates cisplatin-induced caspase-independent apoptosis in human head and neck squamous carcinoma cells. Int J Cancer. 2008 Feb 2008; 1;122(3): 672-80

Kim WS, Kim H, Kwon KW. et al. Cisplatin Induces Tolerogenic Dendritic Cells in Response to TLR Agonists Via the Abundant Production of IL-10, Thereby Promoting Th2- and Tr1-biased T-Cell Immunity. Oncotarget. 2016; 7(23): 33765-82.

Kogan S, Carpizo D. Pharmacological targeting of mutant p53. Transl Cancer Res. 2019; 5(6): 698-706. https://dx.doi.org/10.21037/tcr.2016.11.74.

Krens LL, Baas JM, Gelderblom H, Gucheaar HJ. Therapeutic modulation of k-ras signaling in colorectal cancer. Drug Discov Today. 2010; (15): 502-516.

Kumar P, Ning Y, Polverini PJ. Endothelial cells expressing Bcl-2 promotes tumor metastasis by enhancing tumor angiogenesis, blood vessel leakiness and tumor invasion. Lab Invest. 2008; 88(7): 740-749.

Kurmyshkina OV, Kovchur PI, Volkova TO. Caspases As Putative Biomarkers Of Cervical Cancer Development. Intech: 2015; 265-430.

Kvansakul M. Viral infection and apoptosis. Viruses, 2017; 9(12), 356.

Lebedeva IV, Su ZZ, Sarkar D, Fisher PB. Restoring apoptosis as a strategy for cancer gene therapy: Focus on p53 and mda-7. Semin Cancer Biol. 2003; (13): 169-178.

Lecis D, Drago C, Manzoni L, et al. Novel SMAC-mimetics synergistically stimulate melanoma cell death in combination with TRAIL and Bortezomib. British Journal of Cancer. 2010; (102): 1707-1716.

Legler K, Hauser C, Egberts J, et al. The Novel Traıl-Receptor Agonist Apg350 Exerts Superior Therapeutic Activity İn Pancreatic Cancer Cells. Cell Death & Disease. 2018; (9): 1-15.

Lomonosova E, Chinnadurai G. Bh3-Only Proteins İn Apoptosis And Beyond: An Overview. Oncogene 2008; (1): 2-19.

Lopez J, Tait SWG. Mitochondrial Apoptosis: Killing Cancer Using The Enemy Within. Br J Cancer. 2015; 112(6): 957-962.

MacKenzie SH, Clark AC. Targeting cell death in tumors by activating caspases. Current cancer drug targets, 2008; 8(2): 98-109.

Maeda T, Yamada Y, Moriuchi R, et al. Fas Gene Mutation İn The Progression Of Adult T Cell Leukemia. J Exp Med. 1999; 189(7): 1063-1071.

McCune JS. Rapid advances in immunotherapy to treat cancer. Clin Pharmacol Ther. 2018; 103(4): 540-544.

Merino D, Kelly GL, Lessene G, Wei AH, Roberts AV, Strasser A. BH3-Mimetic Drugs: Blazing The Trail For New Cancer Medicines. Cancer Cell. 2018; 34(6): 879-891.

Miliotou AN, Papadopoulou LC. CAR t-cell therapy: a new era in cancer immunotherapy. Curr Pharm Biotechnol. 2018; 19(1): 5-18.

Mohammad RM, Muqbil I, Lowe L, et al. Broad Targeting Of Resistance To Apoptosis İn Cancer. Semin Cancer Biol. 2015; 35(0): 78-103.

Mortenson MM, Galante JG, Gilad O, et al. BCL-2 functions as an activator of the AKT signaling pathway in pancreatic cancer. J Cell Biochem. 2007; 102(5): 1171-1179.

Nagata S, Nagase H, Kawane K, Mukae N, Fukuyama H. Degradation Of Chromosomal Dna During Apoptosis. Cell Death & Differentiation. 2003; (10): 108-116.

Neil JO, Michael M, Schwartz P, Hockenbery DM. Promises And Challenges Of Targeting Bcl-2 Anti-Apoptotic Proteins For Cancer Therapy. Biochimica Et Biophysica Acta (Bba). 2004; 1(1705): 43-51.

Ogura A, Oowada S, Kon Y, et al. Redox Regulation in Radiation-Induced Cytochrome C Release From Mitochondria of Human Lung Carcinoma A549 Cells. Cancer Lett. 2009; 277(1): 64-71.

