Original Article	**Acetaminophen affects female fertility-evidence by electron and light microscopic study**

Marwa Mohammed Hassan[1], Reem Helmy Shaker[2], Manal Hamdy El-Kafrawy[2], Manal Hamdi Al-Badawi[1]

Department of Anatomy and Embryology, Faculty of Medicine, [1]Helwan University, [2]Faculty of Medicine for Girls El-Azhar University, Egypt

ABSTRACT

Background and aim of the work: Acetaminophen is considered the most popular used over-the-counter (OTC) pain killer taken during pregnancy. Because of the scanty information regarding the safety profile of this drug during pregnancy This study was conducted to estimate the effect of acetaminophen on the ovary of adult pregnant albino rats and its effect on the offspring.

Material and Methods: Forty adult female rats twenty mothers weighing (180-220 gm) and twenty female offspring weighing (5-10 gm), divided into four groups; the 1st Group (control mothers), 2nd Group (acetaminophen treated mothers), 3rd Group (offspring of the control mothers) and 4th Group (treated mothers' offspring). After 75 days of delivery, blood samples and ovarian sections were taken for assessment of ovarian structure.

Results: The control mother and their offspring group compared to treated mothers group and their offspring showed a decrease in the serum level estradiol, an increased FSH serum level with considerable structural changes, including multiple atretic follicles with a degeneration in its oocytes also there was increased collagen fibers deposition in the medulla and ovarian stroma with a defective carbohydrate metabolism.

Conclusion: Acetaminophen affect female fertility by disturbing ovarian architecture, increase collagen deposition and affect carbohydrate metabolism in the ovary.

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Key Words: Acetaminophen; ovarian follicle, ovary, pregnancy.

Corresponding Author: Marwa Mohammed Hassan, Ph.D, Department of Anatomy & Embryology Faculty of Medicine, Helwan University, Egypt, **Tel.:** 01092635203, **E-mail:** hmarwa777@gmail.com

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INTRODUCTION

Acetaminophen is commonly recommended by a high percentage of pregnant women as it is classified as category B by FDA in 2008. It is taken by patients who not allowed to take non-steroidal anti-inflammatory drugs (NSAID), such as patient suffering from peptic ulcer, bronchial asthma, blood diseases, have sensitivity from salicylate. It is the 1st line of treatment in relieving pain of osteoarthritis in addition to pain in muscle and tendon. Also, it is used as analgesic treatment of Migrain which affect 25% of women in reproductive age.[1] However, there were delayed adverse effects of paracetamol that evidenced by recent studies in animal and human. Prenatal paracetamol use has been shown to have endocrine-disrupting functions[2] which increases the risk of cryptorchidism[2,3] increasing the risk of asthma.[4,5] Even paracetamol in therapeutic doses of have been proven to be harmful for the fetus since it may have important effects on (anti)oxidant balance.[6,7] Rebordosa *et al*. 2009 found relation between exposure to paracetamol in prenatal period and preterm infants in women with pre-eclampsia.[8] Also, It was also found that pregnant women exposed to acetaminophen were more prone to develop fetal neurodevelopmental risks with future ADHD (Attention Deficit Hyperactivity Disorder) in both vivo and vitro studies in rats. In vitro and vivo studies in rats found that on using human fetal testis xenografts, acetaminophen decreased level of testosterone production.[9,10] exposure of rat's embryo to the analgesic's indomethacin or acetaminophen, both acts on PG (prostaglandin) pathways, affects development and number of primordial germ cell in fetuses of male and female rats. This reduction

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impact on number of oocytes in the adulthood with decreased level of fertility. Even worse diminished fertility is transmitted to the following generation which attributed to anti prostaglandin effect of acetaminophen as It was found that PGE2 (prostaglandin E2) affects oocyte development and survival during the most sensitive period of period of follicular growth that affect female fertility throughout its reproductive life.[11]

The present study was designed to study the effect of acetaminophen on the ovary of adult female albino rats after pregnancy and the fertility of their off springs.

MATERIAL AND METHODS

Twenty nulliparous adult female albino rats, weighting 180-200gm aging (10-12) weeks and five males weighing 190-230 gm aging (10-12) weeks were purchased from the Animal House of Faculty of Medicine, Al- Azher University for Boys (Cairo, Egypt). The female rats were put in stainless steel mesh cages under temperature (23ºC±3), and relative humidity with access to food and water by ad libitum and fixed 12:12-hours light/dark cycle. The female rats were kept around ten days with a single male, 4 adult females with an adult male which were separated before by a week. The mating and delivery occur at night, so the females must be examined daily at morning for the presence of the vaginal plugs to be examined by Leishman's stain for the presence of sperms.[12] GD1 (gestational date) was coincide with the day vaginal plug and (PD)1 (pup date) was coincide with the expected day of delivery (GD23).[13]

Note: The pregnancy and lactation are 21 days (3 weeks) each.

Study design:

1st group: Control group: 10 rats administrated every morning water add libitum from 7 dpc (days post coitum) to delivery., **2nd group: Acetaminophen Treated group:** 10 rats treated every morning by 372 mg/kg/day (The human paracetamol therapeutic dose for an adult pregnant female is 60 mg/kg b w.[14] According to animal equivalent dose (AED),[15] the dose administrated to the adult female rat was 372 mg/kg b w /day) administrated by a gastric gavage, from 7 day of post coitum (dpc) till the end of the delivery and to cover the most critically important periods of reproductive development in rat offspring, acetaminophen was taken again from PND 1 to PND 22 after birth,[13] **3rd group:** 10 rats of female offspring of albino rats of control group and **4th group:** 10 rats of female off spring of the adult albino rats that treated by acetaminophen.

The body Weights (BWs) of the mothers were recorded from 7th gestational day to 1st pup day to monitor a decrease or increase in weight gain and the dose had been modified relative to the weight.

Data were collected after delivery like body weight, number, sex and anomalies of pups.

At PD21, females' pups were weaned and kept without receiving acetaminophen. Mothers and their pups were sacrificed at (PND 75) day of age whilst in estrus, evidenced by vaginal smears in the morning.[16] Vaginal smears were checked daily starting from PND 75 along a period of 15 days at constant time in the morning, Estrus's smear was characterized by increased number of cornified epithelial cells and containing some nucleated cells.

Blood sample was collected for hormone analysis, and for preparation to histological examination ovaries were taken. Right ovaries were obtained, rapidly fixed in 3% glutaraldehyde for 3 hours, then they were processed for electron microscopic study. Fixation of left ovaries was done in 10% neutral buffered formalin for three days for preparation of paraffin section.

