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The Effects of Grape Seed on Lipid Peroxidation and Haematological Parameters in Broiler Administered Ionophore Antibiotics*

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Summary: The present study was aimed to determine at the determination of the effects of salinomycin, lasalocid and maduramacin, which are ionophore antibiotics, on lipid peroxidation and certain blood parameters in broilers and to investigate the protective effect of grape seed against the adverse effects of these antibiotics. Ninety six broiler chickens constituted the material of the study. Eight trial groups were established, each group comprising of 12 chicks. The first group was maintained for control purposes and was provided with feed free from any drug or feed additive. The second group received grape seed (0.5%), whilst the 3rd, 4th and 5th groups were given salinomycin (60 ppm), lasalocid (100 ppm) and maduramycin (5 ppm), respectively. The sixth group was given a combination of salinomycin (60 ppm) and grape seed (0.5%), whilst the seventh group received lasalocid (100 ppm) combined with grape seed (0.5%). Finally, the eighth group received maduramycin (5 ppm) and grape seed (0.5%). The feed treatments were continued for a period of 6 weeks. On days 21 and 42 of the trial, blood samples were collected. Levels of plasma malondialdehyde (MDA) and nitric oxide (NO), and the activities of erythrocyte catalase (CAT) and glutathione peroxidase (GSH-Px), and haemoglobin and haematocrit of whole blood were evaluated. The results obtained demonstrated that when incorporated into broiler chicken feed at a level of 0.5% for a period of 42 days, grape seed did not cause any adverse effects on lipid peroxidation and haematological parameters. On the other hand, the administration of salinomycin, lasalocid and maduramycin at treatment doses in feed caused adverse alterations in lipid peroxidation and haematological parameters, which were more pronounced on day 42. Furthermore, it was ascertained that grape seed alleviated the adverse effects caused by salinomycin, lasalocid and maduramycin on lipid peroxidation and haematological parameters ($p< 0.05$).

Key Words: Broiler, haematology, grape seed, ionophore antibiotic, lipid peroxidation

İyonofor Antibiyotiklerler Verilen Etçi Piliçlerde Üzüm Çekirdeğinin Lipid Peroksidasyon ve Hematolojik Parametreler Üzerine Etkileri

Özet: Bu çalışmada iyonofor grubu ilaçlardan salinomisin, lasalosid ve maduramisinin etçi piliçlerde lipid peroksidasyon ve bazı kan parametreleri ile üzüm çekirdeğinin bu ilaçlarca oluşturulan istenmeyen etkileri üzerine koruyucu etkinliğinin belirlenmesi amaçlandı. Çalışmada toplam 96 etçi piliç kullanıldı. Her bir grupta 12 civciv olmak üzere 8 grup oluşturuldu. Kontrol grubu olarak tutulan birinci gruba herhangi bir ilaç ve yem katkısı içermeyen yem, ikinci gruba üzüm çekirdeği (%0.5 oranında), üçüncü, dördüncü ve beşinci gruba sırasıyla salinomisin (60 ppm), lasalosid (100 ppm) ve maduramisin (5 ppm); altıncı gruba salinomisin (60 ppm) + üzüm çekirdeği (%0.5), yedinci gruba lasalosid (100 ppm) + üzüm çekirdeği (%0.5), sekizinci gruba maduramisin (5 ppm) + üzüm çekirdeği (%0.5) 6 hafta süreyle yemle birlikte verildi. 21 ve 42. günlerde hayvanların kanat altı venasından heparinli tüplere kan örnekleri alındı. Plazmada malondialdehit (MDA) ve nitrik oksit (NO) düzeyleri, eritrosit hemolizatlarında katalaz (CAT) ve süperoksit dismutaz (SOD) aktiviteleri, tam kanda hemoglobin ve hematokrit değerleri incelendi. Çalışma sonucu, etçi piliçlere 42 gün süre ile %0.5 oranında yeme katılarak verilen üzüm çekirdeğinin lipid peroksidasyon ve hematolojik parametreler üzerine herhangi bir olumsuz etkiye yol açmadığı; etçi piliçlere sağaltım dozlarında yeme katılarak verilen salinomisin, lasalosid ve maduramisinin 42 gün süreyle verilmede daha belirgin olmak üzere bazı lipid peroksidasyon ve hematolojik parametrelerinde olumsuz değişimlere yol açtığı; üzüm çekirdeğinin salinomisin, lasalosid ve maduramisince şekillenen lipid peroksidasyon ve bazı hematolojik parametreler üzerine iyileştirici etki gösterdiği belirlendi ($p< 0.05$).

