ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Research Article / Araştırma Makalesi
20(2), 127-133, 2023
DOI: 10.32707/ercivet.1332533

An Experimental Study on the Use of Exosomes against Acetaminophen-induced Uterine and Fallopian Tubes Damage in Rats

Enes KARAMAN[1,a], Munevver BARAN[2,b], Zeynep Burcin GONEN[3,4,c], Ozge GOKTEPE[3,5,d], Gozde Ozge ONDER[3,5,e], Eda OKUR[5,f], Erol KARAKAS[6,g], Arzu YAY[3,5,h]

[1]Mardin Savur Prof. Dr. Aziz Sancar District State Hospital, Gynecology and Obstetrics Clinic, Mardin-TÜRKİYE
[2]Erciyes University, Faculty of Pharmacy Department of Pharmaceutical Basic Science, Kayseri- TÜRKİYE
[3]Erciyes University, Genome and Stem Cell Center (GENKOK), Kayseri-TÜRKİYE
[4]Erciyes University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Kayseri- TÜRKİYE
[5]Erciyes University, Faculty of Medicine Department of Histology and Embryology, Kayseri- TÜRKİYE
[6]Kayseri State Hospital, Gynecology and Obstetrics Clinic, Kayseri-TÜRKİYE
ORCID: [a]0000-0003-1459-0606, [b]0000-0003-0369-1022, [c]0000-0003-2725-9330, [d]0000-0002-8205-2132, [e]0000-0002-0515-9286, [f]0000-0002-0502-7814, [g]0000-0002-8205-2132, [h]0000-0002-0541-8372

Corresponding author: Enes KARAMAN; E-mail: dr.eneskaraman@gmail.com
How to cite: Karaman E, Baran M, Gonen ZB, Goktepe O, Onder GO, Okur E, Karaka E, Yay Arzu. An experimental study on the use of exosomes against acetaminophen-induced uterine and fallopian tubes damage in rats. ErciyesUniv Vet FakDerg 2023; 20 (2): 127-133

Abstract: Acetaminophen (APAP) is an antipyretic and analgesic drug that can be bought and used without a prescription worldwide. A dosage of APAP greater than the maximum recommended dosage can increase the risk of organ damage. Mesenchymal stem cells (MSCs) are isolated from various human tissues and used for therapy, in which beneficial effects are attributed mainly to mesenchymal stem cell-derived extracellular vesicles (MSC-EVs). However, no study has focused on the protective effect of exosomes in combination with APAP. Therefore, the present study was carried out to investigate the protective effect of taking exosomes against APAP toxicity on the fallopian tubes and uterus. Forty female Wistar albino rats (12–14 weeks old) were randomly divided into four equal groups: control, APAP (received 1 g/kg APAP), exosome (received 30 µg of exosomes), and APAP+exosome groups that received simultaneously 1 g/kg APAP and were followed three days later by a tail vein injection 30 µg of exosomes. The uterus and fallopian tubes were removed for histological and immunohistochemical analyses after the animals were sacrificed. The results showed that exosomes' administration after APAP decreased APAP's autophagic effects. Moreover, exosome treatment exhibited a protective effect on the immunoreactivity intensities of autuphagy markers (Beclin-1, p62, and LC3). The treatments with exosomes had no adverse effect on the uterus or fallopian tubes. The administration of exosomes after APAP toxicity can decrease cell death through the autophagy effect of APAP. It is suggested that this compound can decrease the toxic effects of APAP. Further studies are needed to evaluate the molecular mechanism of this hyperanalgesic effect.
Keywords: Autophagy, acetaminophen, exosome, pain, rat

Sıçanlarda Asetaminofen Kaynaklı Uterin ve Fallop Tüpleri Hasarına Karşı Eksozomların Kullanımı Üzerine Deneysel Bir Çalışma

