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Evaluation of the Therapeutic properties of *Celtis occidentalis* Methanolic Extract against Acetaminophen- Induced Ovarian and Testicular Toxicity and Hormonal Imbalances in Albino Rats

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Abstract

Paracetamol (PCM) is a frequently used analgesic and antipyretic agent; however, its overdose can cause toxicity in various organs. This study investigated the harmful effects of paracetamol on the testes, ovaries, and adrenal glands, and the protective potential of *Celtis occidentalis* leaf extract. Sixty albino rats of both sexes were divided into five groups (n=6) and treated for 21 days: control (untreated), PCM (2 g/kg), extract only (200 mg/kg), and two co-treated groups (EP-1: 100 mg/kg extract + PCM; EP-2: 300 mg/kg extract + PCM). After treatment, blood samples were analyzed for hormonal changes, and histological evaluation of gonads and adrenal tissues was conducted. Paracetamol exposure led to a significant elevation in serum cortisol levels in both sexes, along with a marked decline in serum testosterone in males and 17-β estradiol in females. Co-administration of *C. occidentalis* extract significantly reduced cortisol levels and restored testosterone and estradiol concentrations, particularly in the high-dose group. Histopathological observations revealed severe degeneration and disorganization of seminiferous tubules, loss of spermatogonia in males, and follicular atrophy, corpus luteum hyperplasia, and vacuolation in females. Adrenal tissues exhibited hypertrophy and cytoplasmic vacuolation associated with excess cortisol secretion. Treatment with *C. occidentalis* extract ameliorated these alterations, restoring normal tissue morphology. The study concludes that methanolic extract of *C. occidentalis* leaves exhibits strong protective and regenerative potential against paracetamol-induced reproductive and adrenal toxicity.

KEYWORDS

Acetaminophen, *Celtis occidentalis*, Testosterone, Cortisol, 17-β Estradiol, adrenal glands, gonads histology

1.0 INTRODUCTION

Paracetamol, also known as acetaminophen, is a widely used drug for having analgesic and antipyretic properties. It has the benefit of relieving mild to moderate pain and fever, and is also used in combination with opioids for the treatment of chronic pain [1]. Paracetamol show less anti-inflammatory effect and is reported to have interaction

with COX-3. The main mechanism of action of paracetamol is the inhibition of cyclooxygenases that is COX-1, COX-2 and COX-3 and in the involvement of serotonergic pathway. The study shows that paracetamol reduced prostaglandin formation ten times stronger in brain than in the spleen, this show that paracetamol is prostaglandin inhibitor in the brain [2]. The most current study show that inhibition of nitric oxide synthase activity is also involved in the mechanism of actions of paracetamol [3]. Paracetamol is reported to have adverse effects on liver and kidney and causing hepato and renal necrosis [4] and also on male reproductive system [5]. At therapeutic dose, low amount of N-acetyl-p-benzoquinone imine is produced which is detoxified by conjugation with glutathione but at over dose the oxidation of paracetamol to N-acetyl-p-benzoquinone by cytochrome P450 become more and the glutathione level is not enough to detoxify it, so the N-acetyl-p-benzoquinone form covalent bond with thiol (SH) groups in structural proteins which form protein adduct causing oxidative stress, mitochondrial injury, multi-organ death and may be patient death [6]. The oxidative stress produced in the cell may results in cell death. Additionally, N-acetyl-p-benzoquinone imine attaches to cell macromolecules, probably leading to cell death [7]. It is reported that overdose of paracetamol caused testicular toxicity in experimental animals [8]. After treatment with paracetamol the testicles of male Wistar rats were investigated for its morphological changes. Changed and degenerating seminiferous tubules were found. Well and unusual developed rough endoplasmic reticulum was observed in the spermatid and within the tubules the Sertoli cells were reported fragmented [9]. Toxic dose of paracetamol disturbed the arrangement of spermatogonial cells and also cause reduction in the size of seminiferous tubules [10]. Paracetamol interact with reproductive hormones receptor like estrogen, androgen and progesterone receptors which leads to the imbalance of these hormones and ultimately cause infertility [11]. Environmental toxicant has adverse effects on testes. The somatic cells, the Leydig and Sertoli cells, and the germ cells themselves are the three primary target cells for toxins that interfere with spermatogenesis within the testis [12].