Onoda T, Ono T, Dhar DK, et al. Tetracycline Analogues (Doxycycline and COL-3) Induce Caspase-Dependent and -Independent Apoptosis in Human Colon Cancer Cells. Int J Cancer. 2006; 118(5): 1309-15.

Ozaki T, Nakagawara A. Role Of P53 İn Cell Death And Human Cancers. Cancers (Basel). 2011; 3(1): 994-1013.

Ozkaraca M, Ozdemir S, Comakli S, Timurkan MO. Roles of apoptosis and autophagy in natural rabies infections. Veterinární medicína, 2021; 67(1), 1-12.

Palmerini F, Devilard E, Jarry A, Birg F, Xerri L. Caspase 7 downregulation as an ımmunohistochemical marker of colonic carcinoma. Hum Pathol. 2001; 32(5): 461-7.

Pan ST, Li ZL, He ZX, Qiu JX, Zhou S. F. Molecular Mechanisms for Tumour Resistance to Chemotherapy. Clin Exp Pharmacol Physiol. 2016; 43(8): 723-37.

Pandey MK, Prasad S, Tyagi AK, et al. Targeting Cell Survival Proteins For Cancer Cell Death. Pharmaceuticals (Basel). 2016; 9(1): 11.

Perri F, Pisconti S, Scarpati GDV. P53 Mutations And Cancer: A Tight Linkage. Ann Transl Med. 2016; 4(24): 522.

Piras V, Hayashi K, Tomita M, Selvarajoo K. Enhancing apoptosis in TRAIL-resistant cancer cells using fundamental response rules. Sci Rep 2011; (1): 144.

Putney SD, Brown J, Cucco C, et al. Enhanced anti-tumor effects with microencapsulated c-myc antisense oligonucleotide. Antisense Nucl Acid Drug Dev. 1999; (9): 451-458.

Radogna F, Cristofanon S, Paternoster L, et al. Melatonin Antagonizes The Intrinsic Pathway Of Apoptosis Via Mitochondrial Targeting Of Bcl-2. J. Pineai Res. 2007; 44(3): 316-25.

Ralff MD, El-Deiry WS. Trail Pathway Targeting Therapeutics. Expert Rev Precis Med Drug Dev. 2019; 3(3): 197-204.

Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dubley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer. 2008; 8(4): 299-308.

Roth JA, Nguyen D, Lawrence DD, et al. Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. Nat Med. 1996; (2): 985-991.

Saraste A, Pulkki K. Morphologic And Biochemical Hallmarks Of Apoptosis. Cardiovascular Research. 2000; 3(45): 528-537.

Seitz SJ, Schleithoff ES, Koch A, et al. Chemotherapy-Induced Apoptosis in Hepatocellular Carcinoma Involves the p53 Family and is Mediated Via the Extrinsic and the Intrinsic Pathway. Int J Cancer. 2010; 126(9): 2049-66.

Shanker M, Jin J, Branch CD, et al. Tumor suppressor gene-based nanotherapy: From test tube to the clinic. J Drug Deliv. 2011; 465845.

Sharkey RM, Goldenberg DM. Targeted therapy of cancer: new prospects for antibodies and immunoconjugates. CA Cancer J Clin. 2009; 256(4): 226-243.

Shin MS, Kim HS, Lee SH, et al. Mutations Of Tumor Necrosis Factor-Related Apoptosis-İnducing Ligand Receptor 1 (Traıl-R1) And Receptor 2 (Traıl-R2) Genes İn Metastatic Breast Cancers. Cancer Res. 2001; 61(13): 4942-4946. PMID: 11431320.

Sia J, Szmyd R, Hau E, Gee HE. Molecular Mechanisms of Radiation-Induced Cancer Cell Death: A Primer. Front Cell Dev Biol. 2020; 8:41/full (February).

Silke J, Meier P. Inhibitor Of Apoptosis (Iap) Proteins–Modulators Of Cell Death And Inflammation. Cold Spring Harb Perspect Biol. 2013; 5(2): A008730.

Soengas Ms S, Capodieci P, Polsky D, et al. Inactivation Of The Apoptosis Effector Apaf-1 İn Malignant Melanoma. Nature. 2001; 409(6817): 207-211.

Soung H. Y, Lee J. W, Kim H. S, et al. Inactivating mutations of caspase-7 gene in human cancers. Oncogene. 2003; (22): 8048-8052.