Biochemical Study: Collection of blood sample(2ml) via the retro-orbital sinus from each animal with using 70µl heparinized capillary and then plain sample bottle was used for keeping samples to be ready for estrogen and FSH analysis. Centrifugation of the sample was done at 3000 rpm for five minutes.[17] The serum was analyzed for evaluation of (estradiol and FSH) level. Blood plasma of FSH and estradiol was measured by chemiluminescence immunoassay by using access immunoassay system Measurements were conducted relative to instructions of the manufacturer.

Histological Examination: The Following stains were used; hematoxylin and eosin (H&E),[18] Masson's trichrome stains[19] and Periodic Acid – Schiff`s reaction (PAS).[18]

Right ovarian sections were then processed and embedded in gluteraldehyde. Toluidine blue was used for staining semithin sections and then the sections were photographed. Cutting of ultrathin sections was done on an LKB Ultra tome III, and photographs were taken with by Joel- 1010 (jeol, Tokyo, Japan) transmission electron microscope at 60 KV accelerating voltage. The electron microscopic study was done at the Regional Center for Mycology and Biotechnology Al-Azhar University (Cairo, Egypt) And Electron Microscopic Unit at Histology Department Faculty of Medicine for Girls, Al-Azhar University.

Image analysis: The collagen fibers area percentage of the ovaries of the different groups were measured by using pro plus image analysis software. The data were statistically analyzed and represented using Microsoft excel software.

STATISTICAL ANALYSIS:

Expressing of the data was in the form of a mean ± standard deviation. T- test was done between the control and treated group. The data was elucidated and graphically presented using Microsoft Excel.

P values were considered nonsignificant if greater than 0.05 and were considered significant if less than 0.05. highly significant values were considered if less than 0.01.

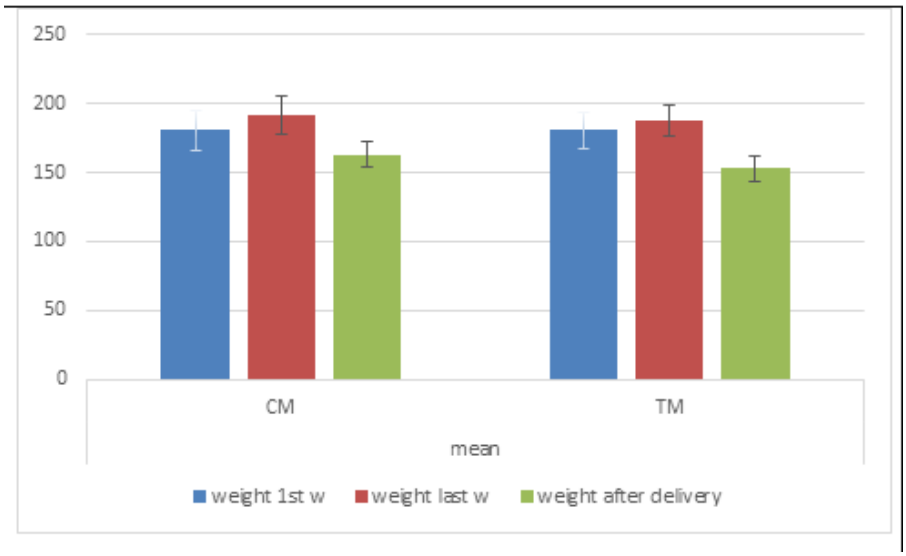
RESULTS

There was no statistically significant in the mean weight of mother in 1st week of pregnancy in the treated group in comparison with the control group (*P=0.96475*) (Table 1). There was no statistically significant in the mean weight of mother in last week of pregnancy in the treated group in comparison of the control group (*P=0.633858*) (Table 1) & (bar chart. 1). There was no statistically significance in the mean weight of the mother after delivery in the treated group compare to the control group (*P=0.116692*) (Table 1) & (bar chart. 1).

Table 1: Mean weight ± standard deviation of the control and the treated mothers in the 1st week of pregnancy, the last week of pregnancy and after delivery

	CM	TM	*P value*
weight 1st w	181±14.335	180.6±13.390	*P=0.96475*
weight last w	191.2±13.971	187.2±11.454	*P=0.633858*
weight after delivery	163±9.110	152.8±9.230	*0.116692*

(*) significant difference from the control ($p \leq 0.05$)



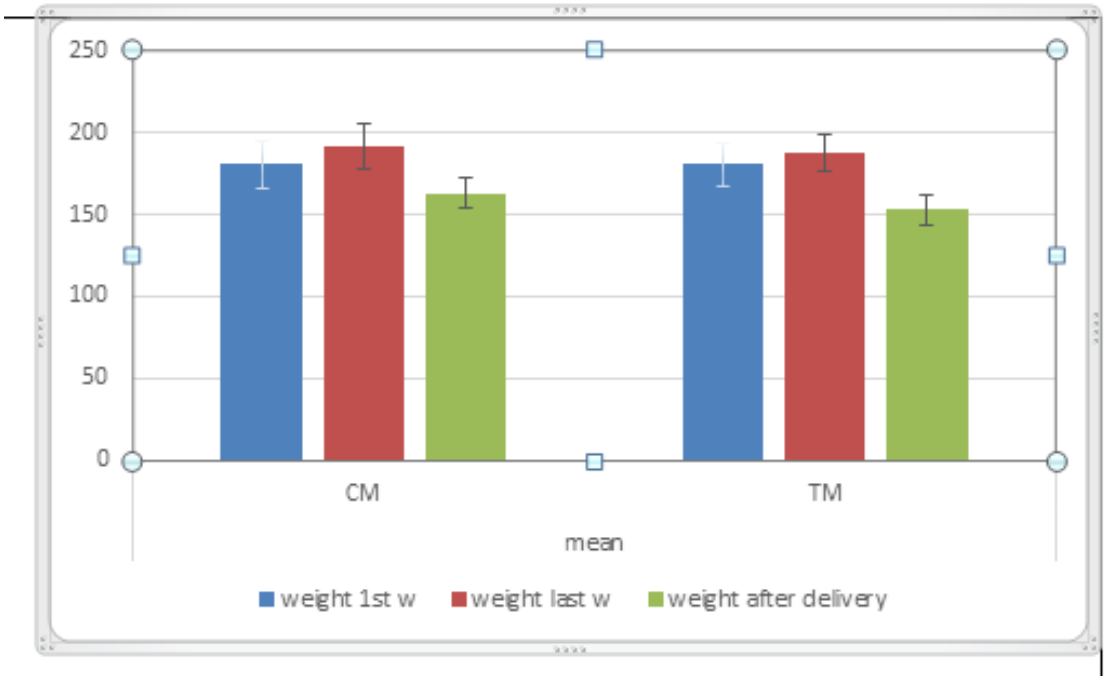
Bar chart 1: Mean weigh t ± standard deviation of the control and the treated mothers in the 1st week of pregnancy, the last week of pregnancy and after delivery

There was no statistically significance in the mean in number of total pups in the treated group compared to the control group (*P=0.186905*) (Table.2) & (bar chart. 2). There was a highly statistically significant decrease in the mean in number of live pups in the treated group compared to the control group (*P=0.002234*) (Table. 2) & (Bar chart 2). There was a highly statistically significant increase in the mean in number of the dead pups in the treated group in comparison with the control group (*P=0.00516*) (Table.2) & (bar chart. 2).

