Effect of Flax Seeds (*Linum usitatissimum* L.) Extract in Male Reproductive System of Albino Rat Treated with Cyproterone Acetate

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**Abstract**

The purpose of this report is to assess the possible protective effects of 20%methanolic extract of flax seeds on cyproterone acetate mediated reproductive dysfunction of adult albino rats. Albino rats (35) placed in a random manner into 7 groups, 5 rats in each. The normal control rat was the first group, while the second control group was given corn oil. Third group received cyproterone acetate (CPA) (10 mg/kg) as (positive control). Fourth and fifth groups were treated with 250 & 500mg/kg of flaxseeds extract respectively. The other protected sixth and seventh groups received CPA (10mg/kg) with flaxseeds extract 250 and 500 mg/kg respectively. All groups treated for 50 days orally by gavages tube. In CPA group, the outcome displayed significant reduction (P≤0.05) in the body weight, sperm parameters and FSH level, a significant raise (P≤0.05) in sperms abnormality and level of testosteron, and non-significant decline (P>0.05) in reproductive organs and the accessory glands/ body weight ratios and LH level as compared to oil control. Treatment with flaxseeds 250 & 500 mg/kg caused decrease in the body weight but it considered as significant raise(P≤0.05) in comparison with CPA group, while group treated with flaxseeds extract 250 mg/kg make on-significant changes (P>0.05) in each of reproductive organs and accessory gland/body weight ratios, LH level record non-significant decrease (P>0.05) and significant decrease(P≤0.05) in testosterone level as compared to control D.W and CPA group whereas treatment with flaxseeds extract 500 mg/kg recorded significant increase in testes and epididymis/body weight ratio as compared to D.W, corn oil controls, and CPA groups. Furthermore, oral administration of the flaxseeds extract with CPA at two doses 250 & 500 mg/kg with CPA could reduce the negative effect of CPA in the body weight but it could not enhance the most of the sperm parameters and histological changes of both testes and epididymis caused by CPA. In conclusion, CPA (10mg/kg) induce degenerative changes in the reproductive organs of male rats and the 20%methanolic extract of flaxseeds were exhibit similar effect of CPA but at less extent in the male reproductive system.

**Keywords:** Flax seeds (*linum usitatissimum* L.), Cyproterone Acetate, Reproductive Male system, Albino Rat.

**Introduction**

The medical importance of coming from plants is associated with the influence of indefinite amount or quantity of chemical compounds which produce a limited physiological activity on human body [1]. These chemical substances are called photochemical. A substantially frequent sample of phytochemicals exists as alkaloids, flavonoids, glycosides, saponins, terpenoids, tannins, and sterols [2]. Besides research for discovery not harmful chemical drug as an efficient oral contraceptive, the raw vegetative drugs are being nearly looked into for their potential efficiency to discover safe and efficient oral drug for regulating human fertility [3]. Medicinal plants had been utilized in a manner based on observation or experience as extracts, fractions or semi-purified compounds [4]. Hence; it has been suitable to employ biologically active botanical materials as fertility-regulating factors of plant source which create interference with the natural forms of reproduction [5]. Flaxseeds (*Linum usitatissimum*) which belong to the family of Linaceae are resident to West Asia and the Mediterranean. Flaxseeds are having an abundance of protein, fat and dietary fibers.
It is frequently grouped into one of the various classes of functional and bioactive and an endocrine food[6]. In the area of functional nutrients, flaxseeds are coming out like any of the important origin of phytochemicals [7] such as cinnamic acids, phenol acids, lignans, and flavonoids which are antioxidants and influence the viability and cell growth. Flaxseed is a fundamental source of soluble fiber, powerful quality protein and has substantial ability as a source of phenolic compounds [8, 7, 9].