Paracetamol increases the weight of adrenal gland [13]. It is reported that paracetamol enhances the thickening of the zona fasciculata and the zona reticularis and increased production of glucocorticoids, cortisol and androgen [14]. *Celtis occidentalis*, a deciduous tree native to America and also found in Africa, Australia or Europe [15]. The bark of *Celtis occidentalis* plant were used as phytomedicines by native American for treating sore throat and aid during menstruation while the wood extract of this plant were used for the treatment of jaundice [16, 17]. This study was conducted to determine the corrosive effect of paracetamol and the therapeutic properties of methanolic extract of *Celtis occidentalis* leave on testes, adrenal glands and ovaries and on the levels of serum testosterone, cortisol and estradiol.

2.0 MATERIALS AND METHODS

2.1 Chemicals and Serum analysis kits

Paracetamol and methanol were purchased from GlaxoSmithKline consumer healthcare Pakistan limited (35-Dockyyard Road, west wharf, Karachi, Pakistan) and Merk company respectively. Different steroidal hormones ELISA kits were purchased from the Chughtai clinical laboratory Lahore. Rat Testosterone, T ELISA Kit (Sensitivity- 0.06 ng/mL, Code: CSB-E05100r & Optimum storage temperature- 2-8°C) [18], Rat Cortisol ELISA Kit (Sensitivity- 1.56 ng/ml, Code: ab108665 ELISA kit & optimum storage temperature- 2-8°C) [19],

and Rat Estradiol, E2 ELISA Kit (Sensitivity- 40 pg/ml, Optimum storage temperature- 2-8°C & code: CSB-E05110r) were used [20].

2.2 Crude extract preparation

The leaves of *Celtis occidentalis* plant were collected from the hilly areas of Maidan Lal Qila, Dir Lower, Pakistan, with latitude and longitude as 34.9519° N, 71.8082° E. The leaves were thoroughly washed with tap water; shade dried and finally grinds into fine powder with electric blender and was soaked in 2.5L of 95% methanol [17]. After 20 days of shaking it was filtered via Whatman filter paper and the evaporated with rotary evaporator and get crude methanolic extract. The remaining solvent in crude extract was decanted using water bath. The dried crude extract was kept in refrigerator at 2-8°C.

2.3 Model Animal

Thirty male and thirty female albino rats weighing 130-150 gram were purchased from National Institutes of Health Islamabad, Pakistan and were housed in the animal house at the University of Malakand. The animals were acclimated for one week and gave free access to food and water and provided suitable laboratory conditions, at temperature (25 ± 3.5°C), humidity (55 ± 2.5) and 12/12 hours light/dark cycle. All individuals of sexes were kept in well protected iron cages for paired experiment with ***n=6*** individual per cage. The cages were kept in isolation and prevent any sexual interaction. The ethical committee of Zoology, University of Malakand approved the experimental work and research activity according to the law with protocol number No: E-SA-11-2009 according to Bye-Laws 2008 Scientific Procedure Issue-1.

2.4 Experimental design

Paired experiments were performed concurrently. Male and female sexes of albino rats were used as model animal for experimentation. Animals of different sex were divided into five groups with six individual in each (***n=6***) in each experiment. The following doses were given to each sex animals on the basis of their body weight for 21 days;

Group 1st: Control group

Group 2nd: Paracetamol group (PCM), received 2g/kg paracetamol

Group 3rd: Extract group (E), received 200mg/kg plant extract only

Group 4th: Extract (EP-1), received extract (100mg/kg) and paracetamol (2g/kg)

Group 5th: Extract (EP-2), received extract (300mg/kg) and paracetamol (2g/kg)

2.4 Dissection of experimental animals

The experimental animals of the paired experiment were anesthetized with isoflurane and dissected on day 21st. 3mL of blood samples were collected using 5 gauge syringes via cardiac puncture from each experimental group of the paired experiments. Body organs such as ovaries, testicles and adrenal glands were also collected thoroughly washed with normal saline and was kept in 10% formalin till further investigation.