Anahtar Kelimeler: Etçi piliç, hematoloji, iyonofor antibiyotik, lipid peroksidasyon, üzüm çekirdeği

Introduction

Consequential to the development of resistance against the majority of chemicals in use, and given their broad spectrum, ionophore antibiotics have found a common use in poultry science (1). Of these compounds, which are added to feed at low concentrations (5-125 ppm) to maintain protection

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from coccidiosis and the treatment of this protozoal infection in broiler production, monensin and salinomycin are also used in large ruminants and swine as growth promoters (32). When administered at normal treatment doses, lasalocid sodium is the least toxic of polyether ionophore antibiotics (20,29). Salinomycin is an antibiotic that have both coccidiostatic and coccidiocidal effects, and when administered at the recommended treatment doses, does not pose any significant toxicity risk for poultry (1,29). Among ionophore antibiotics, maduramycin is a compound with a low treatment index (30).

Lipid peroxidation (LP) is described as a process involving the breakdown of polyunsaturated fatty acids in cell membranes. As a result of LP, it decreases in membrane viscosity and inactivation of membrane-bound receptors and enzymes, also cellular damage could be seen (4,5,7). Malondialdehyde (MDA), one of the most toxic products, leads to mutations in the DNA chain (10-12). Nitric oxide (NO) regulates cellular functions, but may also induces lipid peroxidation. Organisms have developed several defence mechanisms to protect themselves against oxidative damage caused by free radicals (12,13,14,34). Of these mechanisms, superoxide dismutase catalyses the dismutation of the O_2^- anion into H_2O_2 (33). Catalase catalyses the breakdown of hydrogen peroxide into water and oxygen, and prevents hydrogen peroxide from generating OH^- by means of the Fenton reaction. Glutathione peroxidase (GSH-Px) is a metalloprotein, of tetrameric structure, which catalyses the reduction of peroxides (18,22).

Grapes and grape products contain flavonoids such as monomeric flavonols, dimeric, trimeric and polymeric procyanidins, and phenolic acids such as gallic and epigallic acids (23). Particularly grape seeds contain quercetin and resveratrol (3). Proanthocyanidins, also referred as condensed tannins, are mostly found in grape skin and grape seed (24,27). Previous research has demonstrated that proanthocyanidins have biological, pharmacological and therapeutic effects against free oxygen radicals and oxidative stress (16,25,28). Resveratrol is a major bioactive substance involved in the inhibition of free radicals. Resveratrol is found in large amounts in grape seeds, grape skin and grape stalk (23).

The present study aims to investigate the prophylactic effect of grape (*Vitis vinifera*) seed against ionophore antibiotics-induced lipid peroxidation in broiler chickens.

Material and Methods

Experimental design

The trial was performed in compliance with the instructions of the Ethics Board of Veterinary Faculty of Erciyes University, Faculty of Veterinary Medicine. A total of 96, one day-old male Ross MP3 chicks constituted the material of the study. The animals were allocated to 8 groups, each comprising 12 chicks. While the first group was maintained for control purposes, the second group was given grape seed. The third, fourth and fifth groups were given lasalocid (100 ppm), salinomycin (60 ppm) and maduramycin (5 ppm) respectively (32). The sixth group was administered a combination of lasalocid (100 ppm)+ grape seed (0.5%), whilst the seventh group received salinomycin (60 ppm) + grape seed (0.5%). Finally, the eighth group was given a combination of maduramycin (5 ppm) and grape seed (0.5%). These feed treatments were continued for a period of 6 weeks.

