

Research article

Evaluation of The Effects of Carob (*Ceratonia Siliqua* L.) Fruits on the Puberty of Rats

Kılınç Uğurlu A et al. The Effects of Carob (*Ceratonia Siliqua* L.) Fruits on Rat's Puberty

Aylin Kılınç Uğurlu¹, Aysun Bideci², Elvan Anadol³, İpek Süntar⁴, Gülnur Take Kaplanoğlu⁵, Özlem Gülbahar⁶, Zeynep Şafak Teksin⁷, Duygu Dayanır⁵, Tuba Saadet Deveci Bulut⁶, Canan Uluoğlu⁸, M. Orhun Çamurdan²

¹Ankara City Hospital, Pediatric Endocrinology Clinic, Ankara-Turkey

²Gazi University Faculty of Medicine, Department of Pediatric Endocrinology, Ankara-Turkey

³Gazi University, Laboratory Animal Breeding and Experimental Research Center, Ankara-Turkey

⁴Gazi University Faculty of Pharmacy, Department of Pharmacognosy, Ankara-Turkey

⁵Gazi University Faculty of Medicine, Department of Histology and Embryology, Ankara-Turkey

⁶Gazi University Faculty of Medicine, Department of Medical Biochemistry, Ankara-Turkey

⁷Gazi University Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara-Turkey

⁸Gazi University Faculty of Medicine, Department of Medical Pharmacology, Ankara-Turkey

What is already known on this topic?

Natural and organic nutrition, which parents prefer to support their children's immunity and development, can sometimes appear as endocrine disruptors due to the content of the food and the frequency of consumption. This is the first study showing that the use of carob in the prepubertal period causes early puberty and tissue damage by increasing doses.

What this study adds?

We have shown that *C. siliqua*, preferred by parents for organic nutrition, induces early puberty and increases spermiogenesis and folliculogenesis. We discovered that antioxidant mechanisms can affect this and cause tissue damage at high doses.

ABSTRACT

Purpose: This study was planned to determine the effects of carob use on puberty because of the observation of early puberty or pubertal variants due to the long-term use of carob in our clinic.

Methods: Forty-eight Wistar albino rats, on postnatal day 21, were assigned into two groups female (n=24) and male (n=24). Groups were divided into four groups Control, Carob-150, Carob-300, and Carob-600. *Ceratonia Siliqua* L. extract was given to rats in a 0.5% carboxymethylcellulose(CMC) solution. Carboxymethylcellulose (0.5 %) was given to the Control, *Ceratonia Siliqua* L. extract was given 150 mg/kg/day to the Carob-150, 300 mg/kg/day to the Carob-300, 600 mg/kg/day to the Carob-600 by oral gavage. The treatments were performed once daily until the first sign of puberty. Serum FSH, LH, Estradiol, Total Testosterone, Leptin, Glutathione, Glutathione peroxidase, and Malondialdehyde were studied with rat kits by the ELISA method. Testis, uterus and ovary tissue were examined histologically.

Results: The median time of preputial separation in male rats were 38th-31st-31st-31st days, Control, Carob-150, Carob-300, and Carob-600 (p=0.004). The median time of the vaginal opening day in female rats were 39th-31st-34th-31st days in the Control, Carob-150, Carob-300, and Carob-600 (p=0.059). FSH-LH-Testosterone(male)-Estradiol(female)-Leptin levels of groups are similar (p>0.05). Glutathione peroxidase levels were higher in the groups given *C. Siliqua* extract compared to the Control in both male (p=0.001) and female groups (p=0.008).

Testicular and ovarian tissues were concordant with the pubertal period in all groups. As the dose of CS increased, it induced spermatogenesis and spermiogenesis, causing abnormal changes such as ondulation in the basement membrane, capillary dilatation, and increased congestion in male. Edema in the medulla gradually increased with increased dosage, and granulosa cell connections were separated in Carob-300 and Carob-600 in female.

Conclusion: Our study demonstrated that *C. siliqua* caused early puberty and increased spermiogenesis and folliculogenesis. We discovered that antioxidant mechanisms might affect this. Further, it may cause tissue damage at high doses.

Keywords: *Ceratonia Siliqua* L, carob, puberty, antioxidant

Aylin Kılınç Uğurlu, M.D., Ankara City Hospital, Pediatric Endocrinology Clinic, Ankara-Turkey

+90 505 7587125

aylin@ugurlu.org

0000-0003-1265-4952

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INTRODUCTION

In recent years, chemical substances such as sweeteners, flavoring, and preservatives have been used in the foods consumed. These foods are the primary source of chemicals in our daily lives. Natural and organic nutrition can sometimes appear as endocrine disruptors due to the content of the food and the frequency of consumption (1)(2)(3)(4).