2.5 Serum hormones analysis

The blood sample of the individuals in each experimental group was subjected to ultra-centrifugation for separation of serum at 7000 rpm for 10 minutes. After complete separation of the blood cells and serum, the serum was decanted and stored in eppendorf tube at -20°C, and was used for further gonadal and adrenal hormones (cortisol, estradiol, and testosterone) analysis.

2.6 Assessment of serum cortisol

For analysis of serum cortisol, rat cortisol ELISA kit (sensitivity- 1.56 ng/ml, code: ab108665 ELISA kit &

optimum storage temperature- 2-8°C) was used. The serum cortisol level was measured using a salimetrics enzyme ELISA or immunoassay kit and recorded via spectrophotometer at 450 nm. At each time point, the OFC swab containing the absorbed serum was combined with a 3-mL buffer solution for analysis using a Cube Reader and left for 2 minutes. The s/buffer mix samples collected at home were examined in the laboratory with an OFC swab. Two drops of the saliva/buffer mixture from the OFC swab were placed in the cortisol LFD's sample test window. In this process, the liquid flows laterally along the test strip, forming control and test lines that may be seen in the test window. The test line intensity is inversely proportional to the cortisol levels in the sample, providing a quantifiable number to the reader [21].

2.7 Assessment of serum Estradiol

For estradiol analysis, the 1mL serum was added to 50uL lyses buffer. The Rat Estradiol ELISA kit (code: CSB-E05110r) was used for 17-β-Estradiol analysis. The principal assay was to add Estradiol (E2) to the wells pre-coated with E2 monoclonal antibody. The procedure was based on Fang et al. (2022) performed in various steps with slight modification; preparation of a standard solution and washing buffer reagents. The concentration of 17-β-Estradiol was measured using a microplate reader with 280 nm [22].

2.8 Analysis of serum Testosterone

For serum testosterone measurement, the Rat Testosterone, T ELISA Kit (Sensitivity- 0.06 ng/mL, Code: CSB-E05100r & Optimum storage temperature- 2-8°C). Briefly, 15 mL extraction tubes were filled with 0.5 mL of serum, which was then diluted with 1.0 mL of deionized water. After extracting the steroids using 5.0 mL of diethyl ether, the extract was allowed to air dry before being reconstituted in 0.5 mL of 0.15-M phosphate buffer (pH 7.4). The antibody solution (1:2.000) was then added in 50 I.LL to the tubes containing the subjects, controls, and standard curves. There have been prior reports of the antibody's cross-reactivity [23].

2.9 Histo-Physiological Assessment

For histological studies, different body organs (ovaries, testicles, and adrenal glands) were collected and washed thoroughly with normal saline to maintain tonicity and immediately stored and fixed in 10% formalin. The samples were then processed for microscopic histological assessment. The tissues of known size were dehydrated with 60%, 70%, 80%, 90%, and 100% ethanol each for one hour and the dealcoholized with xylol for one hour (two rounds). After Dealcoholization, the tissues were embedded in paraffin wax for 24 hours and placed in incubator at temperature 60°C. Thin section at 4μm thickness was sliced with semiautomatic rotary microtome, fixed on glass slide and then stained with Hematoxylin (basic stain) for 05 minutes and 1% eosin (30-60 seconds). The prepared slides were labeled and studied under high resolution compound microscope [17].

2.10 Statistical analysis

Data was presented in mean ± standard deviation. One way analysis of variance (ANOVA) was used for multiple comparison followed by student t-test and Tucky test using IBM SPSS version 27.0. The least significance level *p*-value < 0.05 was considered.

3.0 RESULTS

Serum testosterone level and serum cortisol level in the first experiment (male rats) and serum cortisol and serum estradiol levels in the second experiment (female rats) were analyzed after paracetamol induced toxicity and the protective effects of *Celtis occidentalis* leaves extract.