Stroh C, Schulze-Osthoff K. Death by a thousand cuts: an ever increasing list of caspase substrates. Cell Death Differ. 1998; 5(12): 997-1000.

Suzuki K, Matsubara H. Recent advances in p53 research and cancer treatment. Journal of Biomedicine and Biotechnology 2011;1-7.

Tait SWG, Ichim G, Green DR. Die another way – non-apoptotic mechanisms of cell death. J Cell Sci. 2014; 127(10): 2135-2144.

Takakuwa T, Dong Z, Nakatsuka S, et al. Frequent Mutations Of Fas Gene İn Nasal Nk/T Cell Lymphoma. Oncogene. 2002; (21): 4702-4705. https://dx.doi.org/10.1038/sj.onc.1205571.

Takasu A, Masui A, Hamada M, Imai T, Iwai S, Yura Y. et al. Immunogenic cell death by oncolytic herpes simplex virus type 1 in squamous cell carcinoma cells. Cancer Gene Ther. 2016; 23(4): 107-113.

Tanioka M, Nokihara H, Yamamoto N, et al. Phase I study of LY2181308, an antisense oligonucleotide against survivin, in patients with advanced solid tumors. Cancer Chemother Pharmacol. 2011, 68(2): 505-511.

Tucker CA, Kapanen AI, Chikh G, et al. Silencing Bcl-2 in models of mantle cell lymphoma is associated with decreases in cyclin D1, nuclear factor- kappaB, p53, bax, and p27 levels. Mol Cancer Ther. 2008; 7(4): 749-758.

Vo TT, Letai A. Bh3-Only Proteins And Their Effects On Cancer. Adv Exp Med Biol. 2013; (687): 49-63.

Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000; (408): 307-310.

Wang HH, Wu ZQ, Qian D, et al. Ablative Hypofractionated Radiation Therapy Enhances Non-Small Cell Lung Cancer Cell Killing Via Preferential Stimulation of Necroptosis in Vitro and In Vivo. Int J Radiat Oncol Biol Phys. 2018; 101(1): 49-62.

Wang L, He L, Bao G, He X, Fan S, Wang H. Ionizing Radiation Induces HMGB1 Cytoplasmic Translocation and Extracellular Release. Int J Radiat Med Nucl Med. 2016; 40(2): 91-9.

Wang YF, Chen CY, Chung SF, Chiou YH, Lo HR. Involvement of Oxidative Stress and Caspase Activation in Paclitaxel-Induced Apoptosis of Primary Effusion Lymphoma Cells. Cancer Chemother Pharmacol. 2004; 54(4): 322-30.

Wong RS. Apoptosis in Cancer: From Pathogenesis To Treatment. J Exp Clin Cancer Res. 2011; 30(1): 87.

Workenhe ST, Simmons G, Pol JG, Lichty BD, Halford WP, Mossman KL. Immunogenic HSV-mediated oncolysis shapes the antitumor immune response and contributes to therapeutic efficacy. Mol Ther. 2014; 22(1): 123-31.

Wu H, Tschopp J, Lin S. Smac Mimetics And Tnfα: A Dangerous Liaison?. Cell. 2007; 131(4): 655-658.

Wyllie AH, Kerr JF, Currie AR. Cell death: the significance of apoptosis. Int. Rev. Cytol. 1980; (68): 251-306.

Xie X, Zhao X, Liu Y, et al. Adenovirus-mediated tissue-targeted expression of a caspase-9-based artificial death switch for the treatment of prostate cancer. Cancer Res. 2001; 61(18): 6795-6804.

Xie Y, Kang R, Tang D. Chapter 2 - Role of the Beclin 1 Network in the Cross-Regulation Between Autophagy and Apoptosis. Editor: M.A. Hayat, Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging, Academic Press. 2016; 75-88.

Yagolovich AV, Artykov AA, Dolgikh DA, et al. Adenovirus-mediated tissue-targeted expression of a caspase-9-based artificial death switch for the treatment of prostate cancer. Biochemistry (Moscow). 2019; (84): 627-636.

Yamada H, Tada-Oikawa S, Uchida A, Kawanishi S. TRAIL causes cleavage of bid by caspase-8 and loss mitochondrial membrane potential resulting in apoptosis in BJAB cells. Biochem Biophys Res Commun. 1999; 256(1): 130-133.