After weaning, parents tend to prefer organic foods like carob for their children. Carob (Harnup-*Ceratonia siliqua* L.) is a plant species belonging to the legumes (*Fabaceae*) family and grows naturally in places where the Mediterranean climate

prevails(5). It consists of two parts, fruit, and seed. Carob is a sweet fruit considered a healthy food by families with the forms of raw fruit, flour, and syrup. These forms are obtained from the fruit part of the carob(6). Carob is also a natural sweetener and a source of vegetable carbohydrates. *C. siliqua* is rich in polyphenols and flavonoids in addition to its carbohydrate, protein, and fat content (7)(8). Due to its rich polyphenol and mineral content, it is used especially for strengthening the immune system. It has been shown in many studies that it has antioxidant, anti-inflammatory, analgesic, and lipid-lowering effects and provides blood sugar regulation (9)(10)(11)(12).

When we questioned the histories of early puberty and puberty variants who applied to our clinic, we noticed that some cases had long-term and regular use of *C. siliqua*. This study was planned to experimentally determine the effects of long-term use of *C. siliqua* on puberty. To the best of our knowledge, this is the first study in the literature that examine the effect of *C. siliqua* on puberty.

METHODS

***Ceratonia Siliqua* L. extract preparation:**

Ceratonia siliqua L. fruits were collected and dried from the Tarsus district of Mersin, Turkey province in 2021. After the fruits (500 g) were separated from their seeds and crushed, 50% aqueous-alcoholic extract was prepared. The extract was concentrated in a rotavapor under reduced pressure and at a temperature not exceeding 40°C. The resulting dry extract was prepared to be given to rats in a 0.5% carboxymethylcellulose (CMC) aqueous solution.

When previous in vivo studies on carob was examined, it was seen that aqueous-alcoholic fruit extracts were administered to animals by oral gavage at doses of 50 mg/kg to 2000 mg/kg. In studies on the reproductive system, it has been reported that the extracts have been studied at dose ranges of 150 mg/kg and 600 mg/kg (13) (14). Considering these studies, 150 mg/kg, 300 mg/kg, and 600 mg/kg doses were selected for Control, Carob-150, Carob-300, and Carob-600, respectively. According to the guideline, the tested extract doses in rats (150, 300, 600 mg/kg) can be converted to a human dose based on body surface area as follows for 0.72 mg, 1.44 mg, 2.88 mg per day for 30 kg weighted children (15). The extracts were prepared and administered to the animals in a 0.5% CMC aqueous solution. The same volume of the vehicle without extract (0,5% CMC) was administered orally to the Control.

Animals and study design

Forty-eight Wistar albino rats weaned on a postnatal day (PND) 21, were assigned into two groups female (n=24) and male (n=24). Animals were kept with a 12-h light and 12-h dark cycle and fed standard rodent chow (Korkuteli Food Industry, Turkey). Female and male groups were divided into Control, Carob-150, Carob-300, and Carob-600. CMC (0.5 %) was given to the Control, *Ceratonia siliqua* L. extract was given 150 mg/kg/day to the Carob-150, 300 mg/kg/day to the Carob-300, 600 mg/kg/day to the Carob-600 by oral gavage. The treatments were performed once daily (6 days/week), at the same hour (between 8:00 and 10:00 AM), until the first sign of puberty. The first sign of puberty for male is preputial separation and for female is the first oestrus stage following vaginal opening. Body weights were recorded, and weight gain was calculated by the formula of Weight gain (%)=(Last day–First day)/First day. Vaginal cytology was performed to determine the estrus stage (cornified epithelial cells) after vaginal opening. Vaginal secretion was collected with a plastic pipette filled with 10 IU of normal saline (NaCl 0.9%) by embedding the tip into the vagina. Dripped the vaginal fluid onto the glass slides was evaluated under the light microscope (Leica CME Microscope, 1349522X, NY, USA, 40x objective lenses) according to Cora et al(16). Female rats in the first estrus stage and preputial separated male rats were euthanized by taking intracardiac blood under ketamine 45 mg/kg and xylazine 5 mg/kg anesthesia.

This study was performed in Gazi University Laboratory Animal Breeding and Experimental Research Center and approved by the Ethical Animal Research Committee of Gazi University. The experimental procedures and animal care are conducted per the EU Directive 2010/63/EU.

Biochemical method:

The collected blood was centrifuged at 3000 rpm for 10 minutes at +4 °C and stored at -80 °C. Serum FSH, LH, Estradiol, Total Testosterone, Leptin, and Glutathione, Glutathione peroxidase (GPx) as an antioxidative marker, and malondialdehyde (MDA) as an oxidative marker were studied with rat kits by ELISA method (Bioassay Technology Laboratory, China).

Histopathological method:

In female rat groups, after euthanasia, ovaries and uterus were dissected, and ovarian (right and left) and uterus weights were measured. After euthanasia, testes (right and left separately) were dissected in male rat groups, and their lengths were measured. Uterus, ovary (single), and testis (single) were fixed in Bouin's fixative, paraffin blocks were obtained, and 4-5 micron thick sections were taken from the paraffin blocks. Sections of 4-5 micron thickness were taken from the prepared paraffin blocks. Sections taken were stained with Hematoxylin-Eosin. The histomorphological changes in the obtained samples were examined with light microscopy in the Leica DM4000 (Germany) computer-assisted imaging system. Their pictures were taken in the Leica-Qwin program and evaluated.