Table 2: Mean number ± standard deviation of (total, live and dead) pups in the control and the treated mothers

	CM	TM	*P value*
Total pups	6.2±0.836	5.2±1.303	*P=0.186905*
Live pups	6±1	2.2±1.643*	*P=0.002234*
Dead pups	0.2±0.447	3±1.581*	*0.00516*

(*) significant difference from the control ($p \leq 0.05$)



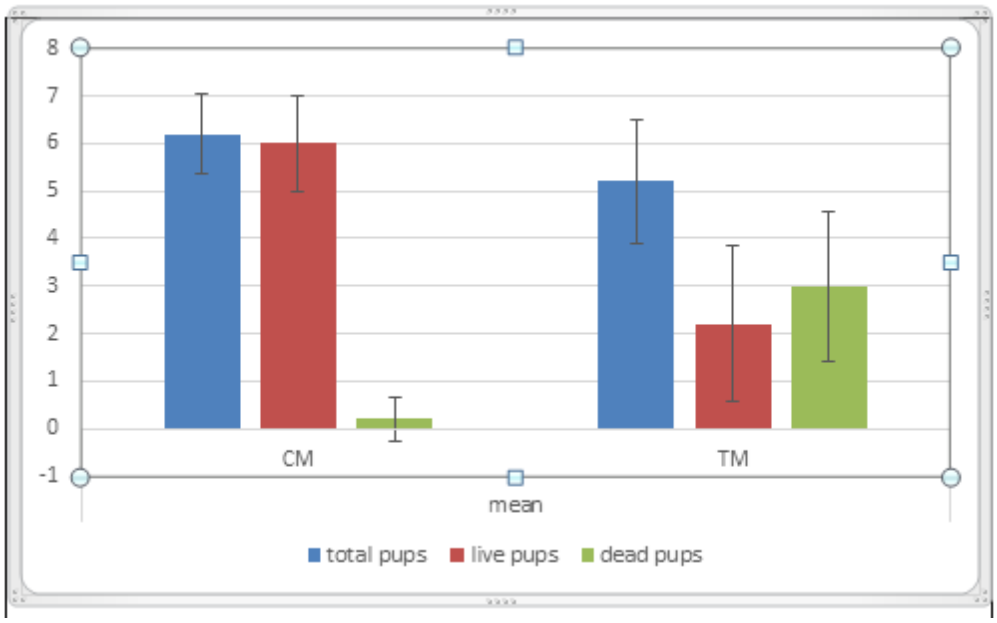
Bar chart 2: Mean number ±standard deviation of (total, live and dead) pups in the control and the treated mothers

As shown in (Table. 3) and (bar chart. 3), There was a highly statistical significant increase in the mean level of FSH in the treated mother group compared to the control mother group (*P=1.76185E-07*) and a highly significant increase in the Mean level of FSH in the pups of the treated mother group in comparison with the pups of the control mother group (*P=1.67386E-06*).

Table 3: Mean plasma level standard deviation of FSH hormone in different groups

	CM	TM	*P value*	CP	TP	*P value*
FSH	0.089±0.020	0.159±0.017*	*P=1.76185E-07*	0.079±0.022	0.157±0.027*	*P=1.67386E-06*

(*) significant difference from control ($p \leq 0.05$)



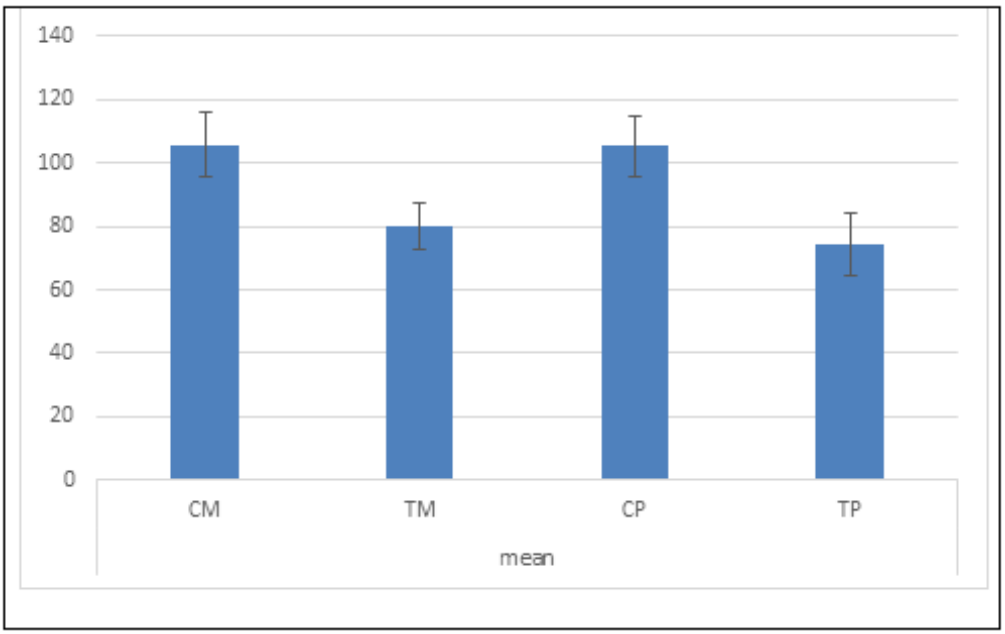
Bar chart 3: Mean plasma level standard deviation of FSH hormone in the different groups

There was highly statistical significant decrease in the mean level of E2 in the treated mother group compared to the control mother group (*P=4.97844E-06*) and highly significant decrease in the mean level of E2 in pups of treated mother group in comparison with pups of control mother group (*P=1.52579E-06*) (Table.4) & (bar chart.4).