Yoon KW, Byun S, Kwon E, et al. Control of Signaling-Mediated Clearance of Apoptotic Cells by the Tumor Suppressor P53. Sci. 2015; (80): 349(6247).

Zhang L, Fang B. Mechanisms Of Resistance To Traıl-İnduced Apoptosis İn Cancer. Cancer Gene Therapy. 2005; (12): 228-237.

Zhao XY, Wang XY, We QY, Xu YM, Lau ATY. et al. Potency and Selectivity of SMAC/DIABLO Mimetics in Solid Tumor Therapy. Cells. 2020; 9(4): 1012.

Zhu H, Yang K, Xie Y, Lin YW, Mao QQ, Xie LP. et al. Silencing Of Mutant P53 By Sirna İnduces Cell Cycle Arrest And Apoptosis in Human Bladder Cancer Cells. World J Surg Oncol. 2013; (11): 22.

TURKISH JOURNAL OF VETERINARY RESEARCH
E-ISSN:2602-3695

INSTRUCTIONS FOR AUTHORS

General Considerations

TJVR strictly obeys the recommendations of the ICMJE, WAME and flowcharts of COPE.

During manuscript application process "Copy Right Transfer Form" signed by all the researchers in the study should be uploaded to the system.

In studies where "Ethical Approval Document" is needed, the relevant document should be scanned in the browser and transferred to the system at the application stage.

Our journal is incorporate the ICMJE recommendations into our review and publication process.

New Manuscript Submissions

Submit manuscripts through the journal's online system. To begin, go to *Manuscript Submission Page* at the following link:

https://dergipark.org.tr/en/journal/1986/submission/step/manuscript/new

Manuscript Categories

Original Research: An Original Research article is a research paper that provides new information derived from original data and usually includes extensive statistical analysis.

Review Article: A Review Article is an in-depth analysis of the current status of a particular topic. It is not an original article with new data but represents a well-balanced summary of a timely subject with reference to the literature.

Letter to the Editor: A Letter to the Editor offers brief comments on published material and should be objective, constructive, and educational.

Case Report: A Case Report is a brief description of a single unique or unusual case. The manuscript should include a title, authors' full names, degrees, and affiliations, a brief text description/discussion without headings, up to 10 references if applicable, and figure legends; note that each figure part (ie, Figure 1A or 1B) is counted as a separate image. No more than 4 images total may be included. It should not contain tables or data analysis. Case Report are generally not peer reviewed and are published at the discretion of the editor based on space available; publication is not guaranteed.

Short Communication: A Short Communication article is a rather short paper reporting preliminary observations, new interpretations of old data, simple new techniques or devices, or points of historical interest.

Organization of The Manuscript

Manuscripts should be organized in the following order, as applicable: title page, structured abstract or summary (when applicable; letters to the editor, review articles and case reports do not have a

structured abstract), full text, acknowledgments, footnotes, references, tables and figure legends. All text elements and tables should be combined in a single document, double- spaced, with line numbering.

Title Page

This first page should contain the complete title of the article, manuscript category, names and credentials of all authors, institutional affiliations of all authors, orcid (all authors of the manuscript must have a orcid number) of all authors and a short running title (≤60 characters). The corresponding author should be indicated, including a complete e-mail address and telephone number.

Structured Abstract or Summary

An abstract is required for Original Research articles. The abstract should be divided into *Objectives, Materials and Methods, Results, and Conclusions* sections. It should be between 250-350 words. Three to 6 key words should be provided at the end. The abstract should not contain figures, tables, or reference citations, trade names, or manufacturer names. Statements of statistical significance should include 'p' values or 95% confidence intervals. The abstract should not include data that are not given in the full text.

In place of an abstract, a summary is required for Letters to the editor, Review Articles, and Case Reports. Content should adhere to the guidelines for abstracts but should not be divided into sections. It should not exceed 200 words and should give a brief overview of the article. Three to six keywords should be provided at the end. Your keywords must come from the list of approved keywords (MeSH library) maintained by the Medical Subject Headings section of the U.S. National Library of Science or Tü rkiye B ilim Terim leri web page.

Full Text

For Original Research articles, the full text of the manuscript should be divided into the following sections: *Introduction, Materials and Methods, Results, Discussion, Conclusion*. For other articles, the text may be divided into sections if desired. Microsoft Word is preferred. All text should be double spaced, and all text elements, including tables, should be combined in a single document. Manuscripts should include continuous line numbering.