Statistical analysis

SPSS 26 program was used for statistical analysis. The "Kruskal Wallis" test was used when comparing the medians of four independent groups in the data that did not fit the normal distribution, and the "Mann-Whitney U" test and the correlation "Spearman test" were used when comparing the medians of two independent groups. Bonferroni correction was used in post hoc tests. Statistically, p<0.05 was considered significant.

RESULTS

Male

At the beginning of the study, the mean weight of the male rat groups Control, Carob-150, Carob-300, and Carob-600 were 45±2.9, 47±11, 49±10, 48±8.8 g, respectively. The median time of preputial separation in male rats was 38th, 31st, 31st, and 31st days in Control, Carob-150, Carob-300, and Carob-600. The day of the beginning of puberty was statistically significantly earlier in all groups given *C. siliqua* extract than in the control group (p=0.04). The medians of weight gain (%) were 126%, 72.5%, 60%, 60.1% in Control, Carob-150, Carob-300, and Carob-600. Weight gain (%) was higher in the control group than in all groups given *C. siliqua* extract (p=0.006). A positive correlation was found between weight gain and

the day of the beginning of puberty ($p=0.001$ $r=0.636$). There was no statistical difference between the groups in terms of FSH, LH, Testosterone, and Leptin ($p>0.05$) (Table 1).

The mean $\pm$ SD of Glutathione levels were 166.8 ± 28.8 mg/L, 164.5 ± 27.4 mg/L, 158.9 ± 26.8 mg/L, 178.2 ± 15.8 mg/L in the Control, Carob-150, Carob-300, and Carob-600; MDA levels were 1 ± 0.3 nmol/mL, 0.9 ± 0.2 nmol/mL, 1 ± 0.2 nmol/mL, 1.2 ± 0.1 nmol/mL in the Control, Carob-150, Carob-300, and Carob-600, respectively. There was no difference between the groups regarding glutathione and MDA levels ($p:0.612$, $P:0.144$). The mean $\pm$ SD of GPx, levels were 85.9 ± 11.4 U/mL, 123.4 ± 12.4 U/mL, 128 ± 20.9 U/mL, 144.0 ± 21.7 U/mL in the Control, Carob-150, Carob-300, and Carob-600. It was found to be statistically significantly higher for Group 2 ($p=0.042$) - Group 3 ($p=0.001$) compared to the control group ($p=0.002$) (Figure 1).

Testicular lengths of the Control were longer than the groups given *C. Siliqua* extract. Although weight gain (%) was higher in the Control, a positive correlation was found between weight gain (%) and both right and left testicular length (right testis $p=0.038$ $r=0.425$ / left testis $p=0.019$ $r=0.474$). Left testis length was shorter in Carob-150 and Carob-300 than in the Control, and it was statistically significant ($p=0.011$) (Table 2).

In histological evaluation, the seminiferous tubules of the Control were observed in the usual histomorphology with regular contoured basement membranes. Secondary spermatocytes and spermatids were observed in some of the tubules, while spermatogonium and primary spermatocytes were observed in most of the tubules. No sperm were found in the lumen of any tubule. These findings showed that the spermatogenesis process had just started in the tubules and cells belonging to the later stages of the series did not differentiate. Leydig cells and capillaries in the interstitial area were in normal formation (Figure 2-A).

In Carob-150, the basement membranes of seminiferous tubule contours were regular. This group's secondary spermatocytes and spermatids distribution were similar to the control group. Leydig cells and capillaries in the interstitial area were observed in normal structure and distribution (Figure 2-B).

In Carob-300, the most striking image was ondulation in the basement membranes of the all seminiferous tubule. In this group, the length of the seminiferous epithelium was elongated in most tubules, and secondary spermatocytes, spermatids and sperms were present in all tubules. Congestion and dilatation were detected in the capillaries in this group. Leydig cells were observed in their usual structures (Figure 2-C).

The seminiferous tubule basement membrane ondulation, seen in the previous group, was much more common and prominent in Carob-600. In all tubules, thickened seminiferous epithelium containing every cell type of spermatogenic series and sperm. Accordingly, the seminiferous tubule walls were significantly thicker. In this group, capillary congestion and dilatation in the interstitial area were more common and prominent. Leydig cells were detected with their typical histological structures near these capillaries (Figure 2-D).

Testicular tissue was concordant with the pubertal period in all groups. As the dose of carob extract increased; it was observed that it induced spermatogenesis and spermiogenesis, did not cause structural and numerical changes in Leydig and Sertoli cells, and caused abnormal changes such as ondulation in the basement membrane, capillary dilatation, and increased congestion.

