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The prospective impact of paracetamol medication on female Wistar rats' reproductive health (biochemical, genotoxic, and histological analysis)

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Abstract

Paracetamol is a commonly purchased and administered non-prescription analgesic medication on a global scale. The goal of this study was to evaluate the impact of paracetamol on the female reproductive health. The study involved random allocation of healthy Wistar rats into three groups (n=6/group). The control group was administered 0.5 ml of physiological saline, the low-dose group received 82 mg/kg paracetamol, and the high-dose group received 164 mg/kg paracetamol, all groups received paracetamol for 30 days. After 24 hours following the final dose, the rats were euthanized under anesthesia. The level of hormones, oxidative stress biomarkers, DNA damage and histological analysis were performed. The findings revealed that there was no significant alteration in overall rats' weight and reproductive organs weight. The biochemical findings indicated that paracetamol exerted an impact on the levels of reproductive hormones and disrupted the normal balance of malondialdehyde, resulting in a notable reduction in overall antioxidant activity. Furthermore, a significant level of DNA fragmentation was observed. Paracetamol induced degeneration of ovarian follicles, and loss of columnar morphology in uterine epithelial cells, indicating the occurrence of apoptosis. This study indicates that administering paracetamol at a low dose of 82 mg/kg and a high dose of 164 mg/kg for 30 days adversely affects female reproductive health, perhaps increasing the risk of infertility.

Keywords: Paracetamol, Female Wistar rats, Reproductive health, Hormones, Oxidative stress markers, Comet assay…

1. Introduction

Reproduction is one of the most important biological processes that is essential for the maintaining the continuity, survival and spread of the race. The process of producing healthy, viable children is intricate and requires the cooperation of several reproductive system tissues, organs, and hormones in order to facilitate fertilization [65]. On the other hand, according to recent research, one in seven couples experience infertility problems and seek medical attention to address them in order to become pregnant [34].The World Health Organization (WHO)'s and the International Committee for Monitoring Assisted Reproductive Technology stated that the current definition of infertility is the inability of sexually active partners to conceive after more than a year of unprotected, continuous sexual activity with no known reproductive problem [51].

Infertility has become a huge problem nowadays as many people are suffering and facing it at a high rate, and it h7as always been the primary challenge for reproductive medicine [58]. Studies showed that 50% of the reasons behind infertility problems are found to be from females [35]. Previous studies showed that infertility is caused due to inherited or acquired disorders [20] . There are different causes of infertility, whether medical factors, environmental factors [8], weight changes [62], hormonal imbalance [8] or pharmaceutical drug exposure [24].

Paracetamol drug is one of the most popular drugs nowadays, and it is the widely utilized drug over the counter as a painkiller and antipyretic drug [30] . It is found in many formulations, in all dosage forms, tablets, syrup, suppositories and injection. It is known as the safest drug, and many guidelines recommend it as a go-to treatment used in almost all ages, forming Step One of the WHO analgesic ladders [57]. It is frequently used in adults to treat headaches, menstrual pain, musculoskeletal pain, and dental discomfort [3].

The complex medication paracetamol works through multiple metabolic pathways to provide antipyretic and analgesic effects [14].

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According to toxicity studies, it is found that paracetamol drug has different numbers of mild side effects like nausea, stomach pain, and itching effect. It starts to affect the body's organs like the liver, kidney, brain, heart and male reproductive health and the drug's impact on the quality and quantity of the sperm if it is administrated long-term or in high doses [44]. Many research investigations have highlighted the impact of paracetamol ingestion on pregnancy and fetal health; nevertheless, others indicate that the harmful effects of paracetamol on the female reproductive system remain limited. The objective of this study is to investigate the effects of paracetamol on the reproductive health of female Wistar rats after a 30-day therapy with different dosages.

2. Materials and Methods

2.1. Chemicals

The paracetamol drug was purchased as its raw material in powder form from INAD Pharma Company at the second industrial zone, 6th of October city.

For the comet assay reagents Dimethyl sulfoxide (DMSO) (Qualigens, CPW59), Disodium EDTA (HiMedia (RM1370), Ethidium bromide (Sigma), Histopaque (Sigma (1077-1)), Phosphate Buffered Saline (PBS) (Bioshop, PBS404), Sodium Chloride (NaCl) (Ranbaxy Rankem (S0160), Sodium Hydroxide (NaOH) (BDH-Merck (89021), Triton X-100 (HiMedia (RM 845), Trizma Base (Tris) (Spectrochem (042061), Normal Melting Agarose (NMA) (Bioshop (1L22739)).

2.2. Animals

The experimental techniques and procedures employed in this work adhered to international law governing the care and use of animals in laboratories. They were approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Science -Cairo University (CUFS/ CU/I/F/75/20).

The study utilized female Wistar rats (Rattus norvegicus), with a weight range of 160g-200g. These rats were acquired from The National Authority for Drug Control and Research. The animals were maintained in controlled laboratory environments with ambient temperature (25 ± 2 ºC) and humidity (55 ± 5%). They were subjected to a two-week, 12-hour dark/light cycle as an adaptation phase.

The animals were accommodated in dedicated, well-maintained, and standardized enclosures. Throughout the experimental period, animals had a continuous access to standard laboratory rat chow, tap water, and unrestricted meals.

2.3. *Dosage*

Paracetamol powder (7 grams) was dissolved in 150 ml of physiological saline. The doses (82mg/kg) and (164 mg /kg) b.w were determined according to the human dose. As the max human dose is 400 mg for adults weighing 60 kg, the conversion equation human dose = Animal dose x KmA/KmH is 400/60 = Animal dose x 6/36. Therefore, the animal dose is 411.5 mg/kg. By calculation, the high dose is 1.8 ml, and the average dose is 0.9 ml of drug suspension [48].

2.4. *Experimental Design*

After a period of 2 weeks accommodation, eighteen adult, healthy and fertile Wistar rats were randomly assigned to three experimental groups, each containing six rats (n=6/group). Group I (Control group) received 0.5 ml of normal physiological saline, group II (low dose group) received 82 mg/kg B.W suspended paracetamol drug, while group III (high dose group) administered 164 mg/kg B.W. paracetamol.

The weights of all the rats were documented prior to starting the treatment. The rats were administered paracetamol orally via a gavage tube. In accordance with their body weight, rats were administered daily doses for 30 days, namely from 12:00 to 14:00 pm [54]. On the 31st day, the rats underwent a subsequent weighing. The rats were euthanized 24 hours following administration of the final dose using light anesthesia and intraperitoneal administration of Sodium pentobarbital at a dosage of 50 mg /kg.

2.5. Histopathological Studies

From each group, one uterine horn and one left ovary were obtained. For a whole day, the specimens were kept in a 10% formaldehyde solution. Tap water was used for the washing process, and methyl, ethyl, and 100% ethyl alcohol were gradually diluted to produce dehydration. After being cleared of xylene, the specimens were placed in a hot air furnace set at 56 °C to embed them in paraffin. Using a sledge microtome, the paraffin

beeswax tissue blocks were cross-sectioned at a 5 µm thickness to be examined later under a light microscope, tissue slices were placed on glass slides, deparaffinized, and stained with hematoxylin-eosin [11].