Collection and processing of blood samples

On days 21 and 42 of the trial, blood samples were collected from the animals by wing vein puncture into heparinised tubes. Blood smears were prepared for haemoglobin and haematocrit measurements and blood cell counts. Blood samples were centrifuged at 3000 rpm for 10 minutes for the extraction of plasma. Plasma samples were used for the detection of MDA and NO levels. The erythrocytes were washed three times with PBS, transferred into tubes, and diluted with an equal volume of PBS. Erythrocyte packages were used for haemoglobin, CAT and GSH-Px analyses.

Analyses of samples

Plasma MDA analyses were performed as described by Yoshioka et al. (36). Nitric oxide detection was performed, using the dinitrilization method (9). Erythrocytes were haemolysed by adding 0.3 ml of erythrocyte suspension 1.2 ml of ice-cold tridistilled water (36). The haemolysates obtained were used for haemoglobin measurement, using the ferrocyanomethaemoglobin method (8). Catalase activity was determined using the modification of the ultraviolet method developed by Luck (17). Glutathione peroxidase analyses were performed as described by Paglia and Valentine (21).

Statistical analysis

Statistical analyses were performed using the SPSS 10.0 software package for Windows. Significant differences between trial groups were detected using one-way analysis of variance. Significant groups were ascertained using Duncan's test ($p<0.05$). Results were expressed as mean±sd.

Results

MDA: In the groups that received salinomycin and maduramycin (Groups 3 and 5), it was determined that MDA values were significantly higher than those of the control group (Group 1). The MDA level of the group which received salinomycin was higher than that of the group given a combination of salinomycin and grape seed (Table 1). In the groups administered with salinomycin, lasalocid and maduramycin (Groups 3, 4 and 5), on day 42 of the trial, it was observed that MDA levels were significantly higher than those of the control group. Similarly, the group given a combination of maduramycin and grape seed (Group 8) had MDA levels higher than those of the control group (Group 1). Furthermore, the MDA level of the group administered with lasalocid and grape seed was lower than that of the group given lasalocid alone ($p<0.05$).

NO: In the groups which received lasalocid and maduramycin, upon antibiotic administration for a period of 21 days (Groups 4 and 5), NO values were observed to elevate compared to the control group. On day 42 of the trial, it was observed that NO values of the groups administered with antibiotics (Groups 3, 4 and 5) were higher than those of the control group ($p<0.05$).

CAT: In the groups given ionophore antibiotics alone (Groups 3, 4 and 5), CAT enzyme values on day 21 of the trial were lower than those of the control group**.** The comparison of the control group with the trial groups that were given a combination of antibiotic and grape seed (Groups 6, 7 and 8) demonstrated that, CAT enzyme activity decreased significantly in only the group that received salinomycin + grape seed (Group 6). CAT enzyme values of the antibiotic-treated groups (Groups 3, 4 and 5) on day 42 of the trial were lower than that of the control group (Group 1). Furthermore, the CAT activities of the groups given maduramycin + grape seed (Group 8) and lasalocid + grape seed decreased in comparison with the control group. The CAT activity of the group given salinomycin + grape seed (Group 6) was greater than that of the group given salinomycin alone. A similar situation was observed for the groups given a combination of maduramycin and grape seed (Group 8) and maduramycin alone (Group 5) ($p<0.05$).

GSH-Px: In the groups administered with antibiotics (Groups 3, 4 and 5), on day 21 of the trial, it was observed that GSH-Px values decreased in comparison with the control group (Group 1). On day 42, the GSH-Px values of the antibiotic groups (Groups 3, 4 and 5) also decreased in comparison with the control group ($p<0.05$).

HB: Analyses of blood samples collected on day 21 of the trial demonstrated that the Hb values of the control group (Group 1) and the group given grape seed alone differed significantly decreased. The groups given combinations of salinomycin + grape seed and maduramycin and grape seed (Groups 6 and 8, respectively) had lower Hb values compared to the control group. The Hb values of the group which received lasalocid and grape seed were higher than that of the group given lasalocid alone. Furthermore, the Hb level of the group administered with maduramycin alone was lower than that of the group that received a combination of maduramycin and grape seed (Group 8). The Hb level of the group given grape seed alone (Group 2) on day 42 was significantly higher than that of the control group (Group 1). Similarly, the Hb level of the group given maduramycin alone (Group 5) on day 42 was higher than that of the control group ($p<0.05$).