The mean seminiferous tubule thickness for the groups (Control, Carob-150, Carob-300, and Carob-600) was 234.3 ± 34.5 , 258.9 ± 46.4 , 301 ± 36.2 , 383.8 ± 76.4 , respectively. The seminiferous tubule thickness was significantly higher in Carob-300 and Carob-600 than in Control and Carob 150 ($p=0.001$).

Female

At the beginning of the study, the mean weight of the female rats was 42.5 ± 4.7 , 50 ± 9.5 , 49 ± 6.7 , 51.1 ± 4.5 g, Control, Carob-150, Carob-300, and Carob-600. The median time of the vaginal opening day in female rats was 39th, 31st, 34th, and 31st days in the Control, Carob-150, Carob-300, and Carob-600, respectively. The day of the beginning of puberty was earlier in all groups given *C. Siliqua* extract compared to the Control, but it was not statistically significant ($p>0.05$). The medians of weight gain (%) were 110%, 77.5%, 65.8%, and 64.5% in the control group, Control, Carob-150, Carob-300, and Carob-600. Weight gain (%) was higher in the Control than in all groups given *C. Siliqua* extract, but it was not statistically significant ($p>0.05$). A positive correlation was found between weight gain and the time of the beginning of puberty ($p=0.001$ $r=0.682$). There were no statistical differences between the groups in terms of FSH, LH, Estradiol, and Leptin levels ($p>0.05$) (Table 3).

The mean $\pm$ SD of Glutathione levels were 161.7 ± 19.7 mg/L, 162.3 ± 31.1 mg/L, 149.3 ± 18.8 mg/L, 141.5 ± 9.2 mg/L in the Control, Carob-150, Carob-300, and Carob-600, and MDA levels were 0.9 ± 0.5 nmol/mL, 1.0 ± 0.0 nmol/mL, 1.0 ± 0.3 nmol/mL, 0.9 ± 0.2 nmol/mL in the Control, Carob-150, Carob-300, and Carob-600. There was no difference between the groups regarding glutathione and MDA levels ($p:0.277$, $p:0.976$). The mean $\pm$ SD of GPx, one of the antioxidant marker, were 79.1 ± 14.3 U/mL, 121.4 ± 25.5 U/mL, 123.9 ± 32.1 U/mL, 113.7 ± 25.8 U/mL in the Control, Carob-150, Carob-300, and Carob-600. Glutathione peroxidase levels were higher in the groups given *C. Siliqua* extract compared to the Control ($p=0.008$) (Figure 1).

There was no difference between the groups regarding ovarian length, weight, and uterus weight. ($p>0.05$) (Table 4).

In histological evaluation, the ovarian tissue of the Control was observed in normal histomorphology compatible with puberty. While all follicles belonging to the developmental stage were seen in the sections, no corpus luteum formation was observed in any section. (Figure 3-A)

In Carob-150, vasodilatation and edema were evident in the medulla. Typical follicle structures at the developmental stage in the cortex were observed. The corpus luteum was not observed in this group either. (Figure 3-B)

In Carob-300, vasodilatation and medulla edema findings increased compared to Carob-150. (Figure 3-C). In the multilaminar primary follicle structure, separation and pericellular edema were observed in the junctional units between the granulosa cells (Figure 3-D in 3-C). We concluded that this type of follicles may lead to atresia.

In Carob-600, the corpus luteum structure was detected in many areas. Degenerative changes were detected in the granulosa cell layer in the multilaminar primary follicle structure. Interstitial edema was observed among the granulosa cells. Edema

and congestion in the medulla were detected at the highest level in Carob-600. Similarly, the corpus luteum structure was observed only in this group. (Figure 3-E)

Ovarian tissue was concordant with the pubertal period in all groups and primary, antral and tertiary follicles were observed in all groups. The corpus luteum was seen only in Carob-600. Edema and congestion in the medulla gradually increased in all groups starting from the group that received the lowest dose of carob extract. The separation of granulosa cell connections was detected in Carob-300 and Carob-600.

Uterine tissue was observed in normal structure with all layers in the Control and Carob-150 (Figure 4-A). However, relatively minimal edema was observed in the lamina propria in Carob-150 (Figure 4-B). In Carob-300, unlike the other groups, vasodilatation and edema in the lamina propria and congestion in the muscle layer were observed in this group. (Figure 4-C). In Carob-600, the mitosis activation, which is the symbol of proliferation, and edema in the lamina propria, more prominent in the juxta epithelial region. As a result of proliferation, it was determined that the mucosa corrugated towards the lumen. Vasodilatation was very prominent in this group (Figure 4-D). These findings showed that carob extract affected the uterine cycle dose-dependently, and histological changes mimicked the proliferation phase in the Control, Carob-150, and Carob-300 and the secretory phase in Carob-600. It was observed that especially high dose carob extract accelerated the cycle in rat uterus.