Anti-androgen can operate direct impact on fertility that inhibits action of testosterone and dihydro testosterone (DHT) in the tissue by negative feedback and this lead to the closure of the androgen receptor and it can remain high level in the blood and this may lead to inhibition of luteinizing hormone (LH) and then lost of the testosterone [10]. Cyproterone acetate (CPA) was a strong steroidal ant androgen accompanied by progestational action. It had been utilized by one self but some time sa companied by Ethinyl Estradiol or Estradiol Vale rate for the therapy of females suffer from disturbance a companied by and organization such as hirsutism or acne. Cyproterone acetate can compete with dihydro testosterone in order to binding with the receptors of androgen and suppress transport of the hormone receptor complex in the direction of the cell nucleus [11].

Preparation of Drug Suspension
The cyproterone acetate (And rocur) was obtained from a pharmacy and their equipment from the company of a subsidiary of Filiale de Schering AG, Germany as a tablet in the concentration of 10 mg/kg. The tablets were macerated by blender and each tablet dissolved in 10 ml ethanol alcohol and exposed to the air until drought then added corn oil and the concentration for experiments were done according to the doses for human [12].

Preparation of Plant Extract
The mixture of methanol in addition to distilled water was used to extract flaxseeds powder in a proportion of distilled water 80%; methanol 20% (v/v) at an average out of 1gm powder of plant :3gm of the mixture by utilizing an electrical mixer in the same temperature of room for 30 minute. The filtration of the suspension took place through gauze and the concentration of the filtrate was performed in the oven at 45ºC. The storage of crude extracts was at 4ºC and kept in dark until use[13].

Sperm Analysis
Determination of sperm motility, viability, concentration, and abnormality were tested. The caudal epididym is cut up into segments, a cutting was done in the tail of epididymis. The microscope slide was used to compress the sperm fluid.

The sperm motility in epididymis was estimated by estimating motile spermatozoa per unit area which described as a percentage of motility. The concentration of sperms in epididymis was calculated by utilizing the hemohyetometer, also, was described as million/ml of suspension. As well, the viability of sperm estimated by utilizing Eosin/Nigros in dye as previous illustrated [14].

Serum LH, FSH and Testosterone Hormones Measurement
The cardiac puncture was used to obtain the blood samples of the rats in experimental groups after anaesthetizing them with diethyl ether.

The blood samples were spun in a desktop centrifuge at 2500 rpm for 10 minutes. Samples of serum were examined for LH, FSH, and testosterone applying the enzyme-linked immune sorbent assay (ELISA) kits.
Histological studies

Histological studies were performed by collecting the specimens from testes and epididymis of experimental groups. The 10% formalin fixative was used as a fixative for these specimens. Paraffin sections were prepared by dehydration, clearing, embedding in paraffin wax and sectioning by hand microtome (5µ thickness). Finally, staining with hematoxylin-eosin and examined microscopically [15].

Statistical Analysis

For analysis of the data in all experiments, Statistical Package for Social Science (SPSS) system/ version 22 was applied. The results described as mean ± S.E. The analysis of variance (ANOVA), the Least Significant Difference (LSD) test and Duncan was used to compare between means (percentage) in this study.

Results & Discussion

Changes in Body Weight

The present study recorded considerable changes of body weight in experimental rats. The data in the table (1) revealed a clearly significant reduction (P≤0.05) in body weight of the CPA 10mg/kg in comparison with the D.W and corn oil groups. This finding is in concurrence with the previous study recorded decline in the body weight of rats treated with CPA13. Also, other report documenting the oral administration of CPA after 15 days treatment brought about a decline in the fertility and weight of the body and accessory sex glands with a significant reduction of sperm concentration [16].

Adult Male rats treated with ant androgen CPA lead in decrease body weight as compared to initial of treatment because of ant androgen effecting the digestion and absorption processes or effect generally in the body weight leading to losing of weight [17].

The flaxseeds extract in both doses caused a reduction of the body weight at a significant level (P≤0.05) of rats treated for 50 days although the dose of 500 mg/kg had a more negative effect compared to control groups. The previous study reported that flaxseeds have small amounts of anti-nutritional materials as inhibitors of protease (e.g., trypsin inhibitors) that have the ability to reduce the digestion besides protein absorption and as a result reduce the animal growth [18].