HTC: The HTC values of the groups given salinomycin and maduramycin alone (Groups 3 and 4, respectively) on day 21 of the trial decreased the control group. It was observed that the HTC value of the group given a combination of salinomycin and grape seed (Group 6) decreased statistically in comparison the HTC value of the control group. Furthermore, it was determined that, on day 21, the HTC value of the group given maduramycin + grape seed (Group 8) significantly increased compared to the HTC values of the group given maduramycin alone (Group 5) ($p<0.05$).

The trial groups were not seen any significant differencies of eosinophil, basophil, neutrophil, lymphocyte and monocyte counts ($p>0.05$).

Table 1. 21st day MDA and NO levels, and CAT and GSH-Px enzyme activities as mean±sd.

Groups	n	MDA (nmol/ml)	NO (nmol/ml)	CAT (U/mgHb)	GSHPx (U/ mgHb)
Group 1	12	2.54±0.67^{ab}	39.96±9.24^{a}	0.09±0.03^{d}	0.29±0.13^{c}
Group 2	12	2.41±1.02^{ab}	41.43±8.10^{a}	0.08±0.02^{cd}	0.28±0.13^{bc}
Group 3	12	3.49±1.19^{c}	47.69±4.98^{ab}	0.04±0.02^{a}	0.16±0.07^{a}
Group 4	12	3.21±0.77^{bc}	53.17±14.02^{bc}	0.05±0.02^{ab}	0.19±0.09^{ab}
Group 5	12	3.50±0.94^{c}	57.13±14.06^{c}	0.06±0.02^{abc}	0.18±0.13^{ab}
Group 6	12	2.29±0.99^{a}	43.82±4.35^{a}	0.06±0.01^{abc}	0.22±0.10^{abc}
Group 7	12	3.07±0.83^{abc}	47.83±6.64^{ab}	0.07±0.01^{bcd}	0.24±0.07^{abc}
Group 8	12	2.93±0.73^{abc}	47.10±2.96^{ab}	0.07±0.02^{bcd}	0.22±0.06^{abc}
P value		p<0.05	p<0.05	p<0.05	p<0.05

Group 1, control; Group 2, grape seed; Group 3, salynomycine; Group 4, lasalocid; Group 5, maduramicin; Group 6, salinomycin+ grape seed; Group 7, lasalocid+ grape seed; Group 8, maduramicin + grape seed.
^{a,b,c,d}. Different superscripts in the same column indicate significant differences (P<0.05).

Table 2. 42nd day MDA and NO levels, and CAT and GSH-Px enzyme activities as mean±sd.

Groups	n	MDA (nmol/ml)	NO (nmol/ml)	CAT (U/mgHb)	GSHPx (U/mgHb)
Group 1	12	2.45±0.58^{a}	39.96±7.69^{ab}	0.09±0.01^{e}	0.31±0.15^{c}
Group 2	12	2.36±0.46^{a}	38.44±3.19^{a}	0.10±0.03^{e}	0.29±0.15^{bc}
Group 3	12	3.74±1.15^{c}	49.30±7.91^{cd}	0.05±0.01^{ab}	0.15±0.05^{a}
Group 4	12	3.47±1.12^{bc}	50.53±14.26^{d}	0.06±0.01^{abc}	0.18±0.03^{ab}
Group 5	12	4.03±0.90^{c}	47.64±13.24b^{cd}	0.05±0.01^{a}	0.22±0.06^{ab}
Group 6	12	3.17±1.32^{abc}	42.31±8.62^{abcd}	0.08±0.02^{de}	0.24±0.16^{abc}
Group 7	12	2.71±0.92^{ab}	43.14±8.13^{abcd}	0.07±0.01^{cd}	0.28±0.13^{bc}
Group 8	12	3.35±0.93^{bc}	41.62±4.65^{abc}	0.07±0.01^{bcd}	0.27±0.13^{bc}
P value		p<0.05	p<0.05	p<0.05	p<0.05

Group 1, control; Group 2, grape seed; Group 3, salynomycine; Group 4, lasalocid; Group 5, maduramicin; Group 6, salinomycin+ grape seed; Group 7, lasalocid+ grape seed; Group 8, maduramicin + grape seed.
^{a,b,c,d}. Different superscripts in the same column indicate significant differences (P<0.05).