Table 4: Mean plasma level ± standard deviation of estradiol hormone in different groups

	CM	TM	*P value*	CP	TP	*P value*
Estradiol	105.48±10.187	80.11±7.290*	4.97844E-06	105.15±9.633	74.43±9.963*	P=1.52579E-06

(*) significant difference from the control ($p \leq 0.05$)



Bar chart 4: Mean plasma level standard deviation of estradiol hormone in the different groups.

Histological results

A- Light microscope

Haematoxylin and Eosin (Hx & E) stained sections

1st Group demonstrated normal histological appearance of the ovary in which the ovarian parenchyma was consisted of cortex and medulla with difficult demarcation between these two structures. ovarian cortex contains the different stages of follicular growth which present under the tunica albuginea (Fig. 1 A).

In treated mother (2nd group), atretic follicles could be observed within the cortex (Fig.1C). zona granulosa' cells of the degenerated follicles with vacuolation with exfoliation of some of these cells within the center of the follicles. The medulla contains congested blood vessels. 3rd group showed that there was similarity in histological findings between this group 1st group (Fig. 1B)

4th group Few follicles were observed mostly at their early stages of development. Some of them showed atretic changes. Their granulosa cells showed different degrees of degeneration and some of them were seen detached from the theca layers in some areas (Fig. 1D). The oocytes of such follicles appeared degenerated and shrunken with a vacuolated cytoplasm and faint nuclei. Some follicles were seen with a floating oocyte in the liquor folliculi (Fig. 1D).

Masson's trichrome stained sections:

(1st) group and (3rd) group showed the normal distribution of collagen fibers in stroma of the ovarian cortex between the follicles and in the medulla (Figs. 2A and 2C). (2nd) and (4th) group, revealed an increased accumulation of collagen fibers in the cortical stroma between the cortical follicles in addition to the medulla (Figs. 2B and 2D).

PAS stained sections:

1st group and 3rd group the cells of surface epithelial cells showed a strong PAS reaction in addition to Granulosa cells of growing follicles, the corpora lutea's granulosa lutein cells, cells of interstiaum and in the zona pellucida which surrounds the oocytes (Fig. 3 A and 3C). 2nd and 4th group showed moderate PAS reaction in the surface epithelium and connective tissue septa (Fig. 3B and 3D).

2- Electron microscopic features

1st group the shape of granulosa cells of primary follicles was columnar or cubical. The columnar cells had thick oval nuclei arranged so that their long axes were perpendicular to the circumference follicles. These nuclei were relatively euchromatic. Their cytoplasm contained, rough endoplasmic reticulum and mitochondria with cristae (Fig. 4A).

2nd group shows part of mature Graafian follicle in which the granulosa cells were arranged in many layers around the Liquor folliculi. the granulosa cells were polyhedral in shape with round or oval heterochromatic nucleus, their cytoplasm showed many dilated cisternae of rough endoplasmic reticulum near the nucleus. Other cells were pyknotic (cells with shrinked cytoplasm and clumped chromatin) discharged in the liquor folliculi. (Fig. 4B).

3rd group the granulosa cells arranged in multiple layers with their nuclei are large rounded or oval with few indentations. The euchromatin was seen as small masses of electron dense chromatin mainly along the inner side of the nuclear envelope. Within the nucleus, a single nucleolus was usually seen. The cytoplasm contains rough endoplasmic reticulum, Golgi apparatus, free ribosomes, lysosomes and many mitochondria (Fig. 4C).

4th group the granulosa cells were arranged in many layers around follicular cavity. The granulosa cells were polyhedral in shape with round or oval nuclei. Some of their nuclei had dilated outer nuclear membrane (Fig. 4D). Most of the chromatin was the heterochromatin type was seen as small masses of electron dense chromatin on the internal surface of the nuclear membrane. The cytoplasm contained many free ribosomes, rough endoplasmic reticulum and oval mitochondria with loss of cristae. Some of the granulosa cells had many dilated cisternae of rough endoplasmic reticulum, lysosomes, vacuolation and have many fat globules occupying most of the cytopl asm (Fig. 4D).

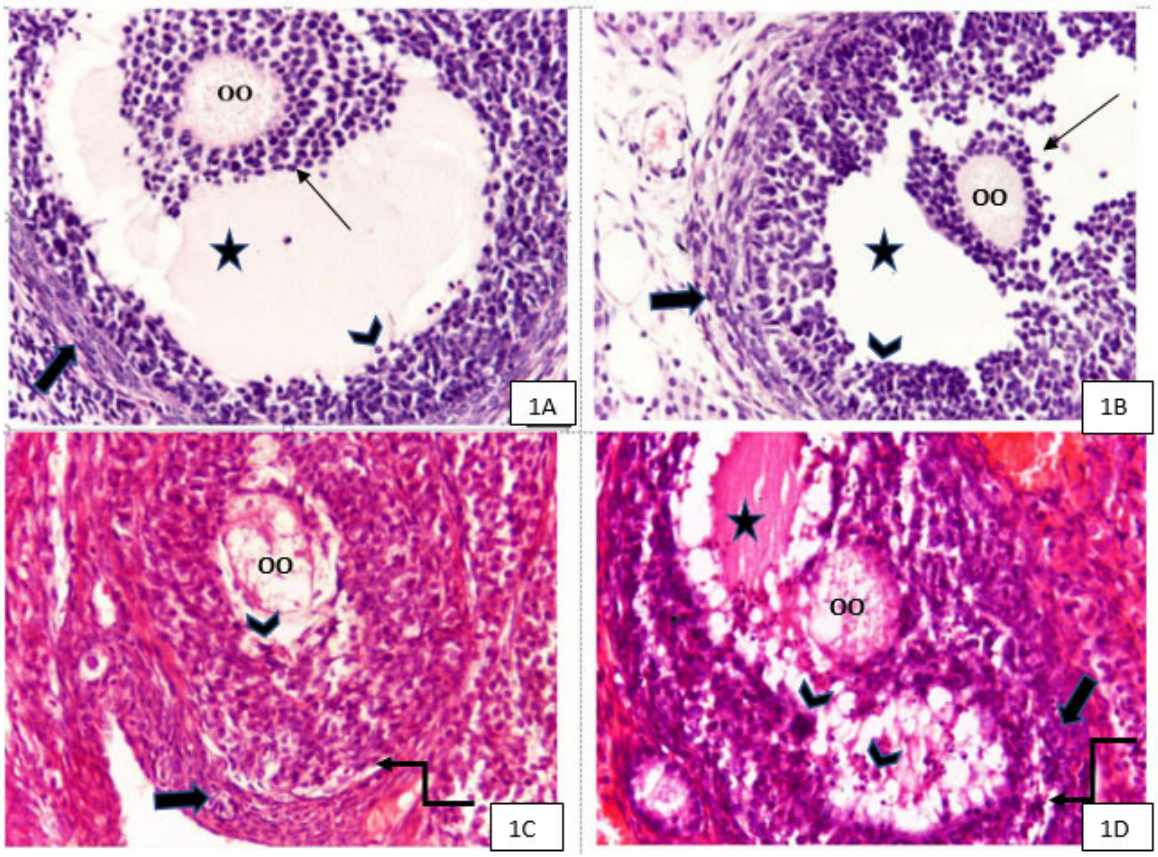


Fig. 1: 1A: A photomicrograph of a transverse section of tertiary (Graafian) follicle of the control mother, showing the granulosa cells (arrow head) arranged around large antrum filled with the liquor folliculi (star), The oocyte (oo) is surrounded by corona radiata cells (thin arrow) with the theca cell layers (thick arrow) at the peripheral part of the follicle (H&E × 400).