Polyphenols as well exhibit the capability to bind and deposit molecules which allow to their diminished digestibility [19]. The interest in consumption of flaxseeds is partially in consequence of enhancement the consumption of polyunsaturated fatty acids and dietary fiber. Dietary fibers possess association directly to the health, decreasing the caloric density of the diet. Flaxseeds exhibited diminishing of fat absorption through fecal excretion in human and animal [20, 21].

The weight of the body raised significantly (P≤0.05) in both groups administrated CPA with flaxseeds 250 or 500mg/kg in comparison with the CPA group and this may as a result of the synergistically influence of photochemical compounds found in 20% metabolic extract of flaxseeds to enhance the body weight.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (mean ± S.E)</th>
<th>Weight Change (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Body Weight (g)</td>
<td>Final Body Weight (g)</td>
</tr>
<tr>
<td>Control (D.W)</td>
<td>226.25 ± 18.05</td>
<td>258.75 ± 16.76</td>
</tr>
<tr>
<td>Control (corn oil)</td>
<td>192 ± 6.22</td>
<td>226 ± 19.32</td>
</tr>
<tr>
<td>CPA 10 mg/ kg</td>
<td>259 ± 12.73</td>
<td>220.25 ± 14.28</td>
</tr>
<tr>
<td>Flaxseeds extract 250 mg/kg</td>
<td>186.50 ± 4.41</td>
<td>189.75 ± 4.71</td>
</tr>
<tr>
<td>Flaxseeds extract 500 mg/kg</td>
<td>215.75 ± 2.89</td>
<td>202.75 ± 3.22</td>
</tr>
<tr>
<td>CPA 10 mg+flaxseeds extract 250mg/kg</td>
<td>183.25 ± 5.6</td>
<td>181.50 ± 5.28</td>
</tr>
<tr>
<td>CPA 10mg+ flaxseeds extract 500mg/kg</td>
<td>180.50 ± 5.89</td>
<td>180.75 ± 8.04</td>
</tr>
</tbody>
</table>
Reproductive Organs and Accessory Gland / Body

Weight Ratios

In table (2), non-significant decrease (P>0.05) was shown in all reproductive organs and accessory gland/body weight ratios in group treated with CPA which were (0.39±0.02)%, (0.21 ± 0.02)% , (0.16 ± 0.01)% and (0.15 ± 0.03)% as compared to corn oil control which were (0.49 ± 0.06)%, (0.25±0.03)%, (0.17±0.01)% and (0.14±0.03)%of testes, epididymis, prostate and seminal vesicle/ body weight ratio respectively.

These results are consistent with the previous study showed that treatment caused decreasing in testes, epididymis, prostate and seminal vesicles weight at the period of 50 days [12]. Other study showed that the CPA 10, 50 and 100 mg/kg dose had the effect of the testes, epididymis and seminal vesicles weight, but with no significant changes in the testes weight [22]. Whereas group treated with flaxseeds extract 250 mg/kg caused non-significant changes (P>0.05) in all reproductive organs and accessory gland/ body weight ratios. Also, a significant elevation in testes/ body weight ratio (0.62 ± 0.06) % as compared to CPA group. Also, flaxseeds extract 500mg/kg caused a significant increase in testes /body weight ratio (0.74 ± 0.08) % and epididymis /body weight ratio (0.36 ± 0.05) %as compared to CPA group and both doses had no significant changes compared to both D.W and corn oil control. A different study, however, had exhibited the influence of phytoestrogens on the reproductive organs weight differently [5].