Tablo 3. 21st and 42nd day HB and HTC values as mean±sd.

Groups	n	Haemoglobin (21st day)	Haematocrit (21st day)	Haemoglobin (42nd day)	Haematocrit (42nd day)
Group 1	12	8.52±1.21^{cd}	31.31±2.82^{b}	9.36±0.68^{a}	35.85±3.15
Group 2	12	6.89±0.68^{ab}	31.91±4.01^{b}	10.71±1.28^{b}	37.81±1.98
Group 3	12	7.52±0.86^{bc}	27.29±2.58^{a}	9.94±0.95^{ab}	35.19±3.38
Group 4	12	7.70±2.08^{bc}	32.71±5.53^{b}	8.89±0.93^{a}	33.67±5.55
Group 5	12	7.42±1.32^{bc}	27.05±5.75^{a}	10.65±1.92^{b}	35.00±3.47
Group 6	12	7.19±0.64^{ab}	28.23±2.53^{a}	10.03±2.09^{ab}	35.57±5.53
Group 7	12	9.02±1.64^{d}	34.05±2.51^{b}	8.78±1.48^{a}	35.97±5.68
Group 8	12	6.26±0.84^{a}	31.49±2.63^{b}	9.01±0.95^{a}	33.84±2.99
P value		p<0.05	p<0.05	p<0.05	p<0.05

Group 1, control; Group 2, grape seed; Group 3, salynomycine; Group 4, lasalocid; Group 5, maduramicin; Group 6, salinomycin+ grape seed; Group 7, lasalocid+ grape seed; Group 8, maduramicin + grape seed.
^{a,b,c,d}. Different superscripts in the same column indicate significant differences.

Table 4. 21st day leukocyte types (%) as mean±sd.

Groups	n	Eozinofil	Bazofil	Nötrofil	Lenfosit	Monosit
Group 1	12	2.50±1.91	17.25±10.30	28.25±4.03	56.25±10.65	2.25±2.87
Group 2	12	1.50±1.29	11.75±1.70	19.25±5.73	66.00±8.60	4.00±2.30
Group 3	12	3.75±3.09	14.00±5.16	22.00±6.32	57.00±14.39	3.75±2.06
Group 4	12	4.00±2.70	12.00±4.69	18.25±4.03	59.50±3.41	3.50±1.73
Group 5	12	5.00±1.41	15.00±5.03	24.00±5.09	56.25±4.78	3.25±1.89
Group 6	12	3.50±1.91	18.75±11.47	17.50±9.43	62.25±10.21	3.50±2.08
Group 7	12	4.00±1.82	12.50±5.00	21.25±2.21	52.75±8.84	5.25±.95
Group 8	12	3.75±1.25	13.00±3.36	21.25±9.42	49.75±8.65	5.00±2.16
P value		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05

Group 1, control; Group 2, grape seed; Group 3, salynomycine; Group 4, lasalocid; Group 5, maduramicin; Group 6, salinomycin+ grape seed; Group 7, lasalocid+ grape seed; Group 8, maduramicin + grape seed.

Table 5. 42nd day leukocyte types (%) as mean±sd.

Groups	n	Eosinophil	Basophil	Neutrophil	Lymphocyte	Monocyte
Grup 1	12	3.50±2.38	13.25±5.73	29.00±7.39	54.25±4.34	3.50±3.00
Grup 2	12	2.50±1.91	19.50±5.25	30.00±8.48	44.00±16.08	6.50±10.50
Grup 3	12	2.75±3.20	16.00±4.89	18.75±5.61	59.25±6.70	2.25±1.70
Grup 4	12	2.50±1.73	13.00±4.76	22.50±11.12	50.00±5.88	8.25±12.60
Grup 5	12	2.00±2.44	12.25±4.78	29.75±6.94	57.50±13.91	2.00±2.30
Grup 6	12	2.25±2.06	17.75±6.44	22.25±8.18	53.75±6.39	3.50±2.38
Grup 7	12	4.50±1.91	21.00±4.76	20.00±1.63	42.50±24.13	5.00±2.58
Grup 8	12	2.25±2.06	17.75±6.44	22.25±8.18	50.75±5.85	3.50±2.38
P value		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05