Histopathological study was also carried out to examine the toxic effect of paracetamol and protective effects *Celtis occidentalis* leaves extract on testes and adrenal glands in the first experiment and on adrenal glands and ovaries in the second experiment.

3.1 Analysis of serum testosterone and serum cortisol levels male rats

Serum testosterone level and serum cortisol level were analyzed at the 21st day of activity. The level of testosterone and the level of serum cortisol are expressed in nmol/L and ng/ml respectively. PCM administration in group II caused significant increase in both the levels of serum testosterone and serum cortisol compared with control respectively. In only extract group the levels of serum testosterone and serum cortisol are considered non-significant to that of control group. In PCM and extract combined groups (EP-1& EP-2) the levels of both serum testosterone (EP-1), (EP-2) and serum cortisol (EP-1), (EP-2) decreased significantly compared to PCM treated group.

Table-1. Effect of paracetamol and paracetamol combined with methanolic extract of *Celtis occidentalis* leaves extract on serum testosterone and serum cortisol level.

Groups	Testosterone level (nmol/L)	Cortisol level (ng/ml)
Control	5.22±1.02***	9.24±1.31***
PCM	30.54±2.55	20.88±1.99
E	6.01±0.85***	9.62±1.19***
EP-1	18.90±1.52*	17.72±1.12ns
EP-2	7.27±0.93***	11.55±1.21***

*P<0.05, **P<0.01 & ***P<0.001: significance level, and ns: non-significant. Vales are expressed as mean ± standard.

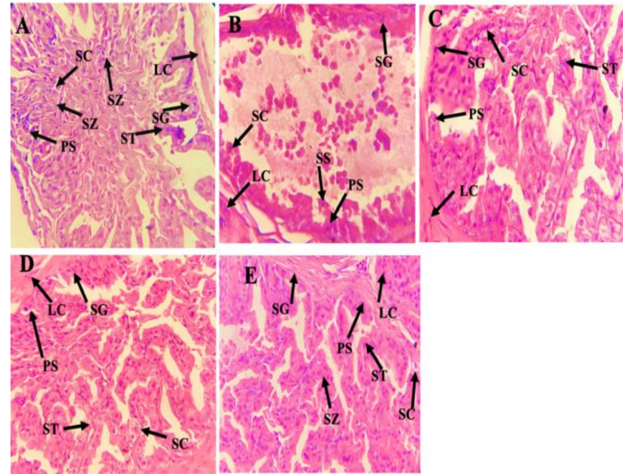

Fig. 1: Histological structures of seminiferous tubules (Magnification 40X). **(A)** Control group, arrows indicates that spermatogonia (SG) along with primary spermatocytes (SC), supportive Sertoli cells (SC) and Leydig cells (LC) were densely arranged and the spermatids (ST) and spermatozoa (SZ) were embedded closer to the lumen. **(B)** PCM, arrows indicating that dense organization of spermatogonia is disorganized, Sertoli cells looks like normal. Spermatozoa and spermatids were not observed while primary and secondary spermatocytes are reduced in number which makes the lumen look larger also the cells of epithelial layer were observed in the lumen. Collagen fibers accumulation was observed. **(C)** Ext-200mg/kg, arrows indicating that spermatogonia were slightly condensed with presence of primary spermatocytes, Sertoli cells and spermatids are slightly greater in number compared to PCM group. **(D)** EP-1, arrows indicating that spermatogonia were arranged slightly dense, density of the Sertoli cells and spermatids has been increased so the lumen looks smaller. **(E)** EP-2, arrows indicating that spermatogonia were densely arranged spermatids density has been increased so the lumen looks smaller, quite similar to the control group. No accumulation of collagen fibers was observed.