Acknowledgments

On a separate page preceding the references, contributions made by colleagues who are not authors of the paper should be acknowledged. This page should also include acknowledgments of published material reproduced with permission, previous presentations of the work at meetings, related grant support received, and disclosures of relevant commercial interests.

Footnotes

Footnotes are to be used when referencing each of the following types of information:

- Abstracts
- Conference presentations
- Online databases
- Personal communications

- Products, drugs, equipment, and other materials
- Statistical and computer software
- Theses and dissertations

Specific products, equipment, or drugs should be included in the footnotes only if they were essential to the outcome of the report or study. Products, equipment, and drugs that are commonly used materials in veterinary medicine need not be footnoted.

Footnotes should be cited in the text as superscript letters and listed alphabetically after the Acknowledgments section and before the references. If more than 26 footnotes are required, continue the sequence with double letters (eg, aa, bb, and cc). For products and equipment, provide complete information in the footnote, including manufacturer's name and location (ie, city, state, and country).

References

Each reference must be cited with first author's surname and publication year (Ceylan, 2004; Ceylan and Ekin, 2006; Ceylan et al., 2008) in the text.

In references list, when more than 6 authors are included in a reference, ***et al*** replaces the names of all authors after the first 3. Inclusive page ranges should be given where applicable. Accepted work in press includes the journal title or the publisher if it is a book. Material not accepted for publication cannot be included as a reference and should be cited parenthetically in the text as unpublished data, including all participants' initials and surnames and the year. Abbreviations of journal titles should conform to those used in the *National Library of Medicine* (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals). The style and punctuation of references should follow the formats illustrated by the following examples:

Journal Article with Up to 6 Authors

Stephane A, Luo W, Zhou X. Management of congenital cholesteatoma with otoendoscopic surgery: Case Report. Turkiye Klinikleri J Med Sci. 2010; 30(2):803-807.

Journal Article with More Than 6 Authors

Stephane A, Luo W, Zhou X, *et al*. Management of congenital cholesteatoma with otoendoscopic surgery: Case Report. Turkiye Klinikleri J Med Sci. 2010; 30(2):803-807.

Journal Article Published Online Ahead of Print

Cummisford K, Sundar S, Hagberg R, Mahmood F. Realtime three-dimensional transesophageal echocardiography and a congenital bilobar left atrial appendage [published online ahead of print June 24, 2009]. J Cardiothorac Vasc Anesth. doi:10.1053/j.jvca.2009.04.005.

Abstract of a Paper Presented at a Meeting

Shipp TD, Bromley B, Benacerraf BR. Comparison of the fundal endometrial diameters using 3-dimensional rendering for those with embedded and non-embedded intrauterine devices [abstract]. J Ultrasound Med. 2009; 28(suppl):47.

Whole Book

Solcia E, Capella C, Kloppel G. Tumors of the exocrine pancreas. Tumors of the Pancreas. 2nd ed. Washington: Armed Forces Institute of Pathology; 1997. p.145-210.

Edited Book

Underwood LE, Van Wyk JJ. Normal and aberrant growth. In: Wilson JD, Foster DW, eds. Wiliams' Textbook of Endocrinology. 1st ed. Philadelphia: WB Saunders; 1992. p.1079-1138.

Website

Animal and Plant Health Inspection Service. Bovine spongiform encephalopathy (BSE). Available at: www.aphis.usda.gov/lpa/issues/bse/bse.html. Accessed Feb 18, 2003.

Tables

Tables must be numbered consecutively with Arabic numerals in the order in which they appear in the manuscript. Each table should appear on a separate page and should contain a title that includes the table number. No vertical lines or borders may appear in a table. Tables must contain all necessary information so that they may stand alone, independent of the text. Data given in the text should not be duplicated in tables. All tables must be cited in numerical order in the text. Tables should be included in the text document and should not be submitted as graphics.

Figure

For initial submissions, figure legends should be provided at the end of the text document. Figures must be numbered consecutively with Arabic numerals in the order in which the figures appear in the article. All figures should be uploaded in jpeg or tiff format at least 300 dpi resolution with the name of the figure (eg, Figure 1), separately. For figures that include multiple panels, each panel should be sequentially labeled with a capital letter in the same corner of each panel. If a figure contains 2 or more rows of panels, the letter labels should be applied sequentially from left to right in the first row, then from left to right in the second row, and so on. All letters and rows used on the figures should be same size and character.