1B: A photomicrograph of a transverse section of the outer cortex of a control pup ovary showing the presence of secondary follicle with cavity filled with the liquor folliculi (star) with the granulosa cells surrounding it (arrow head) (most of it have a dark nucleus) in addition to the theca cell layers (thick arrow) which is the outer most layer of the follicle, the oocyte (OO) was cut tangentially away from the nucleus surrounded by corona radiata cells (thin arrow). (H&E × 400).

1c: A photomicrograph of a transverse section in the cortex of eight weeks treated mother ovary showing the presence of multilaminar follicle (star), in which the granulosa cell showing vacuolation with pyknotic nuclei (arrow head), the granulosa cells separated by a gap (elbow arrow) from the theca cells (thick arrow), the oocyte was degenerated with a vacuolated cytoplasm (oo) (signs of atretic follicle). (H&E × 400).

1D: A photomicrograph of a transverse section of the treated pup ovary showing degenerated oocyte (oo), deeply stained granulosa cells (arrow head) became vacuolated and separated from each other with loose and apoptotic bodies (arrow head) were observed and separated in the liquor folliculi (star). A gap (elbow arrow) was observed between the granulosa and the theca cell layers (thick arrow). (H&E × 400).

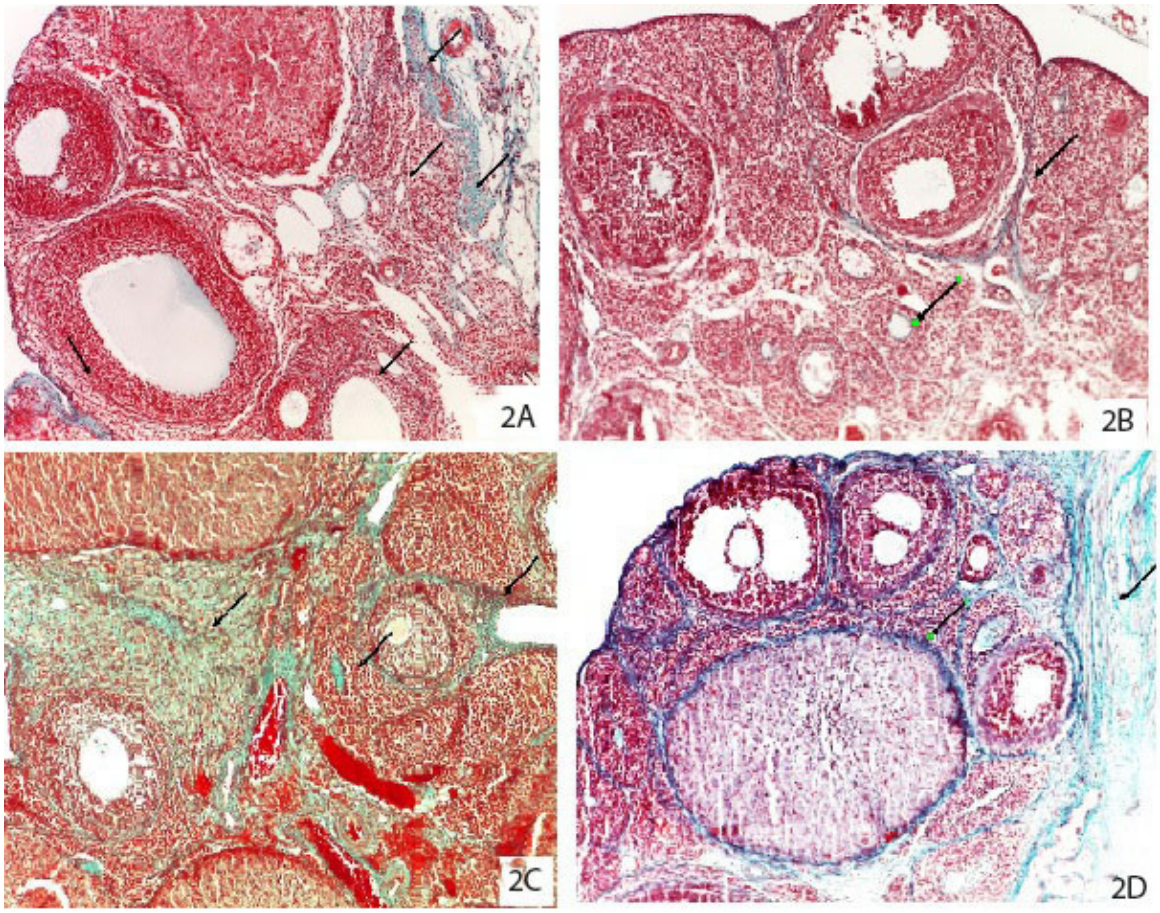


Fig. 2: 2A: A photomicrograph of a section in the ovary of the control group, showing the normal distribution of the collagen fibers deposition (arrow) in the medulla and the stroma of the ovarian cortex around the ovarian follicles. (Masson's trichrome X 100).

2B: A photomicrograph of a section in the control pup ovary, showing a normal distribution of the collagen fibers deposition (arrow) in the medulla and the stroma of the ovarian cortex around the ovarian follicles. (Masson's trichrome X 100).

2C: A photomicrograph of a section in the ovary of the treated mother group, showing increased deposition of the collagen fibers (arrow) in the medulla and the stroma of the ovarian cortex around the ovarian follicles. (Masson's trichrome X 100).

2D: A photomicrograph of a transverse section of the treated pup ovary showing congested blood vessels surrounded by collagen fibers(arrow) of increases density and distribution in the medulla. (Masson's trichrome X 100).