Group 1, control; Group 2, grape seed; Group 3, salynomycine; Group 4, lasalocid; Group 5, maduramicin; Group 6, salinomycin+ grape seed; Group 7, lasalocid+ grape seed; Group 8, maduramicin + grape seed.

Discussion and Conclusion

The toxicity of ionophore antibiotics in poultry has been previously investigated. In a study conducted by Singh and Gupta (26), chicks were fed on feed containing 5 and 10 ppm of maduramycin for a period of 21 days. Accordingly, these researchers determined that haemoglobin levels decreased in both groups on day 14 whilst total erythrocyte counts and polymorph nuclear leukocyte volume decreased in the group fed on 10 ppm of maduramycin on day 21 of the study. Arun et al. (2), in a study in which they administered broiler chickens with 5 and 8 ppm of maduramycin for a period of 6 weeks observed that haematologically the group given 8 ppm of maduramycin developed serious macrocytic anaemia. Yarsan (35) reported that when high doses (220 ppm and 330 ppm) of monensin were given to broiler chickens, MDA levels increased. Apart from their above mentioned effects, ionophore antibiotics, due to processes resulting in the intracellular accumulation of sodium and calcium, lead to the release of neuromediators, in particular noradrenalin. They also lead to the acceleration of the rate of intracellular oxidation processes, further resulting in the generation of active oxygen groups, thereby, causing degenerative disorders in tissues and organs, primarily heart and skeletal muscle, which may cause necrosis (15,31).

In the present study, when compared to the control group, in the group that received salinomycin alone, the observation of increased MDA levels and decreased erythrocyte CAT and GSH-Px enzyme activities on day 21 and increased MDA and NO levels and decreased CAT and GSH-Px enzyme activities on day 42 demonstrated that salinomycin does induce lipid peroxidation. Levels of MDA and NO on day 42 being higher than those measured on day 21 and GSH-Px activity having decreased on day 42 compared to day 21 suggest that the lipid peroxidation-inducing effects of salinomycin become more severe with the advance of time. The lipid-peroxidation-inducing effects of salinomycin have been investigated. In a study conducted by Mezes et al. (19), the administration of salinomycin at a dose of 60 mg/kg in feed for a period of 28 days was observed to result in increased. Levels and decreased GSH-Px activity, on the other hand in another study carried out by Dworschak and Prohaszka (6) in pigs given high doses of salinomycin, it was determined that blood MDA levels were elevated. The results obtained in the present study are in agreement with those obtained in previous research.

It is reported that the administration of ionophore antibiotics at high doses has negative impact on certain haematological parameters (19,35). In the present study, in broiler chickens administered with salinomycin, the observation of only a decrease in the haematocrit value measured on day 21 when compared to the controls is noteworthy. No statistically significant differences were observed in other parameters (haemoglobin,

eosinophil, basophil, neutrophil, lymphocyte and monocyte counts), which suggests that when administered in feed at the recommended dose (60 ppm) for a period of 42 days, salinomycin does not cause any major adverse effect on haematological parameters. In the present study, compared to the control group, while no statistically significant increases were observed in the MDA levels of the group administered with lasalocid (Group 4) on day 21, it was observed that MDA levels increased on day 42, NO values increased on days 21 and 42, activity of CAT decreased on days 21 and 42, and GSH-Px levels decreased in both periods. Based on these findings, it can be suggested that when administered to broiler chickens at a dose of 100 ppm, lasalocid induces lipid peroxidation.