<table>
<thead>
<tr>
<th>Groups</th>
<th>Testis / body weight ratio (%)</th>
<th>Epididymis / body weight ratio (%)</th>
<th>Prostate/body weight ratio (%)</th>
<th>Seminal Vesicle / body weight ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ( D.W)</td>
<td>0.49±0.05</td>
<td>0.21±0.02</td>
<td>0.20±0.04</td>
<td>0.20±0.04</td>
</tr>
<tr>
<td>Control(corn oil)</td>
<td>0.49±0.06</td>
<td>0.25±0.03</td>
<td>0.17±0.01</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>CPA (10 mg/ kg)</td>
<td>0.39±0.02</td>
<td>0.21±0.021</td>
<td>0.16±0.01</td>
<td>0.15±0.03</td>
</tr>
<tr>
<td>Flaxseeds extract 250 mg/kg</td>
<td>0.62±0.06 bc</td>
<td>0.2±0.03</td>
<td>0.16±0.02</td>
<td>0.15±0.02 ab</td>
</tr>
<tr>
<td>Flaxseeds extract 500 mg/kg</td>
<td>0.74±0.08 c</td>
<td>0.36±0.05</td>
<td>0.21±0.06</td>
<td>0.2±0.02 ab</td>
</tr>
<tr>
<td>CPA 10 mg+ flaxseeds extract 250mg/kg</td>
<td>0.48±0.02 ab</td>
<td>0.23±0.02</td>
<td>0.16±0.01</td>
<td>0.14±0.01 ab</td>
</tr>
<tr>
<td>CPA10mg/kg+ flaxseeds extract 500mg/kg</td>
<td>0.41± 0.1 a</td>
<td>0.3±0.04 bc</td>
<td>0.16±0.02</td>
<td>0.12± 0.01 c</td>
</tr>
</tbody>
</table>

Sperm Parameters Assay

Data in the table (3) show the investigation of the parameters of sperm, such as total sperm concentration, the total number of motile sperm, percentages of sperm viability and abnormal sperm of the cauda of epididymal plasma were performed in the control and treated animals.

The total number of sperm/ epididymal fluid in control rats was 71.25×106/ml and 62.5×106/ml, the percentage of sperm motility /ml epididymal fluid was 55±2.24% and 49.2±0.58% with the viability of90±1.54%, 91±1.05%, grade activity 2.63±1.19 and 1.63±1.19, sperm concentration in testes 60×106/ml and 50×106/ml and percentage of abnormal sperms5±1.36% and 6.25±1.07%were recorded in D.W and corn oil groups respectively. The present results recorded a significant decline (P≤0.05) in all types of sperm parameters in CPA group; these results are consistent with study showed a decrease in the concentration of sperm in testes and epididym is the percentage of sperm motility and viability of sperm and increase of abnormal sperm [11].

Also, this finding is in agreement with the previous report documenting the oral administration of CPA decreased the fertility and weights of accessory sex glands with significantly reduced of sperm concentrations after treatment [15].

The concentration of sperms in testes and epididym is and the viability of sperms showed a significant decline (P≤0.05) while significant increasing (P≤0.05) in sperm abnormality were observed in each group as compared with control D.W. Also, the results of the present investigation showed that CPA given orally to
male rats for 50 days increased significantly (P≤0.05) the percentage of all sperm abnormalities (without a head, pin head, round head and two tails) as compared to control D.W and corn oil groups. The loss of regular shape of sperm because physiological and genetically changes may occur [22]. A significant decline in the percentage of sperm aberrations in groups gave a treatment with flaxseeds extract 250mg/kg group, CPA with both doses of flaxseeds extract 250&500mg/kg groups compared to CPA group. The period of spermatogenesis in rats 53.2 days [23], this period may be enough to show a negative effect of CPA on sperm parameters which may be due to oxidative stress caused by CPA [24].

The reactions produced from free radical because of break and accumulated of protein and inhibit of enzymes and per oxidation of lipid then destroy of sperm membrane [25]. Besides, the previous study suggested that the oxidative stress is a major contributor to damage the sperm DNA and abnormal morphology of sperm come together with raised production of ROS can be used as an applicable sign of possible damage to the DNA of sperm [26]. Production of lipid per oxidation, reactive oxygen species, oxidative damage of DNA, total antioxidant capacity and DNA fragmentation were measured as markers of oxidative stress in human semen [27].