DISCUSSION

In our study, we tried to determine whether *C. siliqua* induces puberty precocious. We found that *C. siliqua* accelerated the time of puberty in male and female rats. There are publications in the literature on the relationship between *C. siliqua* and the fertility of male and female adult rats. In these publications, *C. siliqua* extract applications were made after exposure to gonadotoxic agents such as doxorubicin, cyclophosphamide, lead, and monosodium glutamate (13)(14)(17)(18). In these studies, an increase in gonadotropins, testosterone, and estradiol was detected after different doses of *C. siliqua* after contact with gonadotoxic agents. In our research, gonadotropin and sex steroid (total testosterone in males, estradiol in females) levels of the control group and the *C. siliqua* extract applied groups were similar. The gonadotropin levels of the control group and *C. siliqua* extract applied groups suggest that the onset of puberty is associated with the central activity.

In the male Carob groups, the onset of puberty were earlier and the puberty entry days were the same in all three groups. The histological appearance of the testicular tissue was concordant with the pubertal period in all-male *C. siliqua* extract applied groups; increasing doses of carob extract induced spermatogenesis and spermiogenesis and increased seminiferous tubule thickness. And as the dose increased, the tissue was damaged.

The onset of puberty was also earlier in female Carob groups. And ovarian tissue was consistent with the pubertal period in all-female groups given *C. siliqua* extract, and primary, antral and tertiary follicles were observed in all groups. As the dose increased, it was observed that the follicle structures of the oogenesis seen in the ovarian tissue were at a more advanced stage. We observed that increasing *C. siliqua* extract dose accelerated the cycle in the rat uterus. It was concluded that this may have occurred in response to the increased hormone level due to ovarian-induced folliculogenesis. In our study, we found that while *C. siliqua* caused early puberty; it increased spermiogenesis and folliculogenesis on tissue. Like our findings, there are publications in the literature that *C. siliqua* increases spermiogenesis and folliculogenesis after different dose applications in adult rat studies given *C. siliqua* extract after exposure to gonadotoxic agents (13)(14)(18). Some publications mention an increase in the number of Sertoli and Leydig cells in the tissue, but we did not observe an increase in cell numbers in our study (13)(14).

One of the main factors leading to puberty in rats is excess weight gain. Increasing leptin levels with the increase of adipose tissue is a trigger to initiate puberty (19)(20). Weight gain percentage was calculated to evaluate the effect of weight gain on puberty since increasing doses of extract increased the calories taken. Weight gain (%) of the control groups was higher in both female and male rats compared to the extract-given groups. However, the beginning of the puberty of the control group was later than the groups given *C. siliqua* extract (21)(22). Leptin is an effective hormone at the onset of puberty. In humans, weight gain and an increase in leptin cause delayed puberty in boys and early puberty in girls (23)(24). In our study, leptin levels were similar between the groups, and there was no statistical difference. Our study showed that despite the increased calorie intake, weight gain is not one of the factors that cause early puberty.

Fertility markers that were affected at hormonal and tissue levels negatively after exposure to gonadotoxic agents showed improvement after *C. siliqua* extract was administered. It is emphasized that this effect occurs through the rich polyphenol, vitamin, and mineral content of *C. siliqua* and antioxidant mechanisms. Arachidonic acid and aspartic acid found in *C. siliqua* increase the synthesis of annular adenosine monophosphate and cAMP, stimulating testosterone production (25). In addition, *C. siliqua* can exert its antioxidant effect through its content's polyphenols (gallic acid-tannin) and through iron, manganese, zinc, copper, selenium, and vitamin E, which are cofactors of antioxidant pathways. Antioxidants are the most crucial weapon of the human body to eliminate the oxidative stress created by free radicals. Antioxidants are substances that can scavenge free radicals and prevent cell damage (26). Glutathione peroxidase, one of the enzymatic antioxidants, is an important antioxidant that breaks down hydrogen peroxide into the water in the mitochondria and cytosol. GPX activity is selenium-dependent, and there are eight identified GPXs (27). GPX plays a role in cell differentiation and proliferation in gametes. GPX4 is located primarily in the testis, and its expression pattern in the testis suggests that it may be related to the onset of puberty. In the rat study by Roveri et al (28), it was reported that with the stimulation of gonadotropins, GPX increased in the testicular tissue of rats and stimulated spermatogenesis (29).

In our study, GPX levels, one of the antioxidative markers, were statistically significantly higher in males and females. At this point, it can be thought that antioxidant processes also play a role in the progression of puberty (30). In addition to inducing puberty at high doses, *C. siliqua* also caused histopathological changes such as ondulation in the basement membrane in males and degeneration of intercellular junctions in granulosa cells in females. In the presence of these findings, we determined that high doses may cause damage to gametes. When antioxidants are taken in high doses, they become pro-oxidants in the tissue and cause tissue damage and death (31)(32).

The limitation of our study is that if we could analyze kisspeptin and neurokinin B levels, which are mediators involved in the onset of puberty, we think that it would be more supportive of the central onset of puberty. Also, we could show

hyperplasia of gonadotroph cells in the rat pituitary histopathologically. Still, it could not be studied because the rats were in a small age group, and the tissue was difficult to dissect.