Öz: Asetaminofen (APAP), dünya çapında reçetesiz satın alınabilen ve kullanılabilen bir antipiretik ve analjezik ilaçtır. Önerilen maksimum dozdan daha yüksek bir APAP dozu, organ hasarı riskini artırabilir. Mezenkimal kök hücreler (MSC'ler), çeşitli insan dokularından izole edilir ve yararlı etkilerin esas olarak mezenkimal kök hücreden türetilen hücre dışı veziküllere (MSC-EV'ler) atfedildiği terapi için kullanılır. Bununla birlikte, hiçbir çalışma eksozomların APAP ile kombinasyon halinde koruyucu etkisine odaklanmamıştır. Bu nedenle bu çalışma APAP toksisitesine karşı eksozom almanın fallop tüpleri ve uterus üzerindeki koruyucu etkisini araştırmak amacıyla yapılmıştır. Kırk dişi Wistar albino sıçan (12-14 haftalık) rastgele dört eşit gruba ayrıldı: kontrol, APAP (1 g/kg APAP aldı), eksozom (30 µg eksozom aldı) ve APAP+eksozom grupları eş zamanlı olarak 1 g/kg APAP ve ardından üç gün sonra kuyruk damarından 30 ug eksozom enjeksiyonu yapıldı. Hayvanlar sakrifiye edildikten sonra histolojik ve immünohistokimyasal analizler için uterus ve fallop tüpleri çıkarıldı. Sonuçlar, eksozomların APAP'tan sonra uygulanmasının APAP'ın otofajik etkilerini azalttığını gösterdi. Ayrıca, eksozom tedavisi, otofaji belirteçlerinin (Beclin-1, p62 ve LC3) immünoreaktivite yoğunlukları üzerinde koruyucu bir etki sergiledi. Eksozomlarla yapılan tedavilerin uterus veya fallop tüpleri üzerinde herhangi bir olumsuz etkisi olmamıştır. APAP toksisitesinden sonra eksozomların uygulanması, APAP'ın otofaji etkisi yoluyla hücre ölümünü azaltabilir. Bu bileşiğin APAP'ın toksik etkilerini azaltabileceği öne sürülmektedir. Bu hiperanaljezik etkinin moleküler mekanizmasını değerlendirmek için ileri çalışmalara ihtiyaç vardır.
Anahtar kelimeler: Ağrı, asetaminofen, eksozom, otofaji, sıçan

Geliş Tarihi/Submission Date : 21.03.2023
Kabul Tarihi/Accepted Date : 16.06.2023

Introduction

The paraminophenol class of drugs includes acetaminophen (APAP: N-acetyl-p-aminophenol; also known as paracetamol), a non-steroidal anti-inflammatory drug. Its chemical name is N-acetyl-p-aminophenol or 4-hydroxyacetanilide ($C_8H_9NO_2$). The most often recommended antipyretic and analgesic, APAP, generally known as paracetamol, is used mostly for fever, migraine, neuralgia, joint pain, etc. (Zhang et al., 2016). Sexual performance, fertility index, implantation index, and number of implants all decreased after receiving repeated oral treatments for 30 days with APAP (500 and 1000 mg/kg) (Ratnasooriya and Jayakody, 2000). Research on rodents shows that APAP reduces the total number of adult follicles and fetal ovarian germ cells, which affects fertility (Holm et al., 2016; Johansson et al., 2016). Little is known about APAP in girls and its potential relationship to the reproductive system.

The human body consists of mesenchymal stem cells (MSCs) in a variety of tissues. To support numerous physiological processes, MSCs exhibit niche-dependent multiline age differentiation and produce therapeutic exosomes. MSCs thus promise targeted therapy and have the capacity to support both cell-based and cell-free therapies (Li et al., 2019; Mehta, 2021). MSCs are found in a range of tissues throughout the human body. MSCs exhibit niche-dependent multiline age differentiation and produce therapeutic exosomes to support a variety of physiological activities. MSCs can therefore support both cell-based and cell-free therapies and promise targeted therapy. Extracellular vesicles (EVs), which transport a variety of organic molecules, are used by cells to communicate with neighboring cells and distant organs (such as protein, genomic DNA, and RNA). These vesicles are classified depending on their biogenesis and size (Czernek et al., 2020). Exosomes are known as heterogeneous collections of vesicles, and they also carry the phenotypic state of the cell from which they were produced (Sharma et al., 2020). They are released from cells and enter bodily fluids, where they affect the behavior of neighboring cells (Czernek et al., 2020). Exosomes contain a lipid bilayer and, like cells derived from them, transport molecules like DNA, protein, and RNA. Exosomes share a lipid bilayer structure with the cell from which they originated (Kalluri, 2016; Yin et al., 2019). They can freely move throughout the body's blood vessels (Sharma et al., 2020). Through the use of signals including growth factors, proteases, and cytokines, they allow communication between nearby or distant cells (Harris et al., 2015).