2.6. Serum Hormone Analysis

After a heart puncture, the blood was extracted and centrifuged for 5 mins for 3000 rpm to get a clear serum from all groups (n=5, from each group) and the sera obtained were used for analyzing reproductive hormones Follicle stimulating hormone (FSH) by using FSH (rodent) Elisa kit from Abnova Company(Taiwan), Luteinizing hormone (LH) by using ELISA kit from Novus Biologicals Company from USA (NBP2-61257), Progesterone (P4) by using Rat/mouse ELISA kit from Demeditec diagnostics company (Germany). Estrogen (E2) using Mouse Rat Estradiol ELISA Kit, Catalog number: EK7003 from BOSTER biological technology antibody and ELISA Experts Company.

2.7. Oxidative Stress Markers Investigation

To investigate oxidative stress, the right ovary and uterine horn of rats from all groups (n=5) were washed with ice-cold saline to eliminate blood, weighed and homogenized in a cold phosphate-buffered saline (PBS) solution (pH 7.4) at a ratio of 5-10 ml per gram of tissue with a mechanical homogenizer on ice. Following homogenization, the mixture was centrifuged at 4,000 revolutions per minute for 20 minutes eliminate debris and obtained the supernatants that preserved at -80°C for further estimation. The kits used for the oxidative stress assays were all purchased from BioVision Incorporated, 155 S. Milpitas Boulevard, Milpitas, CA 95035 USA; where Lipid Peroxidation (MDA) kit catalogue # K739-100 used, Superoxide Dismutase (SOD) used kit catalogue #K335-100, Catalase (CAT) used kit Catalog #K773-100, Glutathione (GSH) used BioVision's Reduced Glutathione Kit.

2.8. Comet Assay

Pieces of the ovary tissue and uterus from all rat groups (n=3, from each group) were minced and homogenized with a cold mincing solution. The cell suspension was filtered through nylon mesh.10 µl cell suspension was mixed in an Eppendorf tube with low-melting point agarose (LMPA;75 µl) and placed on a microscope slide pre-coated with normal-melting-point agarose (NMPA)**.** Slides were immersed in lysing solution for at least 2 hours at 4°C. The DNA was then unwound by placing the slides in gel electrophoresis for 20 minutes. The alkaline electrophoresis process ran for 20 minutes at 4°C, 21 V, and 270 mA [21] . Neutralization was applied, and slides were stained with ethidium bromide and then analyzed using an Axio fluorescence microscope (Carl Zeiss, Germany). Estimation of DNA was carried out using the X40 objective. A Comet 5.0 analysis system developed by Kinetic Imaging, Ltd. (Liverpool, United Kingdom) connected to a charge-coupled device (CCD) camera was used to measure the length of DNA migration Tail length (µm), the percentage of migrated DNA (DNA %), and to calculate the tail moment (TM). Almost 50-100 randomly selected cells were analyzed per slide [41].

2.9. Statistical Analysis

For every sample group, the data was shown as the mean value and the standard error (mean ±SEM). The one-way analysis of variance (ANOVA) test was used to evaluate the statistical differences between the groups. Tukey's multiple comparison post hoc analysis was then used, using SPSS software, for multiple comparisons between groups. When the p-value was less than 0.05, the statistical significance limits were set.

3. Results

3.1. *Effect of Paracetamol Drug on Weight Change*

The rats exhibited a reduction in body weight following administration of the paracetamol medication. However, for both experimental groups, the observed changes were statistically. Insignificant compared to the control group (Table 1).

3.2. Histopathological findings

3.2.1. Ovarian tissue

Rats of control group showed normal structure of ovarian stroma and developing follicles was revealed. Several large and Graafian follicles were detected with numerous corpora lutea. Both newly formed and large previously formed corpora lutea were observed. A few large atretic follicles were also seen (Fig.1).

Table 1: Effect of paracetamol administration on rats' body weight and absolute weight of some reproductive organs.

Parameters Groups	Body weight change (g)	Right ovary (g)	Left ovary (g)	Uterus (g)
Control	16.09±4.41	0.045±0.005	0.046±0.005	0.517±0.089
Low dose (82 mg/kg)	9.45±1.92	0.042±0.004	0.049±0.005	0.53±0.068
High dose (164 mg/kg)	6.818± 2.77	0.037±0.004	0.053±0.006	0.48±0.71
F-value	2.202	2.202	0.37	0.12
P-value	0.128	0.128	0.689	0.885

Data was expressed as Mean ± Standard Error Mean (M± SEM), (n=6)

While in rats administrated low dose of paracetamol, the ovarian section appeared apparently normal as well. The ovarian surface was loaded with several developing follicles, large-sized previously formed corpora lutea, and a few recently formed corpus luteum. A few sections exhibited dispersion of the formed tissue elements by mild oedema (Fig.2).

Concerning the high dose group, ovarian tissue of female rats appeared normal, with the ordinarily present structures of follicles and corpora lutea. Some individuals showed perivascular oedema (Fig.3). Overall, the histological examination of ovarian tissue obtained from female rats that had been administered either low or high doses of paracetamol revealed the presence of atretic follicles as well as degenerated graafian follicles. Degenerated follicles, edema, pyknotic cells, vacuolation, and degenerated oocytes were some of the additional findings that were observed in both treatment groups.