From the other point of view, spermatozoa are very vulnerable to damage along with the extreme concentration of ROS because of the elevated amount of polyunsaturated fatty acids inside their plasma membrane. The structure of lipid matrix in the spermatozoa membranes is destroyed by the lipid per oxidation and accompanied by the loss of sperm motility and defect of spermatogenesis [28]. Also, significantly decreased the viability of sperms in all experimental groups in comparison with the normal and negative groups the vitality was in flaxseeds extract 250 mg/kg and CPA with flaxseeds extract 500 mg/kg groups were significantly increased in comparison with the positive CPA group. The viability of sperms is regarded as the applicable parameters to distinguish between infertility and fertility state of males [29]. The findings of this investigation showed that the concentration and motility of sperms were significantly decreased (P≤0.05) in the groups give a treatment of flaxseeds extract 250 & 500 mg/kg alone or with CPA as compared to control.

Also, a significant decrease (p<0.05) in the sperm abnormalities recorded in male rats give a treatment of flaxseeds extract 250 & 500 mg/kg alone or with CPA as compared to the CPA-treated group. Accumulating evidence suggests that flaxseeds are a rich source of different types of compounds presenting biological activity such as polyphenol, flavonoids, linolenic acid, oleic acid, polysaccharide, lignans and soluble fiber which are of special interest [30]. Flavonoids, involving is of lavones had the expanding benefit in alternative medicine. These substances possess a broad extend of hormonal and non-hormonal actions in vitro or in vivo.

These propose potential mechanisms for the possible physiological influence of food having an abundance of is flavones in humans [31, 32]. Flaxseeds rich in phytoestrogen asgeniste in which represses tyrosine kinase enzymes, that therefore brings about the reduction of sperm concentration and motility [33]. With respect to the elucidated finding, the probability of various impacts of phytoestrogens on the productive system of male as a result of anti-estrogenic and estrogenic influences, as the function of phytoestrogens by way of receptors of estrogen that possess both antagonistic and agonistic characteristics. Relying on the location and the form of phytoestrogen, the impacts may be different [34]. Exogenous estrogen Exposure or repression of endogenous estrogen, each of two, throughout adulthood or development causes induction of functional and structural alterations in the male reproductive tract. Neonatal rat's exposure to estrogenic compound decreases the concentrations of sperms and test osterone in plasma [35, 36].

Table 3: Changes in sperm parameters in experimental rats treated with CPA and 20% methanolic extract of flaxseeds (Mean ± S.E)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Epididymis million/ml</th>
<th>Motility %</th>
<th>Grade activity</th>
<th>Testes million/ml</th>
<th>Abnormality %</th>
<th>Viability %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (D.W)</td>
<td>71.28 ± 3.31</td>
<td>85.00 ± 2.24</td>
<td>2.63 ± 0.19</td>
<td>60.00 ± 2.74</td>
<td>8.00 ± 0.63</td>
<td>95.00 ± 1.45</td>
</tr>
<tr>
<td>Control (corn oil)</td>
<td>62.50 ± 2.50</td>
<td>49.2 ± 0.88</td>
<td>1.63 ± 0.19</td>
<td>50.00 ± 4.18</td>
<td>6.25 ± 1.07</td>
<td>91.00 ± 1.05</td>
</tr>
<tr>
<td>CPA 10 mg/kg</td>
<td>38.50 ± 3.37</td>
<td>17.60 ± 1.94</td>
<td>1.50 ± 0.22</td>
<td>42.50 ± 4.61</td>
<td>29.25 ± 1.16</td>
<td>71.25 ± 3.31</td>
</tr>
<tr>
<td>Flaxseeds extract 250 mg/kg</td>
<td>50.00 ± 7.07</td>
<td>30.00 ± 3.16</td>
<td>1.50 ± 0.22</td>
<td>43.75 ± 2.91</td>
<td>15.50 ± 0.39</td>
<td>82.50 ± 2.50</td>
</tr>
<tr>
<td>Flaxseeds extract 500 mg/kg</td>
<td>36.75 ± 2.42</td>
<td>26.20 ± 4.29</td>
<td>1.63 ± 0.19</td>
<td>37.50 ± 2.50</td>
<td>13.50 ± 1.96</td>
<td>76.25 ± 1.85</td>
</tr>
<tr>
<td>CPA 10 mg/kg + Flaxseeds extract 250 mg/kg</td>
<td>55.75 ± 1.88</td>
<td>11.30 ± 3.44</td>
<td>1.00 ± 0.16</td>
<td>46.00 ± 3.84</td>
<td>16.00 ± 1.88</td>
<td>71.35 ± 3.31</td>
</tr>
<tr>
<td>CPA 10 mg/kg + Flaxseeds extract 500 mg/kg</td>
<td>52.50 ± 6.89</td>
<td>17.60 ± 3.51</td>
<td>1.50 ± 0.22</td>
<td>46.75 ± 3.99</td>
<td>13.75 ± 1.05</td>
<td>76.75 ± 1.85</td>
</tr>
</tbody>
</table>