It is worrying that research has associated APAP exposure with reduced primordial follicle pools and, hence, reduced fertility. Until now, no studies have investigated the efficacy of concurrent exosome administration on potentially enhancing therapeutic outcomes. So, the purpose of this investigation was to determine whether co-administering exosomes and APAP to female rats had any analgesic activity.

Material and Methods

Animal care

The Institutional Animal Care Committee of Erciyes University approved the study according to a protocol that was followed for this study (approval number 23/030). We used male Wistar albino rats in this work (250–300 g). All of the animals included in the study had normal behaviors, were healthy, and belonged to the same species and gender. Rats that had been previously used in other experiments were not included. The animals were given unlimited access to water and food while being kept in a 12-hour cycle of light and darkness. The animals were kept for at least three days prior to use. The animals were put to death immediately following the experiment using a dose of ketamine.

Obtaining dental pulp MSCs derived exosomes

The secretomes of the dental pulp derived MSCs were collected. Exosomes was isolated using a standard commercial kit (ExoQuick-TC Exosome Precipitation Solution Kit). Briefly, after centrifuging the secretomes obtained for exosome isolation at 3000 x g for 15 minutes, the supernatants were placed into sterile tubes. 10 ml of supernatant were mixed with 2 ml of ExoQuick-TC solution, and the mixture was then incubated for 12 hours or overnight. A 30-minute centrifugation at 1500 x g was then performed on the ExoQuick-TC/supernatant mixture. After the supernatants were removed, pellet-like exosomes could be seen at the tube's bottom.

Experimental design

Different treatments were given to various groups, as follows: Control group: administered as usual with saline. APAP group: On the first day of the trial, rats were gavaged with a single dosage of 1 g/kg of APAP (Baravalia and Chanda, 2011). Exosome group: 30 µg of exosomes were given in 100 µl from the tail vein (Salkın and Basaran, 2023). APAP+exosome group: Each rat received a single dose of 1 g/kg of APAP by gavage, followed three days later by a tail vein injection 30 µg of exosomes. At 72 hours after the last application in the study, the animals were profoundly anesthetized by ketamine and xzylazin (60/10 mg/kg), intraperitoneally, before euthanization. The organs of interest the fallopian tubes and uterus were immediately detached and subsequently processed for histological evaluations.

Histopathological studies

In order to evaluate the uterus and fallopian tubes defects of each experimental group histologically, tissue samples taken at the end of the experiment were fixed in a 10% formaldehyde solution. Tissues kept in formaldehyde for 72 hours were washed in running tap water, passed through a series of increasing grades of alcohol, cleared with xylol, then embedded in paraffin and paraffin blocks were prepared. 5 µm sections taken from paraffin blocks containing rat tissues were taken on polylysine-coated slides. The prepared slides were graded in alcohol (100%, 96%, 80%, 70%, 50%) and then rinsed in water after being deparaffinized with xylol using the standard histological staining technique. The sections were processed through an increasing alcohol series, stained with hematoxylin-eosin (H&E) and Masson's trichrome (MT), passed through xylol, covered with a coverslip by dripping entellan, and examined under a light microscope to identify the general histological structure (Olympus BX51, Tokyo, Japan).

Immunohistochemical procedure

The immunohistochemistry (IHC) studies to evaluate Beclin-1, p62, and LC3 activities use the avidin-biotin peroxidase method. Following deparaffinization, the cross sections were incubated in citrate buffer (pH 6.0) and 3% hydrogen peroxide (Lab Vision, Thermo Scientific, Fremont). To avoid non-specific binding, an Ultra-V block (Lab Vision, Thermo Scientific, Fremont) was used. Following the blocking procedure, tissue sections were incubated overnight with Beclin-1 (Novus Biologicals, Littleton, CO), p62 (Novus Biologicals, Littleton, CO), and LC3 (Cell Signaling Technology, Danvers, MA) primer antibodies. The tissue samples were then exposed for 10 minutes to secondary antibodies (Lab Vision, Thermo Fisher Scientific, Fremont). The streptavidin peroxidase complex (Lab Vision, Thermo Fisher Scientific, Fremont) in combined with DAB enabled made the reaction product detectable. A counterstain was applied using Mayer's hematoxylin. Semi-quantitative Beclin-1, p62, and LC3 immunohistochemical analyses were evaluated in paraffin sections of the uterus and fallopian tubes using a computer imaging system. In the research lab of Erciyes University, two histologists independently and semi-quantitatively assessed immunostaining. The Image J software was used to obtain quantitative measurements. The immunoreactivity intensity for Beclin-1, p62, and LC3 was assessed within 5 fields for each animal at a total magnification of x400.