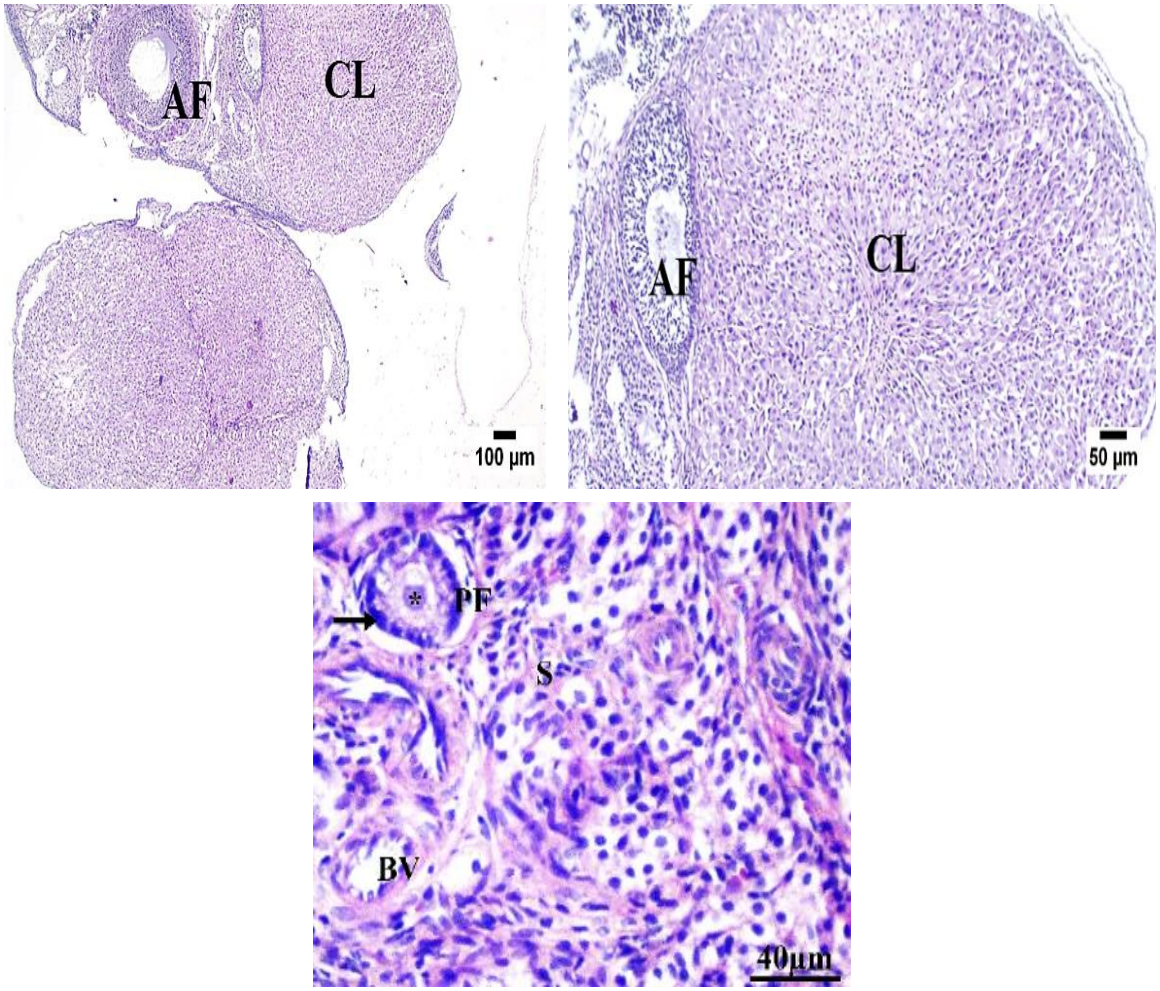

Fig. 1: Photomicrographs showing the histological findings of ovarian tissues from rats of control group. CL=corpus luteum, PF= primary follicle, BV= blood vessel, S= stroma, AF= atretic follicle (stain H&E). 100µm=40X, 50µm=80X, 40µm=600X.

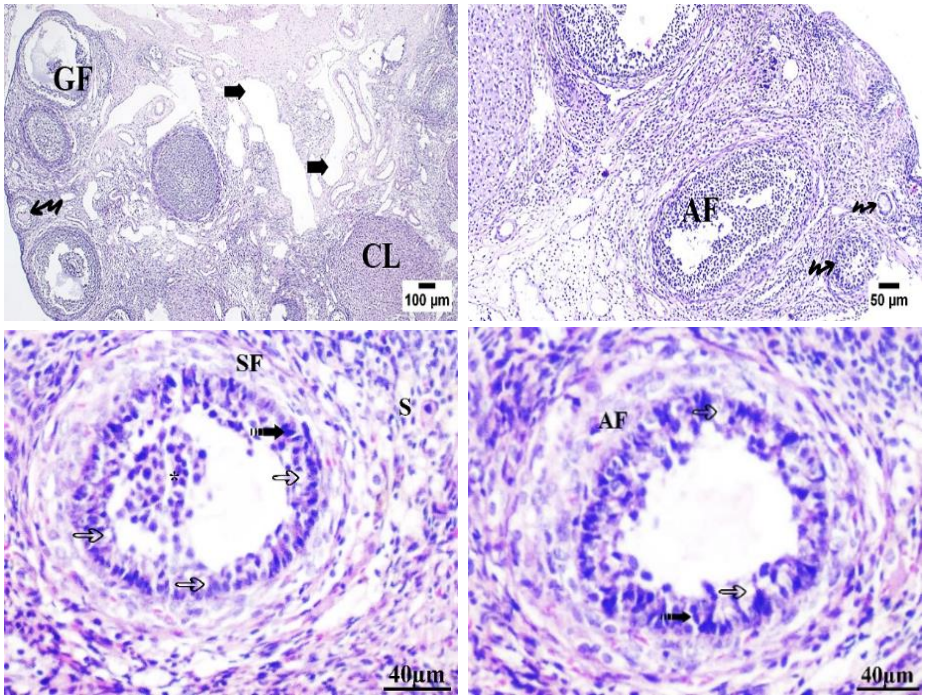

Fig. 2: Photomicrographs showing the ovarian tissue from the female rats treated with low dose (82 mg/kg) of paracetamol.AF=atretic follicle, CL= corpus luteum, GF= degenerated Graafian follicle, Wavy arrow= Degenerated follicle, Bold arrow= edema, SF= secondary follicle, Dotted arrow= pyknotic cell, Hollow arrow= vacuolation, S= stroma, *= degenerated oocyte (stain H&E). 100µm=40X, 50µm=80X, 40µm=600X.

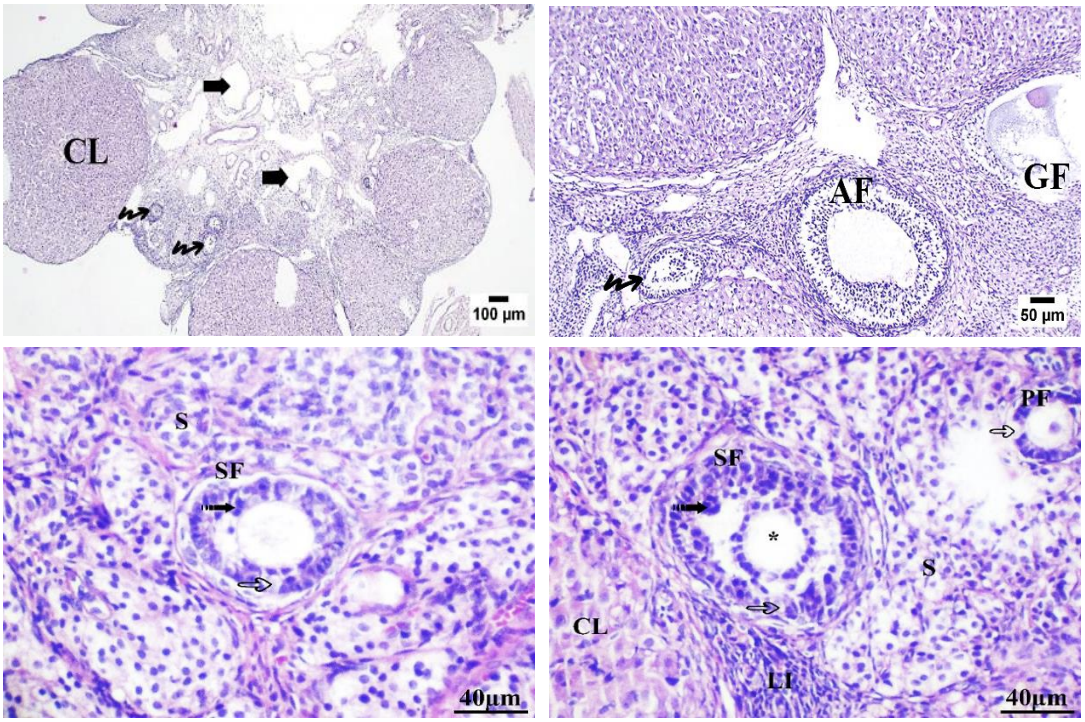

Fig. 3: Photomicrographs showing the ovarian tissue from the female rats treated with a high dose (164 mg/kg) of paracetamol. AF= atretic follicle, CL= corpus luteum, GF= degenerated Graafian follicle, Wavy arrow= Degenerated follicle, Bold arrow= oedema, SF= secondary follicle, PF= primary follicle, Dotted arrow= pyknotic cell, Hollow arrow= vacuolation, S= stroma, LI= lymphocyte infiltration, *= degenerated oocyte Wavy arrow= Degenerated follicle, Bold arrow= oedema, SF= secondary follicle, PF= primary follicle, LI= lymphocyte infiltration (stain H&E). 100µm=40X, 50µm=80X, 40µm=600X.