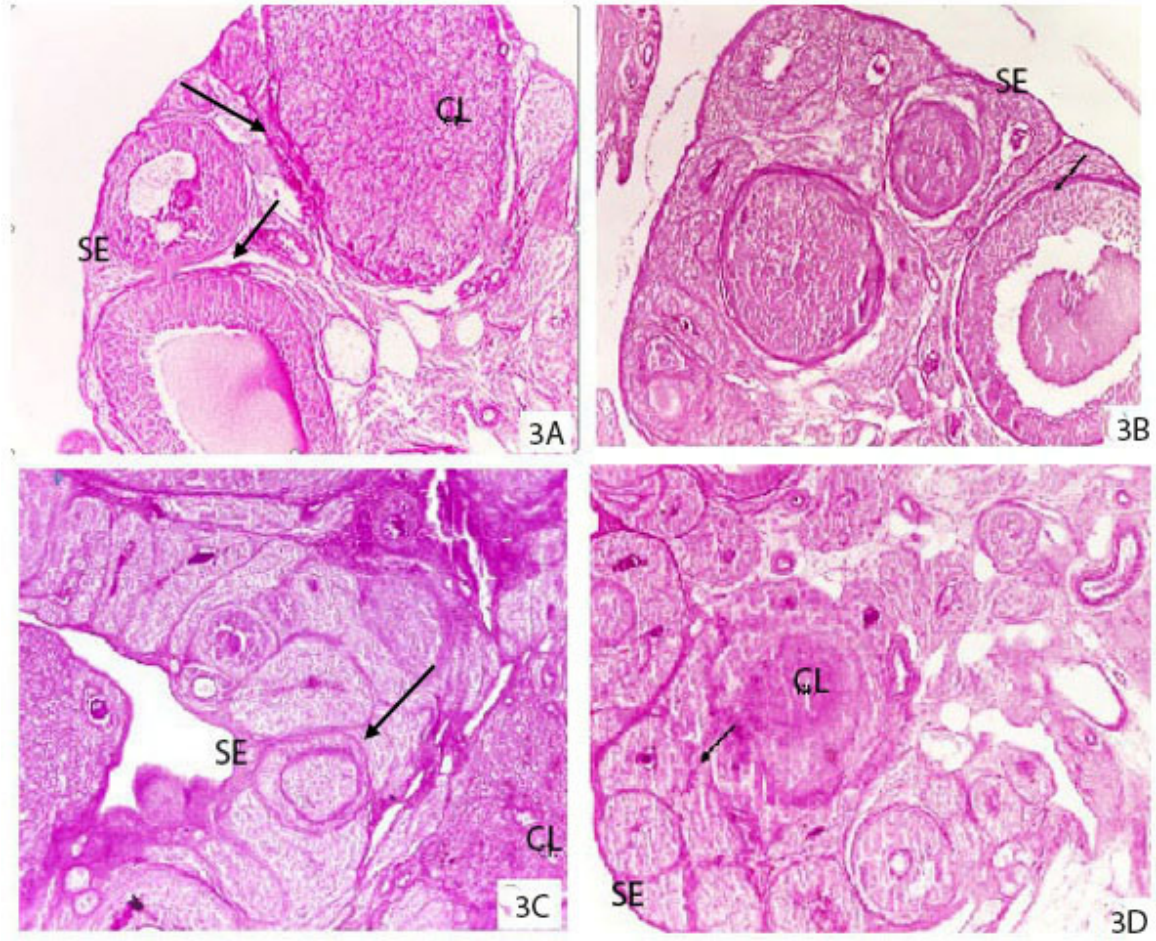


Fig. 3: 3A: A photomicrograph of a transverse section of the cortex and the medulla of the control mother ovary revealing that the surface of the corpus luteum (CL) and the surface epithelium (SE) and the connective tissue septa have strong PAS reaction (arrow). (PAS, X 100).

3B: A photomicrograph of a transverse section of the cortex and the medulla of the control pup ovary revealing a PAS reaction which was strong in the surface epithelium and the surrounding the ovarian follicle (arrow). (PAS, X 100).

3C: A photomicrograph of a transverse section of the treated mother ovary revealing a moderate PAS reaction in the corpus luteum's granulosa lutein cells (CL), the connective tissue septa (arrow) and in the surface epithelium (SE). (PAS, X 100).

3D: A photomicrograph of a transverse section of the treated pup ovary revealing a reaction for PAS which was moderate in the corpus luteum's granulosa lutein cells (CL), the connective tissue septa (arrow) and in the surface epithelium (SE). (PAS, X 100).