CONCLUSION

C. siliqua caused early puberty and increased spermiogenesis and folliculogenesis. We discovered that antioxidant mechanisms might affect this but may cause tissue damage at high doses. Foods consumed due to organic nutrition can become endocrine disruptors when the amount and duration of use increase.

Conflict of interest: The authors declare no conflict of interest.

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Table 1. The hormone levels of the male groups

	FSH (mIU/mL)	LH (mIU/mL)	Testosteron (ng/L)	Leptin (ng/mL)
Control	14.5 ± 7.3	70 ± 45	263 ± 52	3.3 ± 0.3
Carob-150	11.6 ± 5	92.2 ± 29.5	232.6 ± 25.5	2.8 ± 0.3
Carob-300	6.1 ± 2.8	50.4 ± 38.3	227.2 ± 73.3	3.2 ± 0.6
Carob-600	7.6 ± 2.5	68.6 ± 24.5	285.2 ± 21.5	3.2 ± 0.7
p value	0.051	0.273	0.136	0.319

Values represent mean ± SD

Table 2. Testis lengths of the groups

	Right testicular length (mm)	Left testicular length (mm)
Control	14.6±0.9	15±1.1
Carob-150	12.2±2.2	10±4.8
Carob-300	12.1±1.9	11.8±1.4*
Carob-600	12.3±1.6	12.2±2*
p value	0.059	0.011

Values represent mean ± SD

*p < 0.05 vs control

Table 3 The hormone levels of the female groups

	FSH (mIU/mL)	LH (mIU/mL)	Estradiol (ng/L)	Leptin (ng/mL)
Control	15.8 ± 4	65.7 ± 23	99.9 ± 37.4	3.2 ± 0.6
Carob-150	12.3 ± 4.3	101.3 ± 45.3	105.9 ± 30	3.3 ± 0.4
Carob-300	6.1 ± 6	56.6 ± 41.8	89.7 ± 40.4	3.2 ± 0.3
Carob-600	11.4 ± 5.9	95.6 ± 33.6	88.6 ± 28	3.1 ± 0.5
p value	0.051	0.167	0.81	0.963

Values represent mean ± SD.

Table 4. Over lengths / Over and uterus weights of the groups

	Right ovary (mm)	Left ovary (mm)	Ovarian weight (mg)	Uterus weight (mg)
Control	4.7 ± 0.7	4.9 ± 0.7	75 ± 33.9	441.7 ± 240.9
Carob-150	5.1 ± 0.7	5.2 ± 0.5	110.8 ± 17.5	451.3 ± 114
Carob-300	4.4 ± 0.9	4.8 ± 1.4	95.1 ± 32.4	353.7 ± 154.4
Carob-600	4.6 ± 0.9	4.4 ± 0.4	97.7 ± 27.7	384 ± 163.8
p	0.565	0.18	0.22	0.734

Values represent mean ± SD. p < 0.05 vs control

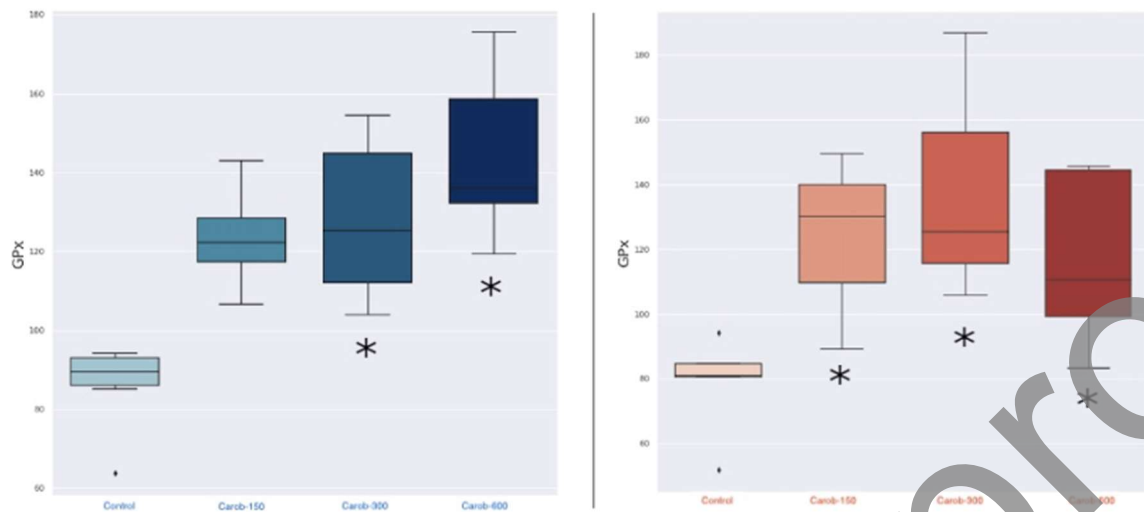


Figure 1: Glutathione peroxidase of male and female rat groups (* $p < 0.05$)