3.3. Uterine tissue

Concerning the effect of paracetamol administration on uterine tissues of rats our results revealed that, uterine wall of control rats appeared histologically normal; lamina epithelial is made up of simple columnar epithelium, and lamina propria was composed of connective tissue containing uterine glands (Fig.4).

In low dose group microscopic examination exhibited an apparently normal uterus in almost all examined sections; one individual showed mild uterine fibroplasia (Fig.5). While in high dose group, the examined uterine sections showed squamous metaplasia manifested by the substitution of the simple columnar epithelium with squamous epithelium. Mucosal and perivascular oedema were also noticed. Some other sections showed marked vacuolation in the epithelial lining with mild fibroplasia (Fig.6). Overall, the histological examination of the uterine tissue retrieved from female rats that had been administered either a low or a high dose of paracetamol indicated a number of significant changes. Lymphatic invasion, glands, hyperplasia, and fibers were some of the characteristic features that were observed. Furthermore, vacuolation, edema and pyknotic nuclei were identified.

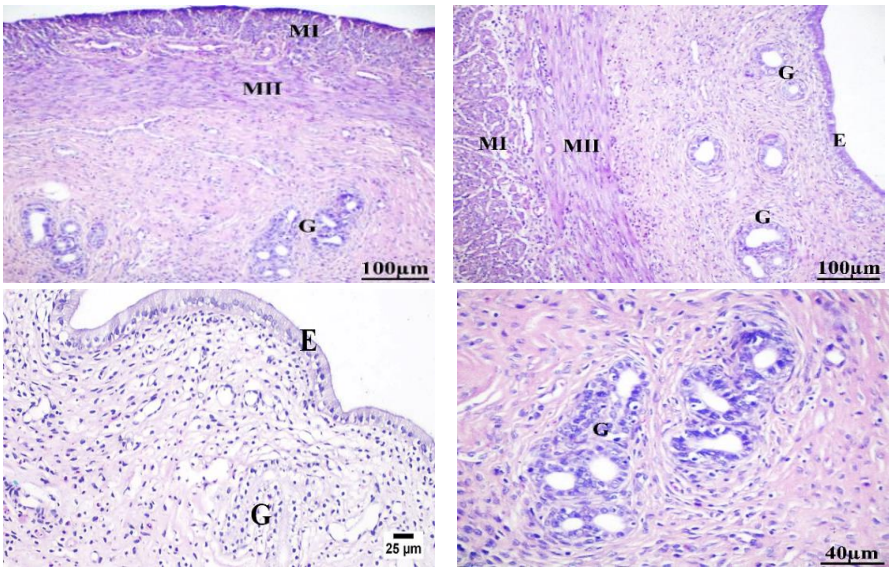

Fig. 4: Photomicrographs showing the uterine tissue from the control group. E= Endothelium, G= gland, MI= Longitudinal muscle layer, MII= circular muscle layer (stain H&E). 100µm=240X, 40µm=600X, 25µm=160X.

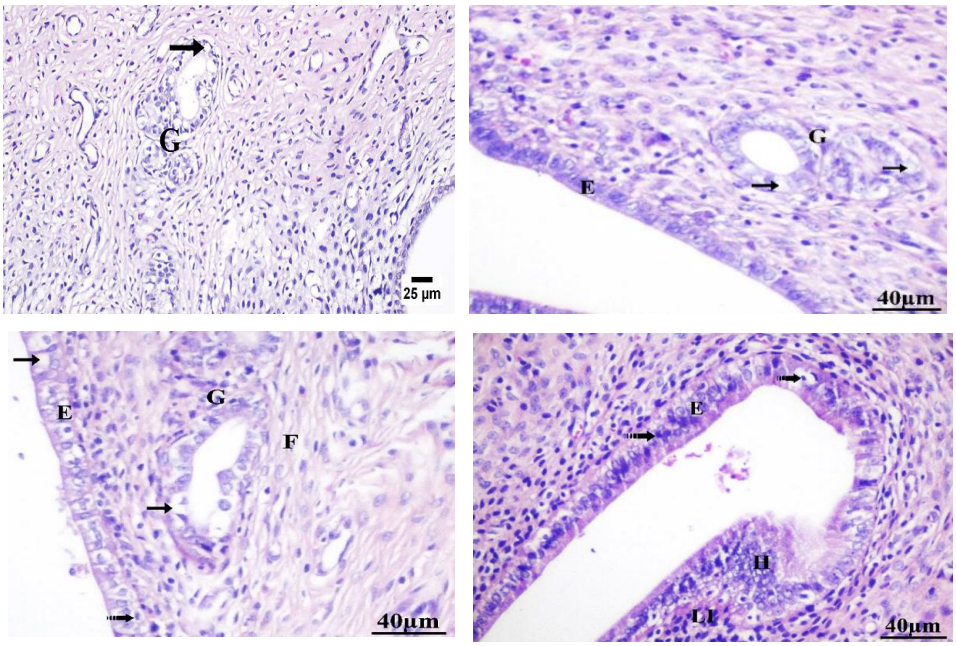

Fig. 5: Photomicrographs showing the uterine tissue from the female treated group treated with low dose (82 mg/kg) of paracetamol. E= endothelium, LI= lymphatic invasion, G= gland, H= hyperplasia, F= fibers. (Stain H&E) .100µm=240X, 40µm=600X, 25µm=160X