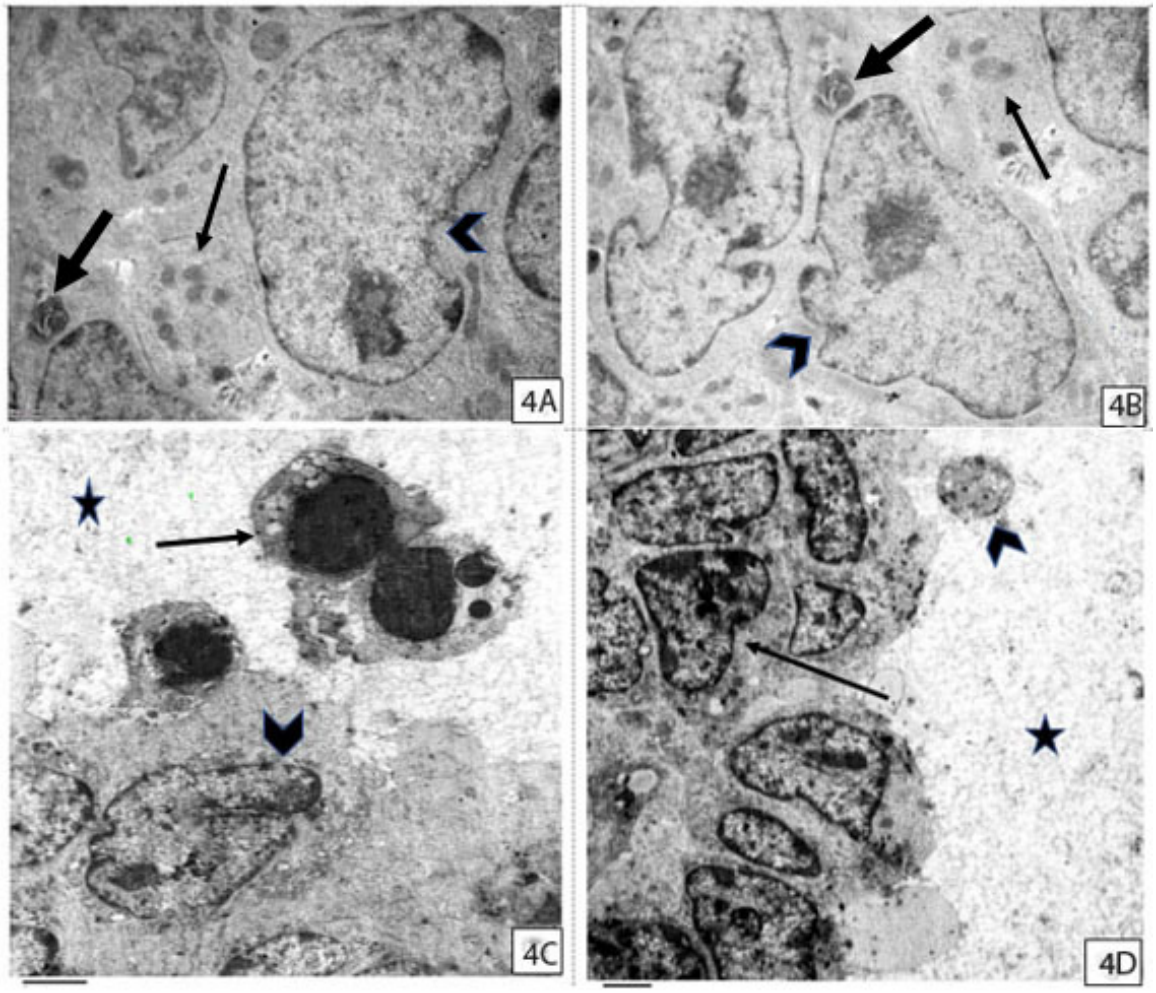


Fig. 4: 4A: An electron micrograph of an adult control rat ovary showing part of the growing follicle. Notice some granulosa cells with their nuclei (arrow head) are large oval with (indentation) and a prominent nucleolus. Their cytoplasm contains many mitochondria (thin arrow) and lysosomes (thick arrow). x 8000.
4B: An electron micrograph of an adult control pup rat ovary showing part of a multilaminar primary follicle. Notice some granulosa cells with their nuclei are large oval with indentation and prominent nucleolus (arrow head). Their cytoplasm contains many mitochondria (arrow) and lysosomes (thick arrow). x 8000.
4C: An electron micrograph of adult treated mother rat ovary shows part of mature Graafian follicle showing Liquor folliculi (star) the granulosa cells arranged in many layers, they irregular in shape with a heterochromatic nucleus (arrow head) and other with a pyknotic cells (arrow) (shrinkage with clumped chromatin) discharged in the liquor folliculi. x 8000.
4D: An electron micrograph of ovary of an adult off spring of the treated mother showing part of the mature Graafian follicle. Notice showing the Liquor folliculi (star) and some granulosa cells with their nuclei are large oval with (indentation) with heterochromatic nuclei (arrow). There was cell with shrinkage cytoplasm and fragmented chromatin (arrow head). x 8000.

DISCUSSION

This study was designated to study the effect of acetaminophen on the development of ovary of albino rats and the fertility of their offspring. The effect of acetaminophen on the female fertility has been studied by many investigators. They found that acetaminophen plays an important role in disrupting reproductive development of the female leading to deficiency in the follicular number in adult life.[20] These studied were very interesting because the mammalian females are born with a particular number of follicles that consumes over their reproductive lifespan and any disturbance in the follicular development during 1st trimester of pregnancy may resulted in disruption of fertility in the adulthood.[13] This supported by the study of which suggested that paracetamol leads to disruption of reproductive development of male.[21,22]

In this study, ovarian sections of mother rats treated with acetaminophen and their offspring revealed considerable changes in the structure of the ovary, including increase number of atretic follicles which had degenerated oocytes. The cells of the zona granulosa of degenerated follicles appeared pulled away from the basement membrane or detached into the follicular cavity. Most of them were degenerated showing vacuolated cytoplasm with or without pyknotic nuclei. These results are consistent with Sun *et al*., (2016) who mentioned that if the follicles contain two or more of the following criteria within a single cross section are considered atretic: degeneration in oocyte, multiple pyknotic nuclei, granulosa cells discharged within the follicular cavity, granulosa cells detached from the basement membrane or irregularity in their arrangement.23

The degeneration that occurred in of ovarian follicles including oocytes may attributed to oxidative stress incriminated by acetaminophen. This in acceptance with the results of Chantong *et al*., 2013, who attributed the damage of the neuronal cells to the increased level of the pro-inflammatory cytokines, thus the increase of IL-1a and TNF-a expression in hippocampus following chronic treatment with paracetamol.[24]

Hormonal analysis results revealed highly statistical significant increment in the mean level of FSH in the treated mother group in comparison with the control mother group and the increase in the mean level of FSH in pups of treated mother group was considered in comparison with pups of control mother group and decrease in the mean level of E2 in the treated mother group was considered significantly high compared to the control mother group and highly significant decrease in the mean level of E2 in pups of treated mother group compared to pups of control mother group, this in acceptance with Rasool and Shah, (2017) who reported that there was negative feedback relationship between of FSH pituitary secretion and estradiol level (one of ovarian hormones) in early follicular phase and this keep level of FSH hormone within normal range. Women who have decreased ovarian reserve will experience elevated plasma basal level of FSH.[25]

The oocytes in most follicles appeared degenerated Vacuolation within the zona granulosa cells of the ovarian follicles, exfoliation of some of these cells within the follicular cavity, and vacuolation within the ovarian stroma and ovarian medulla might be signs of ovarian toxicity and cell degeneration. Also, these may be considered as a type of cellular defensive mechanism against injurious substances. These vacuoles could be responsible for collecting the injurious elements thus prevent the cells from performing their biological activities and this was in acceptance with EL-Deeb *et al*., (2000) and Cheville, (2009).[26,27]

The present study revealed strong PAS reaction in the surface epithelium, connective tissue septa and basement membrane of the ovarian sections of the control group. These results were in consistence with those of Ali *et al*., 2014.[28]

PAS-stained ovarian sections of rats treated with paracetamol showed moderate PAS-positive reaction within some oocytes with complete absence of zona pellucida, whereas other oocytes showed weak reaction with mild reaction in the zona pellucida surrounding them, which appeared interrupted. These results indicated reduction or even depletion of carbohydrates within the oocytes

and their surrounding zona pellucida. These results were compatible with Wu *et al*., 2016, who state that "prenatal exposure to APAP (acetaminophen) in offspring may affect AKT

signaling responsible for insulin-dependent glucose metabolism in liver cells".[29]