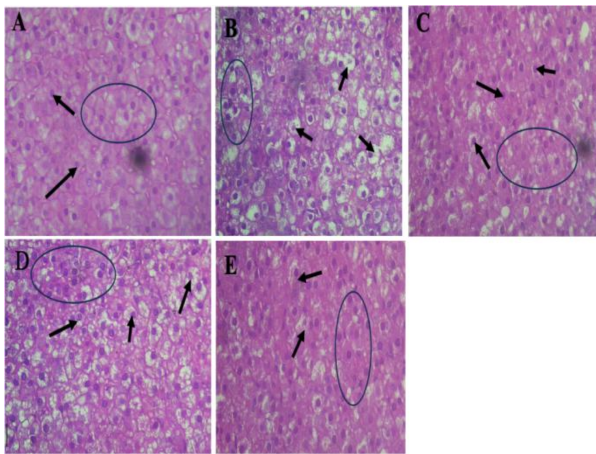

Fig. 2: Histological structure of male adrenal gland tissues (Magnification 40X). **(A)** Control group, the arrow indicates that cells are normal with normal cytoplasm, the circle indicate that cell are not increase in number, or in size. Round and some oval shape nuclei are present. The pale-colored cytoplasm mentioned by arrow demonstrates that the secretion of cortisol is normal and have little lipid droplets. **(B)** PCM, the arrow indicates that hypertrophy is occurred; the cells are enlarged, vacuolated and are filled with more whitish cytoplasm. The circle indicates that cells are increased in numbers and hence hyperplasia occurred. The whitish cytoplasm indicate that the cells are hyper secreted. **(C)** Ext-200mg/kg, a small whitish spot is seen in the cytoplasm indicated by arrows with normal and organized cells approximately similar to that of control group. The pale whitish spot in the cytoplasm demonstrates that there is approximately equal secretion of cortisol to that of control group. **(D)** EP-1, whitish and large cytoplasm is observed, indicated by the arrows and hyperplasia is also observed. **(E)** EP-2, the number of cells and also the size of cells are observed as normal indicated by circles and arrows with normal cortisol secretion as that of control group.

3.2 Analysis of serum cortisol and serum estradiol levels female rats

Serum cortisol and serum estradiol levels were also analyzed at the last day of the experiment. The levels of serum cortisol and serum estradiol are expressed in ng/ml and pg/ml respectively. This study showed that paracetamol administration caused significant increase in cortisol level (from 16.73±0.53 to 36.53±0.93 ng/ml) and decrease in estradiol level (from 209.64±2.05 to 148.14±2.55 pg/ml) in PCM group compared with control group in respective manner. In the only extract treated group (E group) the level of serum cortisol (17.12±1.40 ng/ml) and also the level of estradiol (208.10±1.35 pg/ml) are non-significant to that of control group. The level of serum cortisol in EP-1 and EP-2 (EP-1: 29.31±1.23, EP-2: 19.62±1.22 ng/ml) are significantly decreased towards control. In EP-1 and EP-2 groups, the levels of serum estradiol are increased compared to paracetamol treated group and recovering towards control group.

Table-2. Effect of paracetamol and methanolic extract of Celtis occidentalis leaves extract on serum cortisol level and serum estradiol level.

Groups	Cortisol level (ng/ml)	Estradiol level (pg/ml)
Control	16.73±0.5***	209.64±2.0***
PCM	36.53±0.9	148.14±2.5
E	17.12±1.4***	208.10±1.3***
EP-1	29.31±1.2**	167.59±1.5*
EP-2	19.62±1.2***	190.32±1.4**

*P<0.05, **P<0.01 & ***P<0.001: significance level, and ns: non-significant. Vales are expressed as mean ± standard.