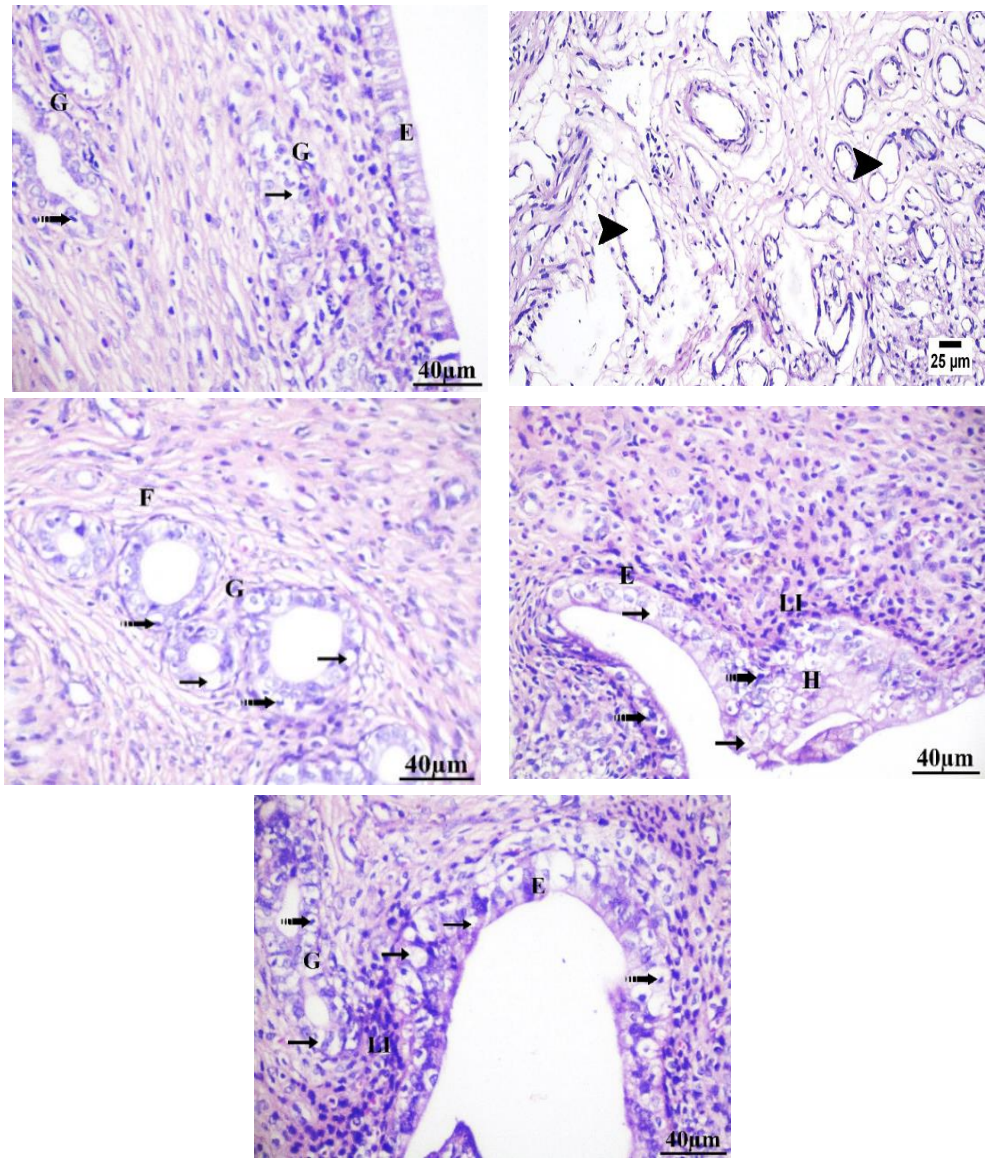

Fig. 6: Photomicrographs showing the uterine tissue from the female rats treated with high dose (164 mg/kg) of paracetamol. E= endothelium, LI= lymphatic invasion, G= gland, H= hyperplasia, F= fibers, Arrow= vacuolation, Arrowhead= edema, Dotted arrow= pyknotic nuclei, (stain H&E).100µm=240X, 40µm=600X, 25µm=160X

3.4. Effect of paracetamol administration on reproductive hormones of female rats:

Regarding to the effect of paracetamol administration on serum reproductive hormones, table (2) pointed out that all serum reproductive hormones (E2, P4, FSH and LH) measured in this study was significantly higher in low and high dose groups comparing with that of control group rats.

Table 2: Effect of paracetamol administration on some reproductive hormones of adult female rats.

Parameters **Groups**	**Estrogen** **pg/ml**	**Progesterone** **pg/ml**	**FSH** **IU/L**	**LH** **IU/L**
Control	3.40±0.34	1.18±0.139	2.125±0.125	2.55±0.19
Low dose (82 mg/kg)	7.8±0.504 [a]	2.52±0.11[a]	5.35±0.45[a]	5.07±0.39[a]
High dose (164 mg/kg)	21.75±1.51[a,b]	3.75±0.19[a,b]	14.9±0.98[a,b]	11.8±0.58[a,b]
F-value	103.6	71.30	111.36	118.5
P-value	0.00	0.00	0.00	0.00

Data was expressed as Mean ± Standard Error Mean (M± SEM), (n=5). Superscript (a, b) are significantly different at P<0.5 as compared with control and low dose respectively.

3.5. Effect of Paracetamol on Oxidative Stress Markers:

3.5.1. Ovarian Tissue

In a dose-dependent manner, administering low and high dosages of paracetamol caused a notable increase in the ovarian MDA concentration and a notable decrease in the ovarian antioxidant levels (GSH, CAT, and SOD) when compared to the control group (Table 3).

3.5.2. Uterine Tissue

Paracetamol administration at low and high dosages induced a significant elevation in the uterine MDA concentration and a significant decline in the uterine antioxidant levels (GSH, CAT, and SOD) in a dose-dependent manner (Table 3).

Table 3: Effect of paracetamol drug on oxidative stress markers in adult female rat ovarian and uterine tissue:

Parameters		Groups				
		Control	Low dose (82 mg/kg)	High dose (164 mg/kg)	F-value	P-value
Ovary	MDA (nmol/mg protein)	0.59±0.05	1.84±0.04[a]	3.34±0.04[a,b]	855.07	0.000
	GSH (nmol/mg protein)	1.60±0.05	1.05±0.06[a]	0.45±0.05[a,b]	88.3	0.000
	CAT (nmol/mg protein)	4.15±0.15	3.1±0.108[a]	1.02±0.133[a,b]	126.8	0.000
	SOD (U/mg protein)	4.67±0.205	3.30±0.12[a]	1.06±0.14[a,b]	107.3	0.000
Uterus	MDA (nmol/mg protein)	0.62±0.05	1.388±0.057[a]	2.455±0.072[a,b]	213.4	0.000
	GSH (nmol/mg protein)	1.38±0.046	1.01±0.1[a]	0.33±0.041[a,b]	48.03	0.000
	CAT (nmol/mg)	3.4±0.19	2.17±0.14[a]	1.08±0.13[a,b]	45.04	0.000
	SOD (U/mg protein)	3.27±0.11	2.27±0.125[a]	0.86±0.17[a,b]	76.26	0.000

Data was expressed as Mean ± Standard Error Mean (M± SEM), (n=5). Superscript (a, b) are significantly different at P<0.5 as compared with control and low dose respectively.

MDA= Malondialdehyde Lipid Peroxidation, GSH= Glutathione, CAT= Catalase, SOD= Superoxide Dismutase

3.6. Effect of paracetamol administration on DNA fragmentation in adult female rats:

3.6.1. Ovarian Tissue

On studying the effect of paracetamol administration on DNA fragmentation % and tail length using comet assay, our results showed a significant increase in tail length, DNA fragmentation % and tail moment in both two treated groups comparing to control one, as well as between high and low dose groups (Table 4 & Fig.7)

3.6.2. Uterine tissue

Concerning the effect of paracetamol on DNA fragmentation, comet assay on uterine tissue of rats showed significant increase in tail length, DNA fragmentation % and tail moment in both treated groups than control one. But there was no significant difference between low and high dose treated groups (Table 4 & Fig.8).

Table 4: Effect of paracetamol drug on the ovarian and uterine DNA of adult female rats:

Parameters		Groups				
		Control	Low dose (82 mg/kg)	High dose (164 mg/kg)	F-value	P-value
Ovary	Tail length	3.44±0.17	4.55±0.171[a]	5.44±0.16[a,b]	35.2	0.000
	DNA %	3.84±0.06	8.05±0.082[a]	14.4±0.059[a,b]	5834	0.000
	Tail moment	0.13±0.006	0.33±0.006[a]	0.84±0.01[a,b]	1981	0.000
Uterus	Tail length	4.1±0.15	5.03±0.14[a]	5.62±0.10[a]	30.89	0.000
	DNA %	5.58±0.07	11.39±0.054[a]	16.22±0.11[a,b]	4025	0.000
	Tail moment	0.24±0.009	0.52±0.01[a]	0.88±0.01[a,b]	1003	0.000

Data was expressed as Mean ± Standard Error Mean (M± SEM), (n=4). Superscript (a, b) are significantly different at P<0.5 as compared with control and low dose respectively.