Ovarian Sections stained by Masson's trichrome in the control group showed normal distribution of collagen fibers in the medulla and ovarian stroma between the cortical follicles. Treesh and Khair, 2014 reported similar findings.[30]

Ovarian Sections stained by Masson's trichrome in the paracetamol group showed increased collagen fibers deposition in the medulla and ovarian stroma surrounding the atretic ovarian follicles. These results were compatible with those of Bai *et al*., 2017, who investigate the induction of the liver fibrosis in mice by long-term treatment of APAP. The deposition of collagen and the infiltration of the inflammatory cells were increased in in mice treated with APAP Liver evident by increase expression collagen's mRNA as (COL)1a1, Col3a1, transforming growth factor-β (TGF-β) and serum contents of COL1, COL3, TGF-β in addition to increase the level of α-smooth muscle actin (α-SMA) expressed by the Liver in APAP-treated mice.[31]

The ultrastructural finding of the present work confirmed the histological findings which were in the form of disintegrated follicles in the treated groups, the granulosa cells showed signs of atresia in the form of loss of architecture and the presence of many pyknotic nuclei. There were dilated outer nuclear membrane and dilated cisternae of rough endoplasmic reticulum observed by electron microscopy in the treated mother group and the treated pup group.

Affection of maternal weights was not significant in treated group in comparison with control in the 1st week, the last week of pregnancy and after delivery, the decrease in the mean weight pup after delivery in the treated group according to statistical significance was high in comparison with the control group. Mazaud-Guittot *et al*., (2013) and Philibert, P. *et al*., (2013) mentioned that If pregnant mothers take paracetamol by chance during the most critical period of development of reproductive system of the fetus, affection of the reproductive function of the childhood may be enough to occur within a few days or weeks.[9,32] Koren, (1994) suggested a critical age for sensitivity relative to exposure of fetal human testis ex vivo to paracetamol and other painkillers.[33] maternal toxicity is considered one of the causes that led to the congenital abnormalities. Any Serious and/or chronic disturbance in the physiology of mother may affect fetal development (Rebordosa, C. *et al*., 2009 Burdan *et al*., 2011; and Scialli *et al*., 2010).[8,34,35]

The decrease in the mean in number of live pups in the treated group was considered statistically significant high in comparison with the control group. There was highly statistically significant in the mean in number of the dead pups in the treated group compared to the control group and this in acceptance with (Black, & Hill, 2003 and Thiele *et al*., 2013) who report that acetaminophen may lead to an increased rate of the perinatal mortality, restriction of intrauterine growth and prolonged gestation and labor.[36,37]

CONCLUSIONS AND RECOMMENDATION

Administration of acetaminophen in therapeutic doses is efficient, well tolerated and safe for most of patients but as any drug has side effects so before prescribing acetaminophen to patients, a balance of benefits and losses should be taken in consideration to ensure the adequate and efficient therapy. the pros and cons of this medication must be considered before prescribing. As well the drug should not be allowed as OTC (Over-the-counter medicine) medication during pregnancy.

ABBREVIATIONS

ADHD (Attention Deficit Hyperactivity Disorder); AKT/ PKB (Protein Kinase B); APAP (N-Acetyl-P-Aminophenol); α-SMA (α-smooth muscle actin); COL (collagen); E2 (estradiol); FDA (Food and Drug Administration); FSH (follicular stimulating hormone); H&E (hematoxylin and eosin); IL-1 (interleukin-1); GD (gestational date); NSAID (non-steroidal anti-inflammatory); OTC (Over-the-counter medicine); PAS (Periodic Acid – Schiff`s reaction); PD (pup date); PGE2 (prostaglandin E2); PND (postnatal date); TGF-β (transforming growth factor-β); TNF (tumor necrosis factor).

CONFLICT OF INTERESTS

There are no conflicts of interest.

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ملخص البحث

خلفية والهدف من الدراسة: يعتبر الأسيتامينوفين من أكثر أنواع الأدويه المستخدمة في علاج الألم التي يتم تناولها خلال فترة الحمل. بسبب المعلومات الهزيلة المتعلقة بسلامة هذا الدواء أثناء الحمل ، أجريت هذه الدراسة لمعرفة تأثير الأسيتامينوفين على مبيض الفئران البيضاء الحامل البالغة وتأثيره على النسل.

المواد والطرق: في هذه الدراسة ، تم تقسيم عشرين من إناث الفئران البالغة البيضاء ذات دورة شبقية منتظمة إلى مجموعتين (10 فئران لكل منهما): المجموعة الاولى (مجموعة الام الضابطة لم تتلقى أى عقار. المحموعة الثانية مجموعة الام المعالجة بالاسيتامينوفين تلقت هذه المجموعة 372 ميلي غرام لكل كيلوغرام في اليوم من العقارعن طريق الفم من اليوم السابع من الحمل حتى الولادة. وتشمل المجموعة الثالثة جراء المجموعة الضابطة و وتشمل المجموعة الرابعة جراء المجموعة المعالجة.

بعد 75 يوما من الولاده تم جمع عينات الدم لتحليل الهرمونات ثم تم التضحية بالأمهات والجراء واستئصال المبيضين لمعاينة تركيب المبيض

النتائج: أظهرت النتائج النسيجية للمبيض للمجموعة الضابطة الأم وجراءها أن معظم الخلايا لها مظهر طبيعي مع بنية طبيعية. أما فى المجموعة الأم المعالجة بالسيتامينوفين وجراءهم كان هناك زيادة فى الحويصلات الرتقية في معظم الفئران في مبيض الأمهات اللاتى تم علاجهم بالأسيتامينوفين. أظهر فحص المقاطع المصبوغة بصبغة البريوديك شيف خلل فى التمثيل الغذائى للكربوهيدرات فى المجموعات المعالجة بالمقارنة بالمجموعات الضابطة.كما أظهرت دراسة المقاطع المصبوغة بصبغة الماسون ترايكروم عن توزيع طبيعى لالياف الكولاجين فى المجموعة الضابطة وزيادة تراكم ألياف الكولاجين فى المجموعة المعالجة.

وقد كشف اختبار هرمونات المبيض لعينات الدم نقص فى هرمون الإستراديول وزيادة فى هرمون الغدة النخامة المحفز لنمو الحويصلات.

الإستنتاج والتوصيات: يستنتج من هذه الدراسة أن عقار الأسيتامينوفين يؤثر على التركيب البنائى للمبيض كما أنه يؤثر على التمثيل الغذائي للكربوهيدرات ويزيد من ترسب الكولاجين في المبيض.