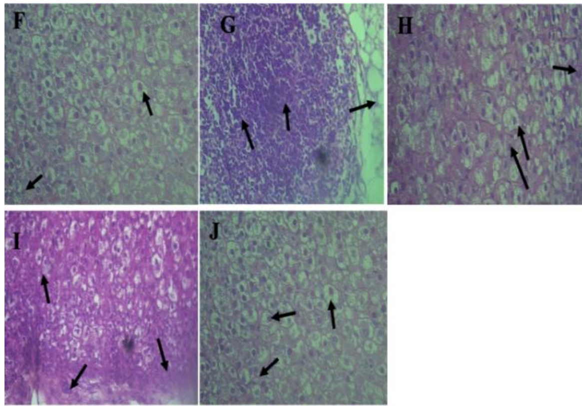

Fig. 3: Histological structure of Adrenal gland tissues of female rats (Magnification 40X). **(F)** Adrenal gland section of Control Group, showed normal cell architecture with nuclei ranges from round to oval in shape. Minimized accumulation of lipid droplets and normal secretion of cortisol were observed in pale-colored cytoplasm. **(G)** Photomicrograph of PCM group, adrenal glands showed high level hypertrophy and hyperplasia. The cortisol was hyper secreted indicated by whitish cytoplasm. **(H)** Photomicrograph section of Extract (E)-group, showed normal cellular architecture with regulated secretion of cortisol. **(I)** Adrenal tissue sections of EP-1, revealed hyperplasia and expanded cells with whitish cytoplasm, indicating a partial recovery of structural alterations. **(J)** Adrenal tissues section of EP-2 indicates recovery of adrenal degenerated tissues by *Celtis occidentalis*. The cells were observed morphologically normal with normal secretion of cortisol.

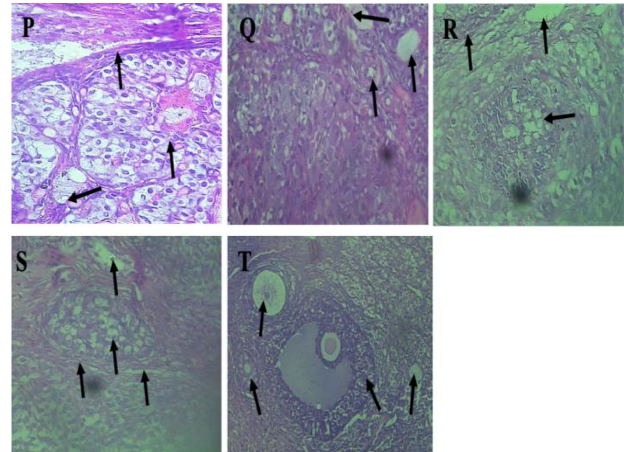

Fig. 4: Histological structures of ovaries (Magnification 40X). **(P)** Control, normal follicles with clear follicular spaces are present and stroma with no proliferated inflammatory cells as indicated by the arrows. No blood hemorrhage occurred in the ovaries and spaces are cleared and without accumulated mucous. **(Q)** PCM, the follicular spaces are filled with mucous and proliferated inflammatory cells are seen in the follicular spaces. Blood hemorrhage occurred in the stroma region. Inflammation occurred in the cells and follicular cells are filled with mucus. The number of follicular cells also decreased. **(R)** E, showed normal tissues histology like control group with has normal structure and the follicular spaces and stroma has normal composition with no inflammatory cells. **(S)** EP-1, the ovary shows slightly normal structure with little accumulation of mucus in follicular spaces and stroma has normal composition with some inflammatory cells in filtered. **(T)** EP-2, the ovary has normal structure and normal composition of follicular spaces and stroma. Follicular spaces are free of mucous. Normally developing follicular cells were observed in the stroma.

4.0 DISCUSSION

The current study were performed for analaysing the effects of acetaminophen and the methanolic extract of *Celtis occidentalis* leaves on male and female reproductive systems and on adrenal gland. In PCM group, a significant

increase in the levels of serum testosterne, serum cotisol and decrease in the level of serum estradiol were observed compared to control group. Treated groups of animals were given the methanolic extract of *Celtis occidentalis* leaves in combination with paracetamol in which the levels of serum testosterone, cortisol and estradiol have different ratios. By comparing the treated goups with normal control group animals it is cleared that the administraion of paracetamol has elevated the levels of serum testosterone and serum cortisol and lowered the level of serum estradiol. In the EP-1 and EP-2 groups, received the methanolic extract of *Celtis occidentalis* leaves in combination with paracetamol, reduced the levels of serum testosterone and serum cortisol and elevated the level of serum estradiol toward normal in a dose dependent manners.