Statistical analysis

PrismTM software version 8.0 (GraphPad Inc., San Diego, CA) was used to analyze the data (GraphPad Software, San Diego, CA, USA). Standard error of the mean (SEM) is used to provide group means for the raw data. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to examine the distribution of the data. Variables with a normal distribution have, one-way variance analysis (ANOVA) was used before a multiple comparison test by Bonferroni. The following significance levels are listed: *P < 0.05; **P < 0.01; ***P < 0.001.

Result

Histological results

Our histological findings showed that the uterine endometrium, myometrium, and serous layers were distinct in the rats from the control group. Rats from the control group were able to distinguish between myometrium, which is composed of smooth uterine muscles, and endometrium, which is composed of ciliated simple columnar epithelium and an underlying lamina propria where the uterine glands and blood vessels are located. In contrast to the other groups, the endometrium, myometrium, and serosa that constitute the uterine histological structures were more difficult to distinguish in the uterus of APAP-treated rats. Comparatively to the untreated rats, ciliated and secretory epithelial cells were not visible, but foamy-like forms with heterochromatic nuclei were in the APAP-treated rats. In comparison to the control group, this group's lamina propria showed less or no uterine glands at a few sites. The fibroblasts, and connective tissue stroma-forming cells in the lamina propria were seen to have edematous regions and extravasation erythrocytes in addition to degenerative changes. The exosome group's rat uterus had the same normal histology as that of the control group, including the endometrium, myometrium, serous layers, and uterine glands. Layers of the rat uterus from the APAP+exosome group were identified as luminal epithelium, lamina propria, myometrium, and serosa. In the luminal epithelium of the uterus of rats in the APAP+exosome group compared to APAP group animals, foamy-like forms containing heterochromatic nuclei were reduced (Figure 1).

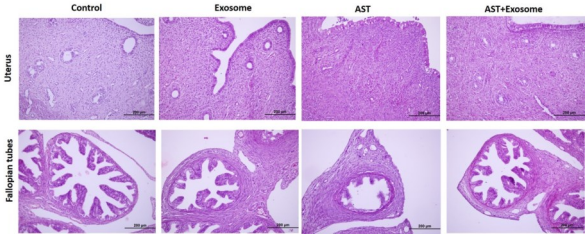

Figure1. Representative micrographs of uterus and fallopian tube stained with H&E are showed at a magnification of ×200. The mucosal folds of the control are covered by simple columnar ciliated epithelium in each tissue. Abnormally loss most of their cilia with the appearance of atypicalpleomorphic nuclei in APAP group. However APAP+exosome group showed the typically normal columnar epithelium covered by numerous cilia (H&E staining).

The control rat fallopian tube was examined using H&E-stained sections, which demonstrated the fimbriae were formed of branched vascular tissue and ciliated columnar epithelium (Figure 1). The stromal and epithelial cells were extensively separated in the damaged tubes. Many cells had pyknotic, darkly pigmented nuclei APAP group. Following exosome therapy, the epithelial cells appeared to return to their usual position, and the pyknotic cells became less noticeable in APAP+exosome group.

In the light microscopic examination of the MT-stained uterus and fallopian tube tissues of the experimental groups, no significant difference was observed in the direction of fibrosis in both tissues (Figure 2).

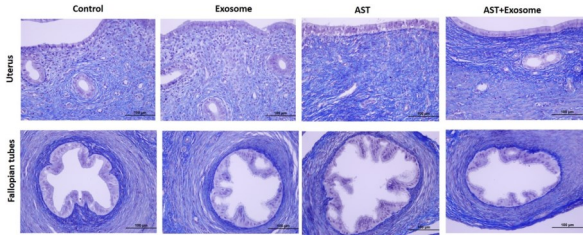

Figure 2. Representative photomicrographs of uterus and fallopian tubes in various groups (MT staining, 390 magnification ×40).

Immunohistochemical results

By using IHC, the semi-quantitative immunoreactivity of the autophagy markers Beclin-1, p62, and LC3 was assessed between the groups. In the endometrium of the rats in the APAP group, we observed that Beclin-1 expression was lower than that of the rats in the control group in surface epithelial cells, glandular epithelial cells, and stromal cells. Rats from the exosome group had similar levels of beclin-1 immunoreactivity in both uterine glands and endometrium compared to the control group (Figure 3).