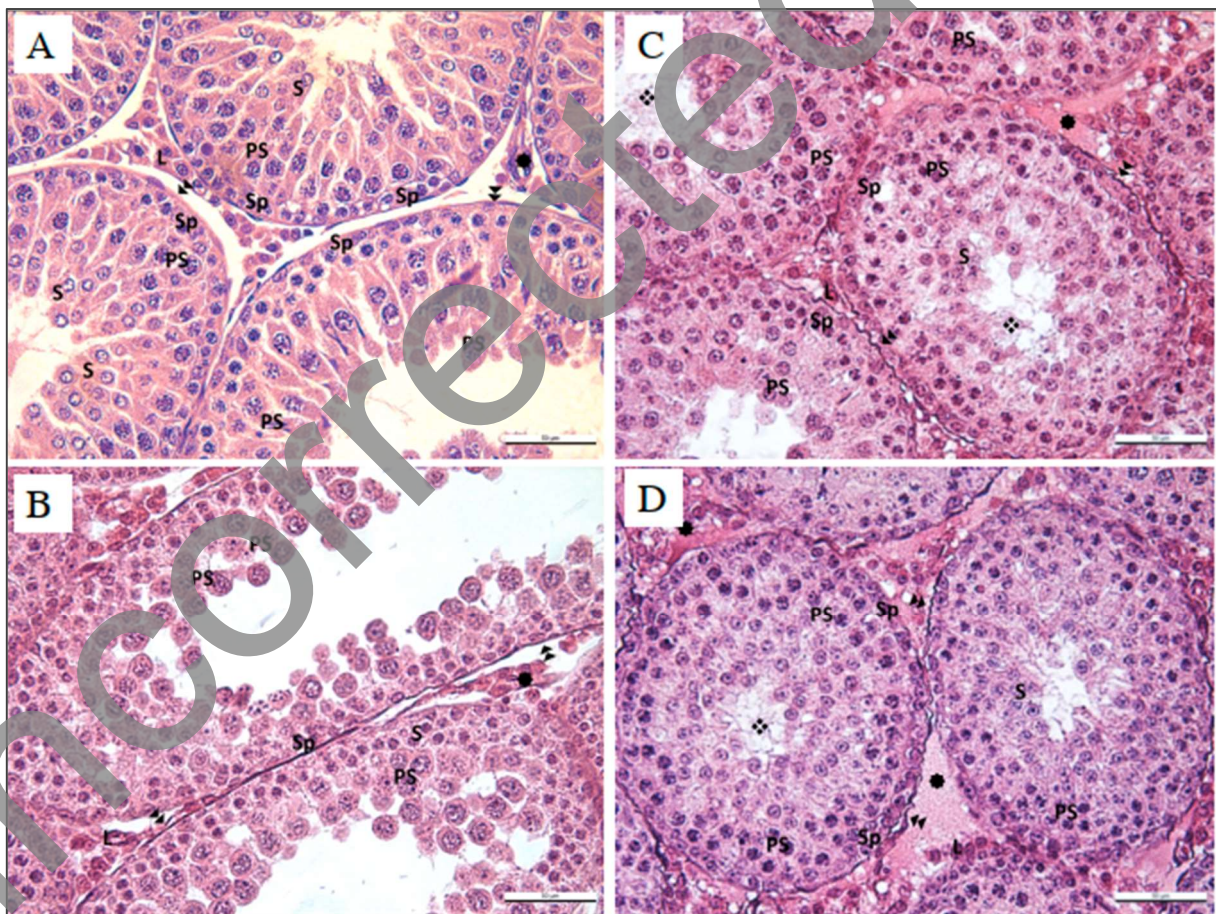


Figure 2. Histological Findings of Testis

A- Control group: Sp: spermatogonia, PS: primary spermatocyte, S: secondary spermatocytes and spermatids, $\llcorner$: basement membrane of the seminiferous tubule in normal configuration, L: Leydig cell, $\bullet$: normal blood vessel (H-E X200).
B- Group 1: Sp: spermatogonia, PS: primary spermatocyte, $\llcorner$: basement membrane of seminiferous tubule in normal configuration, L: Leydig cell, $\bullet$: normal blood vessel (H-E X200).

C- **Group 2:** Sp: spermatogonia, PS: primary spermatocyte, S: secondary spermatocytes and spermatids, ❖: tails of sperm in the stage of spermiogenesis, ⚡: the corrugated basement membrane of the seminiferous tubule, L: Leydig cell, ⬤: congested and dilated blood vessel (H-E X200).

D- **Group 3:** Sp: spermatogonia, PS: primary spermatocyte, S: secondary spermatocytes and spermatids, ❖: tails of sperm in the stage of spermiogenesis, ⚡: densely corrugated seminiferous tubule basement membrane, L: Leydig cell, ⬤: extensively congested and dilated blood vessel (H-E X200).