The various letters indicate the significant difference between groups.

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The Table (4) showed that CPA group had no significant decline (P>0.05) in LH hormone level (39.17 ± 21.25) and significant increase (P≤0.05) in the level of testosterone (2.72± 0.04) while FSH level (2.24 ± 0.01) recorded a significant decrease (P≤0.05) in comparison with corn oil group. These results inconsistent with study showed that the CPA reduce testosterone level and effect in the role and growth of testes and effect to the development of prostate and seminal vesicles[29].Ant androgen competes with binding with their receptors which may the reason of increasing level of testosterone. Steroidal ant androgens possess progestational properties leading to a principal repression by crossing the blood-brain barrier [37].

Anti-androgen can operate direct impact on fertility that inhibits the action of testosterone and dihydrotestosterone (DHT) in the tissue by negative feedback and this lead to the closure of the androgen receptor of their link and it can remain high level in the blood and this may lead to inhibition of Luteinizing hormone (LH)[38]. In flaxseeds extract 250mg/kg group, non-significant decline (P>0.05) in LH level (16.31± 9.51) while flaxseeds extract 500mg/kg produce a non-significant increase (P>0.05) in the level of LH (76.38±28.1) in comparison with both CPA and control groups. Both doses of flaxseeds extract caused non-significant changes in FSH level while flaxseeds extract 250mg/kg group only showed significant decrease in testosterone level (1.25 ± 0.03) as compared to CPA group.

In CPA with flaxseeds extract 250mg/kg group, the results recorded non-significant decrease (P>0.05) in the level of LH (13.06 ± 0.47) and significant decrease (P≤0.05) in the level of FSH (2.15 ± 0.03) as compared with both control and CPA groups while group of CPA with flaxseeds extract 500mg/kg recorded enhancement of LH and FSH levels and recorded non-significant changes in comparison with both control group.

The level of testosterone appeared significantly increased (P≤0.05) (2.59 ± 0.08) in CPA with flaxseeds extract 250mg/kg group as compared to corn oil group while both groups of CPA with flaxseeds extract 250&500 mg/kg had no significant difference as compared with CPA group.

These results not consistent with other study revealed that exposing to flaxseeds in meal postnatal and /or prenatally reduced the weight of prostate, and also raised the level of LH in serum and concentration of sperm in cauda epididymal of rat [3].

<table>
<thead>
<tr>
<th>Groups</th>
<th>LH (mIU/ml)</th>
<th>FSH (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ( D.W)</td>
<td>58.85±15.86</td>
<td>2.31±0.01</td>
<td>2.22±0.37</td>
</tr>
<tr>
<td>Control (corn oil)</td>
<td>75.68 ±27.92</td>
<td>2.34 ±0.001</td>
<td>1.78±0.27</td>
</tr>
<tr>
<td>CPA 10 mg/ kg</td>
<td>39.17 ±21.25</td>
<td>2.24 ±0.01</td>
<td>2.72±0.04</td>
</tr>
<tr>
<td>Flaxseeds extract 250 mg/kg</td>
<td>16.31±9.51</td>
<td>2.33 ±0.02</td>
<td>1.25 ±0.03</td>
</tr>
<tr>
<td>CPA 10mg/kg+ flaxseeds extract 250 mg/kg</td>
<td>76.38±28.1</td>
<td>2.28±0.01</td>
<td>2.14±0.28</td>
</tr>
<tr>
<td>CPA 10mg/kg+ flaxseeds extract 500 mg/kg</td>
<td>13.06±0.47</td>
<td>2.15±0.03</td>
<td>2.59±0.08</td>
</tr>
<tr>
<td>CPA 10mg/kg+ flaxseeds extract</td>
<td>53.52±18.22</td>
<td>2.29 ±0.07</td>
<td>2.14±0.31</td>
</tr>
</tbody>
</table>

The various letters indicate the significant difference between groups.