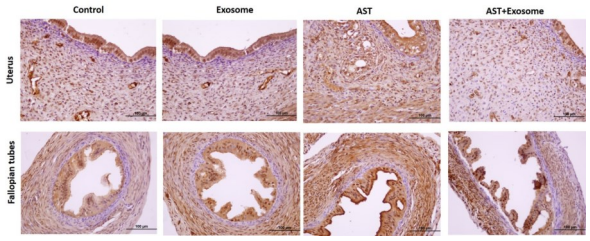

Figure 3. Representative Beclin-1 immunohistochemistry micrographs of uterus and fallopian tubes are showed at a magnification of ×400.

We observed that after APAP administration, Beclin-1, p62, and LC3 immunostaining in surface and glandular epithelial cells and stromal cells in the endometrium increased, while the expression decreased after exosome treatment when we semi-quantitatively compared APAP+exosome with APAP alone (Figure 4).

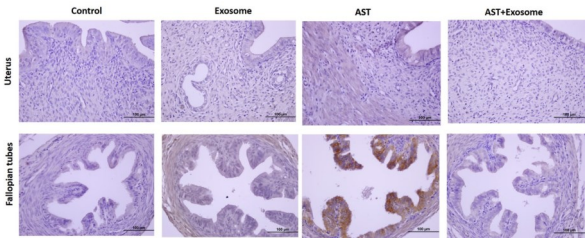

Figure 4. The immunoreactivity of autophagy marker p62 in rat uterus and fallopian tubes from each group. (IHC, magnification ×40).

Rats from the APAP+exosome and APAP groups showed higher levels of LC3 immunoreactivity in glandular epithelial cells, surface epithelial cells, and stromal cells in the endometrium than did rats from the control group. Rats from the exosome group had similar endometrial and uterine gland immunoreactivity to rats from the control group. We found that LC3 immunoreactivity increased after APAP administration in comparison with the control and decreased after exosome administration when we semi-quantitatively compared the APAP+exosome and APAP groups (Figure 5).

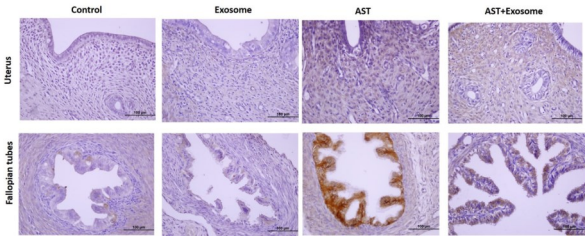

Figure 5. Expression LC3 in uterus and fallopian tubes epithelial cells of the experimental groups (IHC, magnification ×40). The highest intensity expression in APAP group, the lowest in the control group.

The statistical differences between the expression levels of autophagy markers in the uterus and fallopian tube tissues of the experimental groups are shown in Figure 6.

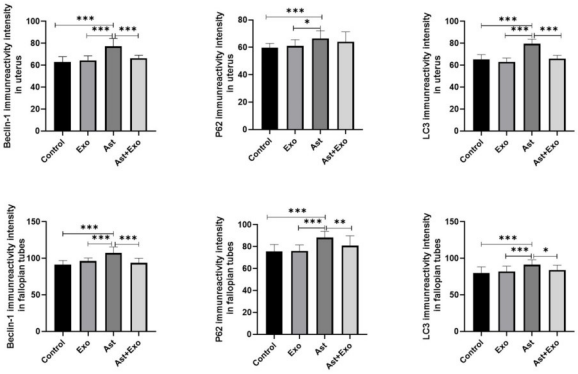

Figure 6. The autophagy markers (Beclin-1, p62, and LC3) in the uterus and fallopian tube were enumerated in immunohistochemical stains. The values in all panels are depicted as mean ± SEM. (*P < 0.05; **P < 0.01; ***P < 0.001).

Discussion and Conclusion

APAP has analgesic, antipyretic, and anti-inflammatory effects on patients with a variety of clinical diseases. Adult fertility and, more broadly, all reproductive functions and processes throughout life are defined by reproductive health. Several research studies have highlighted variations in human reproductive function. The consistent rise in reproductive diseases raises the possibility of environmental and/or lifestyle-related causes (Arendrup et al., 2018). Endocrine disruptors may therefore cause harm throughout generations, particularly in germ cells (Wei et al., 2015), leading to decreased fertility and worse quality gametes, sperm, and oocytes.