In this study elevated level of serum testosterone was obsereved in PCM group. The serum testosterone level in PCM group considerably raised as compared to the control group. In the PCM and EP-1 grpus, the levels of serum testosterone is seen in high concentration, in a previous study it is reported that high levels of testosterone can lead to problems with sperm production and may cause infertility [24]. It is reported that men with very high testosterone levels were more likely to have lower sperm counts and less motile sperm [25], in another study it is reported that too much testosterone can have a negative effect on fertility, as it can lead to lower sperm counts and reduced sperm quality [26]. And this result agrees and confirms the result of the current study in which in the PCM group high serum testosterone level were observed and spermatids and spermatozoa were not observed as mentioned in the photpmicrograph (B) Fig. 1, this may be due to the high level of testosterone. In the current study paracetamol has elevated the level of serum testosterone in PCM group which were significantly recovered by the methanolic extract of *Celtis occidentalis* leaves as in EP-1 and EP-2 groups respectively.

Serum cortisol level were also analyzed in the current study. In our study elevated level of serum cortisol was obsereved in PCM group. This result was described by a previous study in which paracetamol brought a significant increase in the mean zona fasciculata and zona reticularis thickness which could be explained by the hypothalamus-pituitary-adrenal axis (HPA)-controlled increase in ACTH, which leads to hypertrophy in the adrenocortical cells of these zones and increased production of glucocorticoids, cortisol, and androgens secreted from zona fasciculata and zona reticularis [14]. In PCM group, the hypersectoin of cortisol hormone might be due to the hypertrophy and hyperplasia of zona fasciculata as mentioned in photomicrographs (B & G) in Fig. 2 & 3. this could be explained by the central activation of the HPA axis by ACTH causes the adrenal cortex to respond morphologically, molecularly, and physiologically. Along with the release of glucocorticoids, this causes the steroidogenic cytochrome P450 messenger ribonucleic acids to be up-regulated. The result is obvious morphological changes in the adrenal gland that are characterised by hypervascularization, cellular hypertrophy, and hyperplasia [27]. The current study showed that paracetamol has toxic effect on adrenal glands and on the level of cortisol hormone. This was in agreement with previous study in which the elevated level of serum cortisol showed that paracetamol cause hyper activation of adrenal glands might be, by the activation HPA axis or by the hypertrophy and hyperplasia of adrenal gland [28]. In this study the elevated levels of serum cortisol in PCM treated group was significantly mitigated by the methanolic extract of *Celtis occidentalis* leave in

dose dependent manners. High cortisol may mediate Stressful life events, high neuroticism, depression, sleep problems, as well as cardiovascular risk factors, may have an adverse effect on cognitive function, neurodegeneration, and cognitive decline. Additionally, elevated cortisol can increase amyloid peptide toxicity and oxidative stress while having neurotoxic effects on the hippocampus [29]. Patients with high serum cortisol levels had greater rates of cardiovascular disease and left ventricular systolic dysfunction as compared to those with low serum cortisol levels. Higher all-cause mortality was seen in the patients in the high cortisol group than in the low cortisol group [30] [2].

Analysis of serum estradiol showed that administration of paracetamol has reduced seum estradiol level compared to control group. It is reported that paracetamol altered the expression of genes involved in steroidogeneses and reduced the levels of estradiol in human placental cells in a dose dependent manners [31, 32]. Reported study showed that paracetamol lowered estrogen and gonadotropin levels in women who used paracetamol for relieving menstrual pain [33]. Our results showed reduced the levels of serum estradiol in PCM group compared control group due to paracetamol administration which was significantly attenuated by the methanolic extract of *Celtis occidentalis* leaves as in EP-1 group.