**Histological Study of Testes and Epididym is**

The microscopic study of both control D,W and corn oil testes show the typical normal histological structure of testes(figure -1A,B-)and epididymis (Figure 2A,B-).Also, the microscopic study of rat’s testes and epididymis treated with CPA for 50 days revealed some somniferous tubules had degenerative changes, spermatogenesis, and the number of sperms in the lumen of somniferous tubules occurred which comprise significant decline (P≤0.05) in diameter of somniferous tubules and heights of germinal epithelium intestes. As well, diameter and germinal epithelium heights of tubules in cauda epididymis are (Table-5). Furthermore, atrophy was observed in both somniferous tubules and spermatogenic cells. Also the sperms absent in the lumen of most somniferous tubules Moreover, expansion of interstitial space between somniferous tubules was observed but other tubules
showed normal spermatogenesis stages including spermatozoa compared to the control group. Both Leydig and Sertoli cells not affected by CPA at dose 10 mg/kg used in this study (figure -1C, D-). No remarkable changes were found in epididymis is tissue except some degenerated area of some tubules (figure-2C-). Most effects of CPA on spermatogenesis revealed a significant decrease in spermatogonia, spermatids, and Sertoli cells [29]. Flaxseeds groups at two doses 250&500 mg/kg revealed non-significant differences in both somniferous tubules diameter and their germinal epithelium heights of testes in comparison to the control groups and a significant elevation in the diameter of epididymal tubules in flaxseeds extract 500 mg/kg (Table-5).

In addition, spermatogenesis continues to grow into mature spermatozoa in many tubules but in some tubules, the number of germ cells was decreased with degeneration and reduced of sperms in other somniferous tubules lumen (Figure-1 E, F, G, H-). Furthermore, oral administration of the flaxseeds extracts with CPA at two doses exhibit the same histological changes of both testes and epididym is is caused by CPA(figure-1 I,J,K,L) but it could cause some improvement as causing no significant differences in the somniferous tubules diameter and significant increase (p≤0.05) in the germinal epithelium heights of testes in comparison with D.W control group besides significant increase (p≤0.05) in the diameter of tubules in the cauda epididym is is at 250 mg/ kg flaxseeds extract.

All these results statistically considered as significant increase compared to CPA group. Histological section showed that spermatogenesis found in different stages and continue to grow into mature spermatozoa in many tubules but other tubules were still had decreased spermatogenesis with degeneration and reduced spermatozoa in the lumen of other somniferous tubules. In addition, there were no obvious histological changes in epididymal tissues in all flaxseeds treated groups alone (Figure-1D,E) or with CPA at both doses (Figure-F,G). In the present results of flaxseeds extract 250&500mg/kg with or without CPA-treated rats had normal spermatogenesis but in other somniferous tubules it exhibited degeneration of somniferous tubules accompanied by the loss of spermatogenic stages in the lumen of tubules, this due to its active photochemical compounds present in the extract as a phytoestrogen.

This disturbance of spermatogenesis can be independent of the axis of hypothalamus – pituitary-testis and this probably in consequence of disturbance of auto crine and/or paracrine action of estrogen in the testis [39]. It obstructs the action of androgen like a competitive inhibitor of receptors of androgen and as well employs antagion adotrophic and progesterational effect [40]. Other previous study revealed the elevation in number and motility of sperm, diameters of the somniferous tubule, and diminishing normality of sperm reveal the beneficial influence of flaxseeds in the reproductive system of the male. Prenatally and/or postnatal exposure to flaxseeds reduced the prostate weight, besides increasing the level of LH serum and concentration of sperms in caud a epididym is is of rat [29].