APAP is widely used by women. After exposure to APAP during the process of embryogenesis or organogenesis, animal models have demonstrated necrotic and degenerative alterations in the pulmonary, reproductive, and neurological systems (Van den Anker and Allegaert, 2018). The research that is now available also suggests that paracetamol may disrupt several hormonal processes, including steroidogenesis and the depletion of sulfated sex hormones (Cohen et al., 2018), establishing the biological plausibility for any potential change in the embryonic reproductive systems related to paracetamol exposure. When pregnant rats were given indomethacin (0.8 mg/kg/day between 15.5 and 18.5 dpc) or APAP (350 mg/kg/day between 13.5 and 21.5 dpc), the number of germ cells in the fetal ovaries was decreased (by 40–50%). As a result, in utero exposure to APAP and indomethacin impacted the size of the adult ovary and the fertility of F1 females (as determined by the number of pups per generation) (Dean et al., 2016). The sexual development of female offspring was also affected by maternal APAP exposure (350 mg/kg/day from 6 dpc until delivery and from 6 dpc until weaning), which increased plasma estradiol levels, decreased follicle reserve, and impaired sexual behavior in female offspring (Aleixo et al., 2020). In prenatal exposure to APAP in mice (50 or 150 mg/kg/day from 7 dpc to delivery), similar reductions in the number of primordial germ cells were observed at 13.5 dpc, which resulted in a reduced follicular pool in exposed ovaries than in controls. Hence, research suggests that therapeutic APAP dosages have an impact on female fertility (Holm et al., 2016). Growing human epidemiological data over the past 20 years has also raised worry about early life paracetamol exposure and an increased incidence of neurodevelopmental, atopic, and reproductive adverse effects (Bardanzellu et al., 2017). Conversely, the consequences of postpartum ovarian and female reproductive health exposure are unknown. Therefore, the effects of APAP on reproductive health should also be examined. However, we show for the first time that APAP alters the architecture of the uterus and fallopian tubes, which can have toxic effects on female rats.

Exosomes were formerly thought to function as a method for the cell to expel waste. Exosomes were shown to be involved in intercellular communication in the 2000s. Exosomes have been shown to load a wide range of substances and transport them among cells by acting as cargo in the decades that followed (Zhao et al., 2020). Through the application of signals including growth factors, proteases, and cytokines, they enable communication between near or distant cells (Harris et al., 2015). Considering that they include miRNA, exosomes offer a crucial idea in the regulation of changes in cellular activity through genetic material transfer (Valadi et al., 2007). Exosomes behave and function differently depending on where they come from (Börger et al., 2017). Exosomes have an additional benefit over mesenchymal stem cells in that they are a cell-free therapy, preventing immunological reactions and other undesirable effects (Timmers et al., 2011). Because of its better safety profile and reduced immunogenicity compared to using MSCs directly, using exosomes may offer noticeable advantages (Mendt et al., 2019).

In order to promote embryonic implantation during the conceptive cycle and periods of shedding and regeneration during the subsequent non-conception cycle, the endometrium is a dynamic, complex tissue that passes through stages of proliferation and differentiation in succession (Tabibzadeh, 1996). The fallopian tube is essential for several reproductive processes, including sperm transport and capacity, ovary retrieval and transport, fertilization, and the nutrition and transportation of early embryos, in addition to functioning as a passive pathway for gametes and early embryos (Patil, 2009). In order to maintain cellular homeostasis, autophagy mediates the lysosome-mediated degradation of cytoplasmic components such as damaged mitochondria and protein aggre-

gates (Parzych and Klionsky, 2014). In multicellular organisms, autophagy genes play a crucial role in controlling a broad spectrum of vital cellular processes, including cell proliferation, cell death, inflammation, and a wide range of innate and adaptive immune responses (Levine and Kroemer, 2008). As autophagy may be protective or harmful depending on the biological context, the relationship between autophagy and disease pathogenesis is currently a subject of significant research.