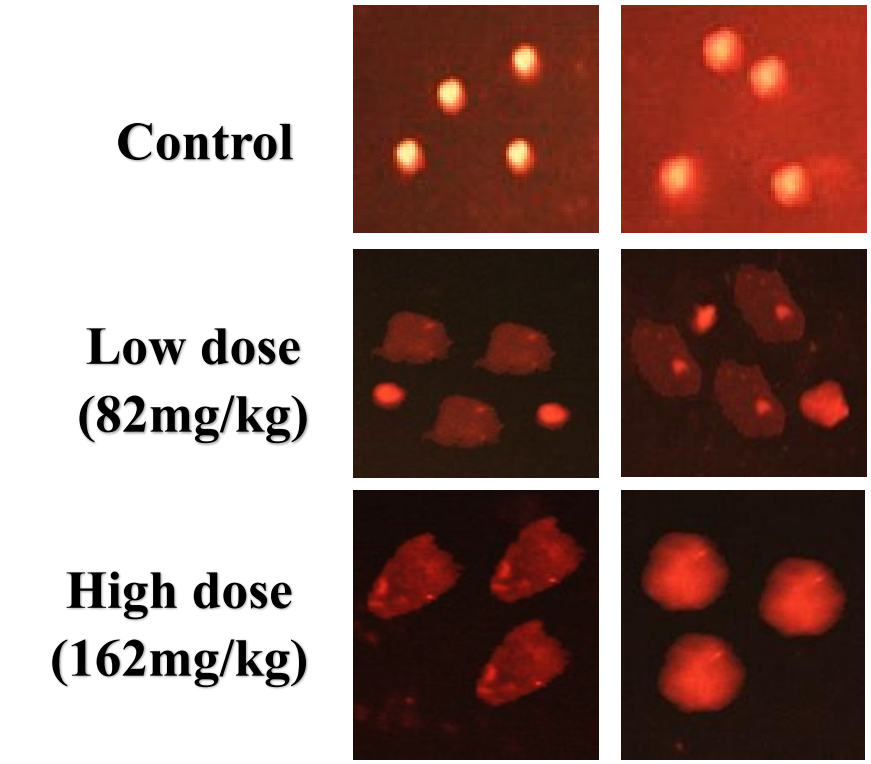

Fig. 7: Photographs of adults femle rats showing the DNA damage degree in the ovarian tissue.

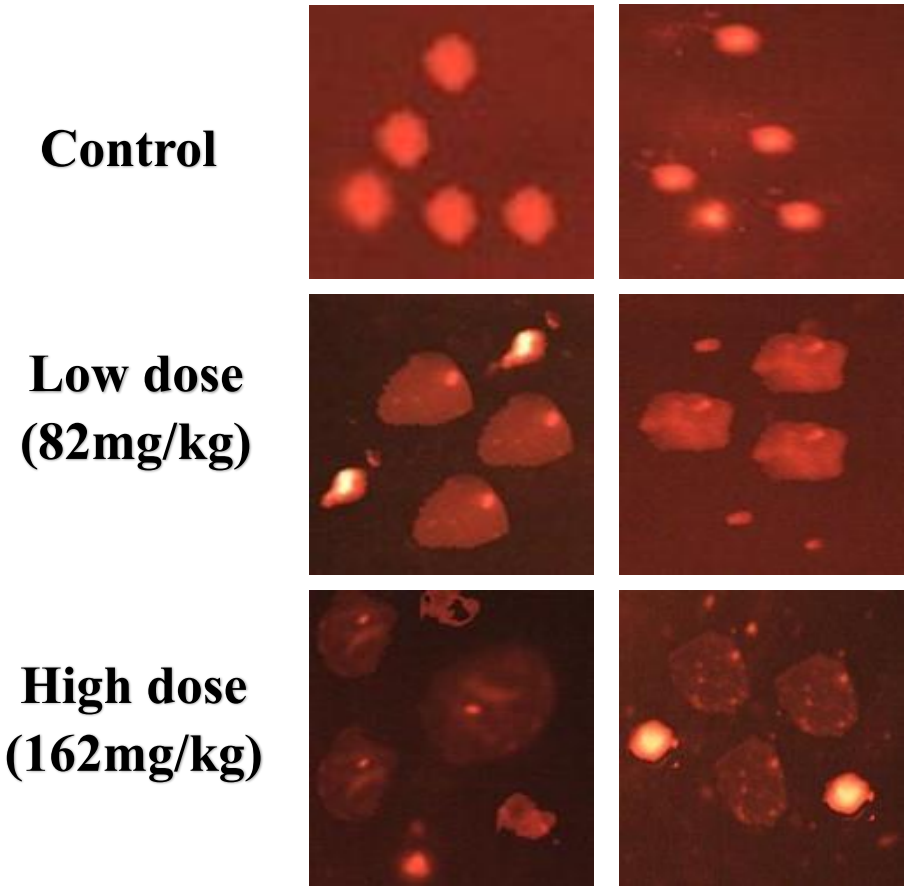

Fig. 8: Photographs showing the DNA damage degree in the uterine tissue.

4. Discussion

Paracetamol (acetaminophen) is a common pain reliever often recommended during early pregnancy due to its perceived safety. While it's considered safer than other options like aspirin, recent studies suggest potential risks, especially for female offspring [37]. Exposure to paracetamol during pregnancy has been linked to early puberty signs like acne, breast development, and pubic hair growth [26]. Additionally, it may affect female reproductive development, as evidenced by reduced anogenital distance in animal models [4]. Different previous studies showed the effect of paracetamol on the pregnant female rat model and the offspring but little information about the repro-toxic effect paracetamol with different doses on the female rat and its effect directly on the female reproductive organs. This current study further investigates these effects on specific parameters in a female rat model.

4.1. Change in Body Weight:

Weight changes, whether due to weight increase or decrease, are typically viewed as harmful. In the current study, when administrated paracetamol drug by an intraperitoneal route in low and high doses (82 mg/kg) and (164 mg/kg) respectively for 30 days showed no change in body weight between the control group and treated groups, whether for low or the high doses of the drug. This data correlates with previous studies' findings [5, 41], where Wistar rats were administrated 350 mg kg/day PAR by gavage daily. The study by [60] also correlated with this present study, showing no change in the body weight of the rats when administrated paracetamol drug by it is different doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg b.wt. In contrast to our findings this study by [31] showed that there was a significant decrease in the body weight for the female rats treated with APAP with a dose of 500 mg/kg, and they returned this for the decrease in food consumption by the rats. Another study estimated that the birth body weight of a baby for a mother consuming paracetamol drug was lower than usual, and they proposed that this may return as paracetamol's ability to reduce prostacyclin production that could result in tocolytic activity that affects the fetus's weight [59].This study [1] showed that rats given APAP (200 mg/kg, ip) for 14 days showed a markedly reduced body weight. In the same context, a study by [40] showed that administering (105mg APAP/Kg b.w) causes weight loss in rats. Accordingly, our results show that there are no differences in the amount of food consumed by the treated groups compared to the control group. As a result, taking paracetamol intraperitoneally for 30 days at concentrations of 82 mg/kg and 164 mg/kg may not have an impact on body weight.