The previous study estimated the effect of the utilize of flaxseeds in the reproductive system and displayed potential opposed impacts of consumption of flaxseeds in males as delay of puberty[41]. Without a doubt, flaxseeds are beneficial in many ways but their consumption in excess amounts can cause some side effects [9]. In conclusion, the results revealed that oral administration of CPA (10 mg/kg/day) induces suppression of sperm production in rats and caused testicular injury characterized by decreased weight of the testes and epididym is is, lowered semen quantity and quality. Flaxseeds were exhibit similar effect of CPA but at less extent in the male reproductive system.

Table 5: Changes in somniferous tubules diameter and germinal epithelium height of testes and epididym is is of experimental rats treated with CPA and 20% methanolic extract of flaxseeds (Mean ± S.E)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Testes</th>
<th>Diameter of somniferous tubules (μm)</th>
<th>Height of germinal epithelium (μm)</th>
<th>Diameter of tubules (μm)</th>
<th>Height of germinal epithelium (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (D.W)</td>
<td></td>
<td>289.9 ± 5.83</td>
<td>92.8 ± 1.46</td>
<td>211 ± 6.6</td>
<td>23.2 ± 0.67</td>
</tr>
<tr>
<td>Control (corn oil)</td>
<td></td>
<td>500.3 ± 5.39</td>
<td>94.8 ± 3.46</td>
<td>208.8 ± 7.46</td>
<td>31.3 ± 0.83</td>
</tr>
<tr>
<td>CPA 10 mg/ kg</td>
<td></td>
<td>259.9 ± 5.74</td>
<td>86 ± 2.19</td>
<td>162.3 ± 3.90</td>
<td>19.6 ± 0.86</td>
</tr>
<tr>
<td>Flaxseeds extract 250 mg/kg</td>
<td></td>
<td>275.5 ± 5.5</td>
<td>96 ± 2.16</td>
<td>216.1 ± 5.45</td>
<td>25 ± 0.92</td>
</tr>
<tr>
<td>Flaxseeds extract 500 mg/kg</td>
<td></td>
<td>281.5 ± 5.55</td>
<td>100.2 ± 1.82</td>
<td>256.8 ± 7.96</td>
<td>22 ± 0.76</td>
</tr>
<tr>
<td>CPA 10mg/kg + flaxseeds extract 250mg/kg</td>
<td></td>
<td>287.7 ± 4.73</td>
<td>103.2 ± 2.15</td>
<td>246.7 ± 5.59</td>
<td>21.3 ± 0.74</td>
</tr>
<tr>
<td>CPA 10mg/kg + flaxseeds extract 500mg/kg</td>
<td></td>
<td>285.1 ± 5.13</td>
<td>99.6 ± 2.49</td>
<td>204.4 ± 4.37</td>
<td>22.4 ± 0.67</td>
</tr>
</tbody>
</table>

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Figure 1: Cross section from testes of (A) D.W. (B) corn oil. (C-D) CPA group. (E-F) flaxseeds extract 250 mg/kg group. (G-H) flaxseeds extract 500 mg/kg group. (I-J) CPA with flaxseeds extract 250 mg/kg group. (K-L) CPA with flaxseeds extract 500 mg/kg group. Degeneration of some seminiferous tubules with the loss of sperm in the lumen (D), increasing in interstitial spaces (I) and normal spermatogenesis (N) in other seminiferous tubules (H&E, 100X)
Figure 2: Cross section from cauda epididymis of (A) D.W. (B) corn oil groups showing normal epididymal tubules. (C): CPA group showing degeneration of some epididymal tubules ( ) and reducing diameters and height of epithelium. (D-E): flaxseeds extract 250 & 500 mg/kg groups respectively showing normal epididymal tubules. (F-G): CPA with both flaxseeds extract 250 & 500 mg/kg groups respectively showing normal epididymal tubules and some tubules with degeneration (H&E, 100 X) [12].

References