Recent studies have demonstrated the importance of autophagy in the physiological and pathological functions of the endometrium, including cyclic menstruation, decidualization, implantation, and disorders such as endometrial hyperplasia, endometrial cancer, and endometriosis (Oestreich et al., 2020). Human uterine epithelial cells from postmenopausal women had higher autophagy levels than those from premenopausal women, suggesting that autophagy began to develop in response to estrogen deficiency (Zhou et al., 2016). This is consistent with the finding that ceasing progesterone or estrogen causes modifications to the menstrual cycle (Choi et al., 2012) by significantly increasing the expression of LC3 in the endometrial Ishikawa cell line. These results suggest that the basal levels of autophagy that are maintained in the cycling endometrium are under the control of ovarian steroid hormone levels. A further interesting observation in this study is that APAP administration, immunostaining of Beclin-1, p62, and LC3 autophagy markers in epithelial and stromal cells in the endometrium increased, while the expression decreased after exosome treatment. Moreover, animals in the exosome group had endometrium and uterine glands that immunoreactive similarly to control rats for Beclin -1, p62, and LC3.

The rat uterus and fallopian tubes exhibited histological and morphometric alterations as a result of exposure to APAP. Exosome treatment can also prevent the degenerative changes caused by APAP in the uterus and fallopian tubes. It appears that APAP has a detrimental impact on the uterine and fallopian tube ultrastructure in rats until it is demonstrated differently. Exosomes can also be used to improve this histology process. Studies utilizing combinations of analgesics should be generalized based on our findings because people frequently take analgesics or are exposed to different cocktails of anti-androgenic or anti-estrogenic chemicals in the environment. Due to the widespread usage of analgesics, more rodent research is needed to evaluate the harmful effects of these drugs.

References

Aleixo JF, Pereira MRF, Montagnini BG, Pereira MJD, Forcato S, Moreira EG, Ceravolo GS, Vieira ML, Kiss ACI, Gerardin DCC. Effect of paracetamol treatment on maternal care and reproductive outcomes in female rat offspring. Reprod Fertil Dev 2020; 32(18): 1311-25.

Arendrup FS, Mazaud-Guittot S, Jégou B, Kristensen DM. EDC IMPACT: Is exposure during pregnancy to acetaminophen/paracetamol disrupting female reproductive development? Endocr Connect 2018; 7(1): 149-58.

Baravalia Y, Chanda S. Protective effect of Woodfordia fruticosa flowers against acetaminophen-induced hepatic toxicity in rats. Pharm Biol 2011; 49(8): 826-32.

Bardanzellu F, Neroni P, Dessì A, Fanos V. Paracetamol in patent ductus arteriosus treatment: efficacious and safe? Biomed Res Int 2017; 2017: 1438038.

Börger V, Bremer M, Ferrer-Tur R, Gockeln L, Stambouli O, Becic A, Giebel B. Mesenchymal stem/stromal cell-derived extracellular vesicles and their potential as novel immunomodulatory therapeutic agents. Int J Mol Sci 2017; 18(7): 1450.

Choi J, Jo M, Lee E, Oh YK, Choi D. The role of autophagy in human endometrium. Biol Reprod 2012; 86(3): 70.

Cohen IV, Cirulli ET, Mitchell MW, Jonsson TJ, Yu J, Shah N, Spector TD, Guo L, Venter JC, Telenti A. Acetaminophen (paracetamol) use modifies the sulfation of sex hormones. EBioMedicine 2018; 28: 316-23.

Czernek L, Düchler M. Exosomes as messengers between mother and fetus in pregnancy. Int J Mol Sci 2020; 21(12): 4264.

Dean A, van den Driesche S, Wang Y, McKinnell C, Macpherson S, Eddie SL, Kinnell H, Hurtado-Gonzalez P, Chambers TJ, Stevenson K, Wolfinger E, Hrabalkova L, Calarrao A, Bayne RA, Hagen CP, Mitchell RT, Anderson RA, Sharpe RM. Analgesic exposure in pregnant rats affects fetal germ cell development with inter-generational reproductive consequences. Sci Rep 2016; 6: 19789.

Harris DA, Patel SH, Gucek M, Hendrix A, Westbroek W, Taraska JW. Exosomes released from breast cancer carcinomas stimulate cell movement. PloS One 2015; 10(3): e0117495.

Holm JB, Mazaud-Guittot S, Danneskiold-Samsøe NB, Chalmey C, Jensen B, Nørregård MM, Hansen CH, Styrishave B, Svingen T, Vinggaard AM, Koch HM, Bowles J, Koopman P, Jégou B, Kristiansen K, Kristensen DM. Intrauterine exposure to paracetamol and aniline impairs female reproduc-

tive development by reducing follicle reserves and fertility. Toxicol Sci 2016; 150(1): 178-89.