The current findings in control group (Fig. 1-A) are in accordance with a previous study in which a section in the normal testis showed normal seminiferous tubules lined by several layers of spermatogonia cells, spermatogonia, and primary spermatocytes, spermatids, separated by Sertoli cells with a narrow interstitium in between. Many spermatozoa in the tubular lumen can be seen [34]. PCM treated group (Fig. 1-B) showed disorganization in the dense form of spermatogonia. Spermatozoa were not seen, and the lumen appeared to be larger because there were less primary and secondary spermatocytes and more epithelial layer cells were observed in the lumen. These results were previously reported in a study in which the animals in high paracetamol group had primary spermatogonia and some spermatocytes without any spermatids in the epithelium of the seminiferous tubules and no spermatozoa in the lumen of the tubules [35]. The mechanism of action of paracetamol action is mainly due to the formation of reactive metabolites (N-acetyl-p-benzoquinone imine) by enzymatic reaction of cyclooxygenases which might reduce the formation of glutathione and induce oxidative stress. It is also reported that paracetamol cause morphological changes in the testes by degenerating the seminiferous tubules [36]. Methanolic extract of *Celtis occidentalis* leaves (300mg/kg) in combination with paracetamol (2g/kg) in EP-2 attenuated the morphological alterations indicated by the arrows that spermatogonia were densely arranged, spermatids have been increased in number so the lumen looks smaller, quite similar to control group, the current finding is similar to a previous study, in which EP-1 and EP-2, showed nearly normal seminiferous tubules with normal spermatogenesis [37]. In the current study Sertoli cells were not affected by PCM at 2g/kg this result is attributed to previous studies in which no change was observed in the Sertoli cells after the administration of paracetamol at high dose [38, 34].

Photomicrograph (A) in Fig. 2 and photomicrograph (F) in Fig. 3 control group indicates that cells are normal with normal cytoplasm, round nuclei are present in the pale-colored cytoplasm, this was in consistent with the results of previous study in which sections from control group revealed the normal histological architecture of the adrenal

cortex. The cells of zona fasciculata were large and polyhedral with pale vacuolated cytoplasm and vesicular rounded nuclei [39]. The hypothalamus-pituitary-adrenal axis (HPA), which regulates ACTH, may be responsible for the hypertrophy of the adrenocortical cells in this zone and the enhanced production of cortisol that are produced from the zona fasciculata [40]. And this confirmed the current study in which in photomicrograph (B) Fig. 2, and photomicrograph (G) in Fig. 3. PCM in Fig. 2 & 3, caused hypertrophy, indicated by enlarged, vacoulated cells filled with more whitish cytoplasm. No change were obseerved in E-group photomicrographs (C & H) in Fig. 3, and the histology of the cells were approximately similar to that of control group. In EP-1, photomicrograph (D & I) in Fig. 2 & 3 respectively, the whitish and enlarge cytoplasm were observed, indicated by the arrows and increased cells gowth were also observed compared to control group. In EP-2, the number of cells and also the size of cells were observed as normal with normal and round nuclei and normal cytoplasm indicated by circles and arrows with cortisol secretion approximately same as that of control group (Fig.2 (E) and Fig. 3 (J)).

It is reported that paracetamol caused complication in ovarian cells. In literature it is reported that paracetamol reduced the numbers of primordial follicles and caused long term alterations in ovarian cells [41]. The same result is also reported in another study in which paracetamol decreased the follicular reserve [42]. In our study it is also observed that paracetamol reduced the numbers of follicular cells, and caused inflammations with mucus accumulation in follicular cells mentioned in Fig. 4 (Q). All types of follicles were normal and visible in the control group compared to paracetamol treated group. In paracetamol treated group degenerative changes were reported compared to control group [43]. These findings are in accordance with the current study mentioned in Fig. 4 (P & Q) respectively. Reported study showed that *Matricaria chamomilla* extract attenuated and increases the number of follicles and elevated the level of estrogen in rat's undergone torsion [44-46]. In the current study in EP-1 and EP-2, the methanolic extract of *Celtis occidentalis* leaves also increased the number of follicles and attenuated them.

5.0 CONCLUSION

It was concluded that the methanolic extract of *Celtis occidentalis* leaves are potently regulate the hormonal levels and attenuate the testicular, ovarian and adrenal gland toxicities. Further research work should be recommended to isolate the novel phyto compounds and their therapeutic properties.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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