Despite the unavailability of research on the effects of maduramycin on lipid peroxidation, as is the case with other ionophore antibiotics, it is most probable that maduramycin also leads to the generation of free radicals as a result of the secretion of catecholamines by adrenal chromaffin cells, the transport of catecholamines and their breakdown (6,35). In the present study, when compared to the control group, it was determined that, the administration of maduramycin alone led to increased MDA and NO levels and decreased CAT and GSH-Px activities on days 21 and 42. These findings have demonstrated that, when administered at normal dose (5 ppm), maduramycin results in hydroperoxide- and NO-induced lipid peroxidation.

It has been reported that maduramycin has adverse effects on haematological parameters. In a study, in which Singh and Gupta (26) fed chicks with 5 and 10 ppm of maduramycin, it was ascertained that blood haemoglobin decreased by day 14, and that in the group that received 10 ppm of maduramycin, total erythrocyte counts and the volume of polymorph nuclear cells decreased by day 21. Arun et al (2) determined that when administered to chicks at a level of 8 ppm for 6 weeks, maduramycin led to severe macrocytic anaemia. In the present study, similar to the groups administered with salinomycin and lasalocid, in the group that received maduramycin, percentile rates of leukocyte types did not display any statistically significant alterations in comparison to the control group. The only noteworthy alterations were an increase in haemoglobin values on day 42 and a decrease in haematocrit values on day 21. The decrease observed in day 21 haematocrit values was in agreement with previous literature reports. However, it was ascertained that, on day 42, haematocrit values drew closer to the values of the control group. In addition to this finding, the haemoglobin level determined to have decreased on day 21, having displayed an increase on day 42 may have arisen from the administration period of maduramycin and differences due to the chicks belonging to different poultry breeds or might be considered as a defence mechanism of the body acting against the adverse effects of maduramycin.

It was determined that, on day 21 of the trial, in the group that received a combination of salinomycin+grape seed, MDA level was significantly lower than that of the group given salinomycin alone associated with statistically insignificant decrease in NO levels and increase in catalase and GSH-Px activities ($p>0.05$). Based on, in particular, the alterations observed in MDA levels, it could be suggested that grape seed provides partial protection against lipid peroxidation induced by salinomycin. In the group that was administered with both grape seed and salinomycin, no statistically significant differences were observed in MDA and NO levels and GSH-Px activity on day 42, and the only significant change was an increase in CAT activity ($p<0.05$). Catalase breaks down hydrogen peroxide (H_2O_2) into oxygen and water, and thereby, protects cells against H_2O_2–induced oxidative damage (12). This finding suggests that grape seed has protective effect against potential H_2O_2-induced oxidative damage caused by salinomycin in poultry.

The comparison of the group given a combination of grape seed+lasalocid and the group administered with lasalocid alone demonstrated that grape seed resulted in a statistically insignificant decrease in MDA and NO levels on days 21 and 42 of the trial which was associated with a statistically insignificant increase in CAT and GSH-Px activities ($p>0.05$). These results demonstrated that when given to chickens at a rate of 0.5% in feed, grape seed provides inadequate protection against lipid peroxidation induced by lasalocid.

The comparison of the group given grape seed+maduramycin (Group 8) with the group administered with maduramycin alone (Group 5) showed that grape seed only caused a statistically significant decrease in NO levels on day 21 of the trial. In the group that received grape seed+maduramycin, of the values measured on day 42 of the trial, only CAT activity displayed an increase. Furthermore, the comparison of the

control group with the group that received maduramycin alone demonstrated that maduramycin caused a statistically significant increase in NO levels. In the group, which was administered with both grape seed and maduramycin, the decrease observed in NO levels on day 21 and the increase observed in CAT activity on day 42 demonstrated that grape seed was particularly effective against NO-induced lipid peroxidation and that grape seed induced the synthesis of the CAT enzyme, which entered the consumption phase (12).

In conclusion, the present study demonstrated that when administered to broiler chickens at a rate of 0.5% in feed for a period of 42 days, grape seed did not induce any adverse effect on lipid peroxidation parameters, on the other hand, when added to broiler feed at treatment doses and particularly for a period of 42 days, salinomycin, lasalocid and maduramycin caused lipid peroxidation. Finally, it was ascertained that when administered in combination with salinomycin, lasalocid and maduramycin, grape seed displayed regulatory effects on certain lipid peroxidation and haematological parameters.

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