Johansson HK, Jacobsen PR, Hass U, Svingen T, Vinggaard AM, Isling LK, Axelstad M, Christiansen S, Boberg J. Perinatal exposure to mixtures of endocrine disrupting chemicals reduces female rat follicle reserves and accelerates reproductive aging. Reprod Toxicol 2016; 61: 186-94.

Kalluri R. The biology and function of exosomes in cancer. J Clin Invest 2016; 126(4): 1208-15.

Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008; 132(1): 27-42.

Li QC, Liang Y, Su ZB. Prophylactic treatment with MSC-derived exosomes attenuates traumatic acute lung injury in rats. Am J Physiol Lung Cell Mol Physiol 2019; 316(6): L1107-L1117.

Mehta KJ. Role of iron and iron-related proteins in mesenchymal stem cells: Cellular and clinical aspects. J Cell Physiol 2021; 236(10): 7266-89.

Mendt M, Rezvani K, Shpall E. Mesenchymal stem cell-derived exosomes for clinical use. Bone Marrow Transplant 2019; 54(Suppl 2): 789-92.

Oestreich AK, Chadchan SB, Popli P, Medvedeva A, Rowen MN, Stephens CS, Xu R, Lydon JP, Demayo FJ, Jungheim ES, Moley KH, Kommagani R. The autophagy gene atg16L1 is necessary for endometrial decidualization. Endocrinology 2020; 161(1): bqz039.

Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Signal 2014; 20(3): 460-73.

Patil M. Assessing tubal damage. J Hum Reprod Sci 2009; 2(1): 2-11.

Ratnasooriya WD, Jayakody JR. Long-term administration of large doses of paracetamol impairs the reproductive competence of male rats. Asian J Androl 2000; 2(4): 247-55.

Salkın H, Basaran KE. Effects of non-steroidal anti-inflammatory drug (ibuprofen) in low and high dose on stemness and biological characteristics of human dental pulp-derived mesenchymal stem cells. Connect Tissue Res 2023; 64(1): 14-25.

Sharma P, Diergaarde B, Ferrone S, Kirkwood JM, Whiteside TL. Melanoma cell-derived exosomes in plasma of melanoma patients suppress functions of immune effector cells. Sci Rep 2020; 10(1): 92.

Tabibzadeh S. The signals and molecular pathways involved in human menstruation, a unique process of tissue destruction and remodelling. Mol Hum Reprod 1996; 2(2): 77-92.

Timmers L, Lim SK, Hoefer IE, Arslan F, Lai RC, van Oorschot AA, Goumans MJ, Strijder C, Sze SK, Choo A, Piek JJ, Doevendans PA, Pasterkamp G, de Kleijn DP. Human mesenchymal stem cell-conditioned medium improves cardiac function following myocardial infarction. Stem Cell Res 2011; 6(3): 206-14.

Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9(6): 654-9.

van den Anker JN, Allegaert K. Acetaminophen in the neonatal intensive care unit: shotgun approach or silver bullet. J Pediatr 2018; 198: 10-11.

Wei Y, Schatten H, Sun QY. Environmental epigenetic inheritance through gametes and implications for human reproduction. Hum Reprod Update 2015; 21(2): 194-208.

Yin X, Hou T, Huang B, Yang L, Li F. Aptamer recognition-trigged label-free homogeneous electrochemical strategy for an ultrasensitive cancer-derived exosome assay. Chem Commun (Camb) 2019; 55(91): 13705-8.

Zhang J, Zhang L, Sun X, Yang Y, Kong L, Lu C, Lv G, Wang T, Wang H, Fu F. Acetylcholinesterase inhibitors for alzheimer's disease treatment ameliorate acetaminophen-induced liver injury in mice via central cholinergic system regulation. J Pharmacol Exp Ther 2016; 359(2): 374-82.

Zhao X, Wu D, Ma X, Wang J, Hou W, Zhang W. Exosomes as drug carriers for cancer therapy and challenges regarding exosome uptake. Biomed Pharmacother 2020; 128: 110237.

Zhou S, Zhao L, Yi T, Wei Y, Zhao X. Menopause-induced uterine epithelium atrophy results from arachidonic acid/prostaglandin E2 axis inhibition-mediated autophagic cell death. Sci Rep 2016; 6: 31408.