4.2. *Change in Absolute and Relative Weight*

This investigation recorded relative and absolute weights for the left and right ovaries and the uterus. When comparing the control group with the treated groups, results showed no difference in the relative and absolute weights of the two reproductive organs (ovaries and uterus). The ovaries and uterus weight of the animals in the control and treatment groups did not significantly differ, according to a study by [47] while taking acetaminophen 200 mg/kg orally via gavage for a period of twenty days. Also, a study by [60] showed no change in the absolute or relative weight of the female rat's organs when administrated up to 1000 mg/kg paracetamol drug. In contrast to our findings, another study by [22] showed the results by seeing the effect of a sinarest drug, which is a drug that contains paracetamol. It showed that the weight of the ovaries in females and testis in males significantly decreased**,** and this study returned this due to ova shrinkage (pyknosis). Also, the study by [33] was aligned with our results, as no change in the weight of the ovaries and uterus occurred when the rats were administered 350 mg/kg of paracetamol. This study by [13] showed that ovaries weight of the offspring of a female treated with 350 mg/kg/day of paracetamol decreased, and they returned this for the decrease in the primordial follicles. The study by [38] showed an increase in the liver, kidney and heart organ weight for the female rats administrated paracetamol drug and they returned this to the toxic effect of this drug.

4.3. Serum Hormones

For the hormonal analysis, Mammals' oestrous cycle is regulated by hormones generated by the hypothalamus, pituitary gland, and ovary (hypothalamo-pituitarygonadal axis, or HPG) [36]. The ovarian activity's periodic rhythm, referred to as the oestrous cycle, permits a female mammal to change from a reproductively receptive condition to one of non-receptivity, which eventually results in the development of a pregnancy after mating [47]. Failures in reproduction could be associated with suppressing prostaglandin synthesis [56]. Infertility may arise from a disruption in the sex hormones cascade at any point. So, the current study investigated the change in the female sex hormones (LH, FSH, progesterone and estrogen hormones) and saw the direct effect of paracetamol drug by its different therapeutic doses on the hormonal level. The current study showed a significant increase in the hormonal level for the treated groups in both low and high doses of paracetamol drug when compared to the control group, and this was supported by the previous study [9] where they confirmed

that paracetamol drug directly disturbs the hormone-dependent processes. Also, previous study [18] supported the idea that paracetamol was listed as a potentially endocrine-disrupting pharmaceutical. In contrast [12] showed that paracetamol use for the fertile model caused a decrease in the level of estrogen, and they returned that paracetamol causes placental cell death where the placenta is the primary source of estrogen and progesterone hormones production. In the same context also, the study by [43] showed that the use of over-the-counter analgesics causes an increase in progesterone and FSH and a decrease in estrogen levels. The study by [7] showed that delay in ovulation is one of the adverse effects of acetaminophen in mice, as it lowers the number of implantation sites returned due to disturbance in the hormonal levels.

Regarding progesterone, it is produced from the corpus luteum. The previous study [48] showed the lack of corpus luteum in their histopathology during the metestrus stage after giving the mice 200 mg/kg of acetaminophen orally via gavage for the twenty days, and this is associated with a decline in progesterone level, and this supported our result as here in this current study progesterone level increased in the treated groups with different doses of paracetamol drug. It was returned due to corpus luteum presence in the histopathological examination of the ovaries of the treated groups. The study [45] showed that paracetamol overdose (400 mg/kg b.wt) usage alters the gonadotrophic hormones, causing testosterone hormone disturbance where this hormone level is under the control of luteinizing hormone level balance.so we concluded that administration of the paracetamol drug negatively causes an imbalance in the hemostasis of the hormones, causing a disturbance in the normal physiological body functions of the female and, as a result, affecting her fertility and ova quality and her ability to have a healthy pregnancy.

4.4. Oxidative Stress Investigation

Reactive oxygen species (ROS) may come from granulosa cells, oocytes, cumulus cells, and endometrial cells in the female reproductive system [35]. ROS and antioxidants are in balance in a healthy organism. Oxidative stress results from the overproduction of reactive oxygen species that happens when there is an imbalance [54]. An imbalance between the synthesis of oxidants and antioxidant chemicals, which damages proteins, lipids, and nucleic acid as well as compromising tissue integrity, is known as oxidative stress [55]. It is considered one of the central roles and causes of different pathophysiology disorders, including complications and various problems, and is a prevalent mechanism for cell destruction [15] Almost 90% of Paracetamol metabolism occurs mainly in the liver and is converted into non-toxic metabolites that are execrated through the kidney by glucuronidation and sulfation process; the remaining 10% are metabolized in the liver by oxidation process through an enzyme known as cytochrome P450 that cause a hepatotoxic metabolite called N-acetyl-p-benzoquinoneimine (NAPQI), NAPQI is neutralized by the hepatic GSH under the usual therapeutic dose [23]. Therefore, when paracetamol is administrated by overdose, a high amount of NAPQI production leads to the depletion of the GSH, leading to its inability to detoxify NAPQI, so the availability of a higher amount of NAPQI than normal reactions with cellular protein, causing oxidative stress and lipid peroxidation that are the leading cause of toxicity [29]. The disturbance in the oxidase system appears and is estimated by the change in the oxidative stress markers levels like MDA, GSH, CAT and SOD.

4.5. Ovarian and Uterine MDA and Total Antioxidant Levels

In the current study, we noticed an increase in ovarian and uterine MDA through the administration of paracetamol in low and high doses. These results were corroborated by a previous study that demonstrated how the hazardous metabolite NAPQI forms protein complex structures through interactions with other protein groups. These reactions result in oxidative stress and mitochondrial malfunction, which generate free radicals [25]. Past studies showed that rat hepatocytes' exposure to APAP (100 µM) for one hour caused an increase in the MDA level [55]. In the same manner, [66] reported that administration of APAP (1g/ kg b.w) for nine days for a rabbit model, which caused an elevation in the serum MDA level. Moreover, another study investigating the toxic effect of APAP showed that treatment with (750 mg/kg b.w) causes renal MDA level increase, indicating oxidative impairments for kidney lipids [28]**.** As MDA is a byproduct of lipid peroxidation, we noticed an increase in the MDA level of female rats treated with high and low doses and past studies linked between high dose of APAP and the production of high NAPQI that start lipid peroxidation process causing an increase in the MDA level [35]. Also, this prior study supported the current study conclusion as oxidative stress for the lipids of the cell membrane cause a noticeable increase in lipid peroxidation, which causes changes in MDA as it is the most common aldehyde result from the lipid peroxidation process [61]. Likewise, studies found that ROS production causes activation of the enzymatic antioxidant system as a defensive mode. In the toxicity of ovarian and uterine tissues, catalase (CAT), SOD, and GSH are considered the primary enzymatic antioxidants and serve as the first markers that show the oxidative state [63].Together, SOD and CAT help to neutralize the effects of superoxide radicals as they are the primary cellular defenses against oxidative damage where SOD

dissimulates superoxide radicals into either ordinary molecule oxygen (O_2) or hydrogen peroxide (H_2O_2), (H_2O_2) is then converted to water and molecular oxygen, counteracting the harmful effects of free radicals in the body. This current study showed that the administration of paracetamol drug by low and high doses causes a reduced level in ovarian and uterine SOD and CAT levels. This result was in accordance with a previous study which pointed out that, administration of 2 g/kg of APAP caused a decrease in SOD and CAT levels and an increase in the MDA level of the hepatic cells, and this indicated hepatotoxicity [17]. Also, this prior study [32] showed that injection of paracetamol drug (1500 mg/kg b.w) resulted in a considerable decrease in the activity of the SOD, and they returned this due to excessive generation of the free radicals that overwhelmed the enzyme power. GSH, our current study, showed a decrease in the level when the control group was compared with the treated groups by paracetamol drug. (GSH) It has already been revealed in different studies that when paracetamol is administrated at a rate over the therapeutic dose, it causes different body organs necrosis and mainly the liver as in ordinary therapeutic doses, the dangerous metabolite NAPQI, which is produced when paracetamol is converted, is first detoxified in the liver by conjugating with reduced GSH, and the conjugate is then excreted in the urine. However, high-dose glutathione depletion may result from increased NAPQI synthesis and the necrosis process is caused by NAPQI's covalent binding to liver cell proteins, causing hepatotoxicity [39]. Similarly, another study accepted the noticeable increase in lipid peroxidation and confirmed its accomplishment by a decrease in the GSH, in which NAPQI, a reactive intermediate metabolite of the medication paracetamol, is scavenged by the important free-radical scavenger GSH, a tripeptide presents in many mammalian tissues [61]. The study [6] confirmed that usage of acetaminophen causes a decrease in the GSH level, which causes an increase in the paracetamol metabolite NAPQI, and they suggested that this affects the body cells, causing necrosis and may be a cause of ovarian carcinoma in the long term.