Similarity Index

Similarity index report, created by ithenticate or Turnitin software, must be uploaded during submission. Our journal expects the similarity index to be less than %25 for submitted manuscripts.

Abbreviations

Abbreviations of common clinical and technical terms listed in the *American Medical Association Manual of Style, 10th Edition*, may be used if they appear 3 or more times in the full text and abstract/summary, respectively. Each abbreviation should be defined at first use in the full text and abstract/summary, respectively. Use of other abbreviations is discouraged unless they are essential to the clarity of the manuscript.

Supplemental Materials

Additional materials that are not in themselves essential to the understanding of the article but provide an important expansion of the article contents may be submitted for publication as supplemental materials. Examples include extended descriptions of experimental methods, extended bibliographies, additional supporting data or results (eg, tables and figures), reporting checklists, copies of survey instruments or questionnaires, handouts, forms, and multimedia representations (eg, video clips) of relevant content.

Supplemental materials must be useful to readers and relevant to the article; redundant and extraneous

content will not be accepted. Whether supplemental materials will be accepted for publication is solely at the discretion of the editors. Supplemental materials accepted for publication will not appear in the printed version of the journal but will be posted on the journal's website. Ideally, supplemental materials will be sent with the manuscript to external reviewers for peer review. Whether supplemental materials have or have not undergone peer review will be indicated on the landing page where the supplemental materials are posted. Supplemental materials should be prepared in compliance with the general guidelines for manuscript style. Although supplemental materials may undergo minor copy editing or formatting, they generally will not undergo the same substantive editing provided for manuscripts. Therefore, the authors are responsible for ensuring clarity and accuracy of the content.

Permission to Reproduce Published Material

Written permission from the author and publisher of any previously published figure, table, or lengthy passage of text included in the manuscript must be submitted with the paper. If any form of preliminary publication, other than an abstract of no more than 400 words, has occurred or is contemplated, a reprint or manuscript of the actual or proposed preliminary publication should accompany the manuscript.

Authority/Responsibility

The intellectual content of the paper is the responsibility of the author; the editor and the TJVR accept no responsibility for the opinions and statements of authors. While every effort is made by the editor and editorial staff to avoid any inaccurate or misleading information, they accept no liability for the consequences of wrong information. The authors agree to keep the editor and publisher fully indemnified against any liability or claim that may arise from the publication of incorrect or misleading data.

Ethics in Experimentation

If the work involves the use of human and animal subjects, the author should ensure that the work described has been carried out in accordance with the internationally accepted rules and codes of conduct. The authors should clearly indicate in the manuscript that such guidelines have been followed and the study has been approved by the appropriate ethical committee along with name of committee and reference code.

Editing

Accepted manuscripts will be copyedited to eliminate grammatical and typographical errors and to ensure conciseness and clarity. The corresponding author will receive proofs of the copyedited article before publication.

Permission Requests

Manuscripts are considered for publication with the understanding that they are submitted solely to the Turkish Journal of Veterinary Research and are not under consideration elsewhere.

CONTACTING THE JOURNAL

The Turkish Journal of Veterinary Research Editorial Office may be contacted through the following:

Prof. Dr. Ebubekir CEYLAN

Ankara Üniversitesi Veteriner Fakültesi

İç Hastalıkları AD Dışkapı, 06110 Ankara, TURKEY

E-mail: *ebubekirceylan@gmail.com*, *infotjvr@gmail.com*

Manuscripts are considered for publication with the understanding that they are submitted solely to the Turkish Journal of Veterinary Research and are not under consideration elsewhere.

TURKISH JOURNAL OF VETERINARY RESEARCH
E-ISSN:2602-3695

Article Copyright Transfer Agreement

We, the undersigned researchers, certify that; the article we have sent; is original, wasn't sent to or disapproved of potential publication by any other journal, has not initially published, and we bear the responsibility concerning the Scientific content and Ethical values related to the article, and transfer any kind and form of copyright related to the Article to Turkish Journal Veterinary Research (TJVR) since it is published in the journal, and accept that we will not make any changes wholly or partly in the article and chose ……..……..…… named author as the authorized researcher.

Title of the article: ……..……..……

……..……..……..……

……..……..……..……

……..……..……..……

	Authors Name	Date	Signature
1.			
2.			
3.			
4.			
5.			
6.			

Authorized Researcher

Title, Name-Surname :

Full Address :

e- mail :

Tel, Fax :

Date and Signature :