4.6. Effect of Oxidative Stress on Female Fertility

This prior study showed that damage to the cell membrane brought on by lipid peroxidation also reduces the capacity of follicles and oocytes in the female reproductive system to survive and function [35]. Studies showed that the oxidative stress (OS) caused by the imbalance between ROS production and destruction may affect the intraovarian environment and cause damage to the oocyte quality that may cause apoptosis [64]. As well, another study showed that oxidative stress negatively affects a woman's reproductive life span and significantly affects different physiological processes, from oocyte maturation to fertilization [2]. Moreover, studies have shown that ROS imbalance affects the structure of the uterus and causes disturbances in its functions [52]. In conclusion for the oxidative stress regarding our current study and coloration with previous studies, we concluded that administration of paracetamol drug in high and low doses affects the female reproductive health and fertility as prior study showed that the follicular fluid, peritoneal fluid, and ovaries all contain ROS and antioxidants that have an impact on the development, implantation, fertilization, and quality of eggs.

Furthermore, it has been shown that the process of protein oxidation reduces the viability and functionality of proteins in the developing follicles and oocytes found inside the female reproductive system. On the other hand, the oxidation of amino acid residues within proteins causes the intrinsic functions of the proteins to be disrupted. Additionally, the OS induces uterine complications by impeding the successful implantation of the fertilized embryo. In addition, the occurrence of lipid peroxidation, which can also be induced by OS, leads to a reduction in the permeability and fluidity of the oocyte membrane. Consequently, this diminishes the egg's ability to undergo fertilization [35].

4.7. Comet Assay

In the current study, we estimated the DNA fragmentation degree in the ovaries and uterus of the female model, and in contrast to the control, our findings demonstrated that the administration of paracetamol raised the frequency of tail length, tail moment, and percentage of damaged DNA in the tail for both the ovary and uterine tissue. The findings of our study are consistent with the previous research conducted by [19], which reported that the administration of APAP at a dosage of 300 mg/kg intraperitoneally resulted in an elevation of tail percentage DNA in the liver and kidney of mice. This rise can be attributed to the covalent interactions between NAPQI and mitochondrial proteins. Endonuclease G is released from mitochondria by the above-described binding process, which also causes the mitochondrial cell membrane to permeabilize and break. This endonuclease G subsequently translocate to the nucleus, thereby inducing nuclear DNA damage, as well as hepatotoxicity. Likewise, this study [35] on the uterus and ovaries showed that oxidative stress causes a decrease in mitochondrial activity that causes a decrease in energy generation and an increase in ROS emission. As a result, mitochondrial malfunction compromises the viability and functionality of oocytes and follicles in the female reproductive system. Furthermore, this study [49] showed that it is the main pathway for the formation of DNA damage and that the rise in oxidative stress was the cause of genetic harm. As well, this study showed

that paracetamol drug metabolite NAPQI directly interacts with DNA. Correspondingly, this study showed that DNA damage from oxidative modification results in mutations and defective DNA. As well, damage to DNA affects the viability and functionality of follicles and eggs in the female reproductive system. Additionally, studies showed that DNA damage caused to the uterus negatively affects the implantation process. This procedure is essential to starting a pregnancy healthily as the oxidative stress mentioned before causes alteration for mutations and gene expressions that cause cell apoptosis [35].

4.8. Histopathological Results

Other studies on other body organs showed histopathological changes in the organ, high oxidative stress, and DNA damage [45]. Also, the same study showed that the high oxidative stress results caused by paracetamol overdose cause damage in the leading cells of the testis and cause a kind of disturbance in the spermatogenesis process. Moreover, this was collated with our histopathological results. We returned that this besides may cause disturbance in the ovulation process that affects the fertility and reproductive health of the female. Previous study [27] showed that when paracetamol is administrated in different doses, the ovaries showed no corpus letter, only a few follicles, and some Graffin follicles appear, especially at high doses. Another study by [42] showed that acetaminophen has a significant impact on the female's reproductive development, which results in a follicular deficit in adulthood. Same study showed that when paracetamol administrated at (372 mg/kg/day) caused a noticeable change in the ovary structure, which showed an increase in the atretic follicles that contain degenerated oocytes, returned the vacuolation appeared in the ovarian stroma as a defensive mode for the cell due to presence of cell toxicity. The same study showed the relation between the ovary histology and the hormonal level, as prior studies showed that Women with less ovarian reserve will have higher FSH serum baseline levels, which is aligned with our results. Furthermore, this study showed that oxidative stress induces endometrial inflammation that may reduce the endometrium's ability to accept and nourish the embryo [35].

5. Conclusion

In conclusion, the administration of paracetamol, regardless of dosage, has been found to have adverse effects on female reproductive health. These effects include oxidative stress, hormonal imbalances, and histopathological alterations, which have the potential to compromise fertility and predict undesirable pregnancy outcomes.

Based on our findings, it is recommended that additional study be conducted to examine the potential long-term effects of paracetamol administration on female reproductive health and foetal development. Furthermore, it is imperative to do research that investigates the underlying mechanisms via which paracetamol perturbs hormonal homeostasis and triggers oxidative stress. Gaining a comprehensive understanding of these systems has the potential to contribute to the advancement of more secure pain management techniques for women in their reproductive.

6. Conflicts of Interest

"There are no conflicts to declare".

7. List of abbreviations

AF	Atretic Follicle	MDA	Lipid Peroxidation
APAP	N-acetyl-p-aminophenol	NaCl	Sodium Chloride
B.wt	Body weight	NaOH	Sodium Hydroxide
CAT	Catalase	NAPQI	N-acetyl-p-benzoquinoneimine
CCD	Charge-coupled device	NMA	Normal Melting Agarose
DMSO	Dimethyl sulfoxide	O_2	oxygen
DNA	Deoxyribonucleic acid	OS	Oxidative stress
E2	Estrogen	P4	Progesterone
FSH	Follicle stimulating hormone	PAR	Paracetamol
GSH	Glutathione	PBS	Phosphate Buffered Saline
H&E	hematoxylin-eosin	pH	Potential of hydrogen
H_2O_2	Hydrogen peroxide	ROS	Reactive oxygen species
HPG	hypothalamo-pituitarygonadal	Rpm	Revolutions per minute
IACUC	Institutional Animal Care and Use Committee	SOD	Super oxide dismutase
LH	Luteinizing hormone	Tris	Trizma Base
LMPA	Low melting point agarose	WHO	World health organization

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