It was absent in the CG and G1 and was detected in G2 and G3.

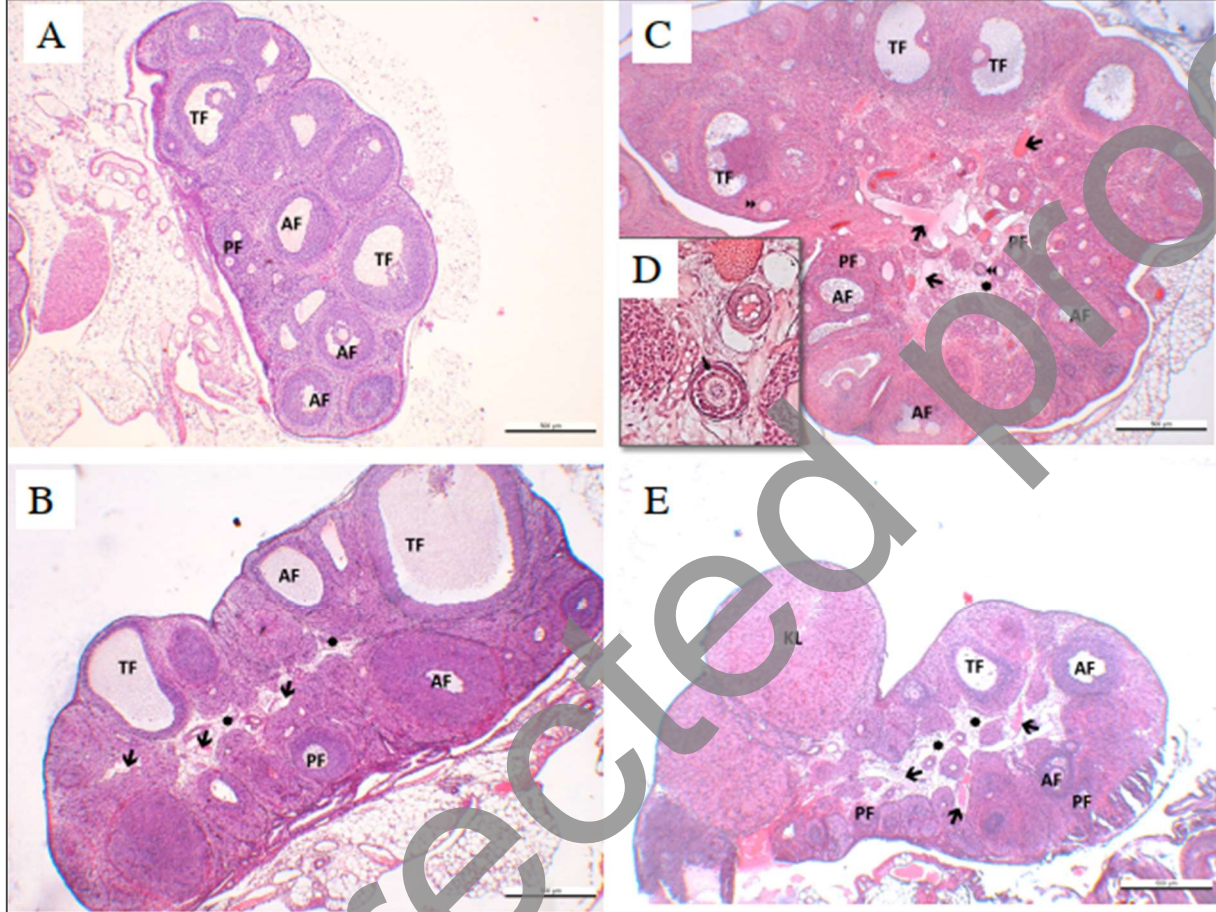


Figure 3. Histological Findings of Over

A-Control: Normal histomorphological ovarian tissue. At various developmental stages, with their normal structures PF: primary follicle, AF: antral follicle, TF: tertiary follicle (H-E X 40).

B- Carob-150: Vasodilation in all areas, especially in the medulla (⚡), edema in the medulla (⬤). At various developmental stages, with their normal structures PF: primary follicle, AF: antral follicle, TF: tertiary follicle (H-E X 40).

C- Carob-300: Vasodilation increased in all areas, especially in the medulla. (⚡). Common edema in the medulla (⬤). At various developmental stages PF: primary follicle, AF: antral follicle, TF: tertiary follicle, particularly in primary follicles, separations between granulosa cells (⚡) (H-E X 40).

D- Separation (⚡) and pericellular edema in the junctional units between granulosa cells, in the multilaminar primary follicle structure

E- Carob-600: corpus luteum (CL), significant vasodilation and congestion in all areas (⚡) at the highest level in this group. Progressive medullary edema. (⬤). At various developmental stages PF: primary follicle, AF: antral follicle, TF: tertiary follicle, particularly in primary follicles, separations between granulosa cells (⚡) (H-E X 40).

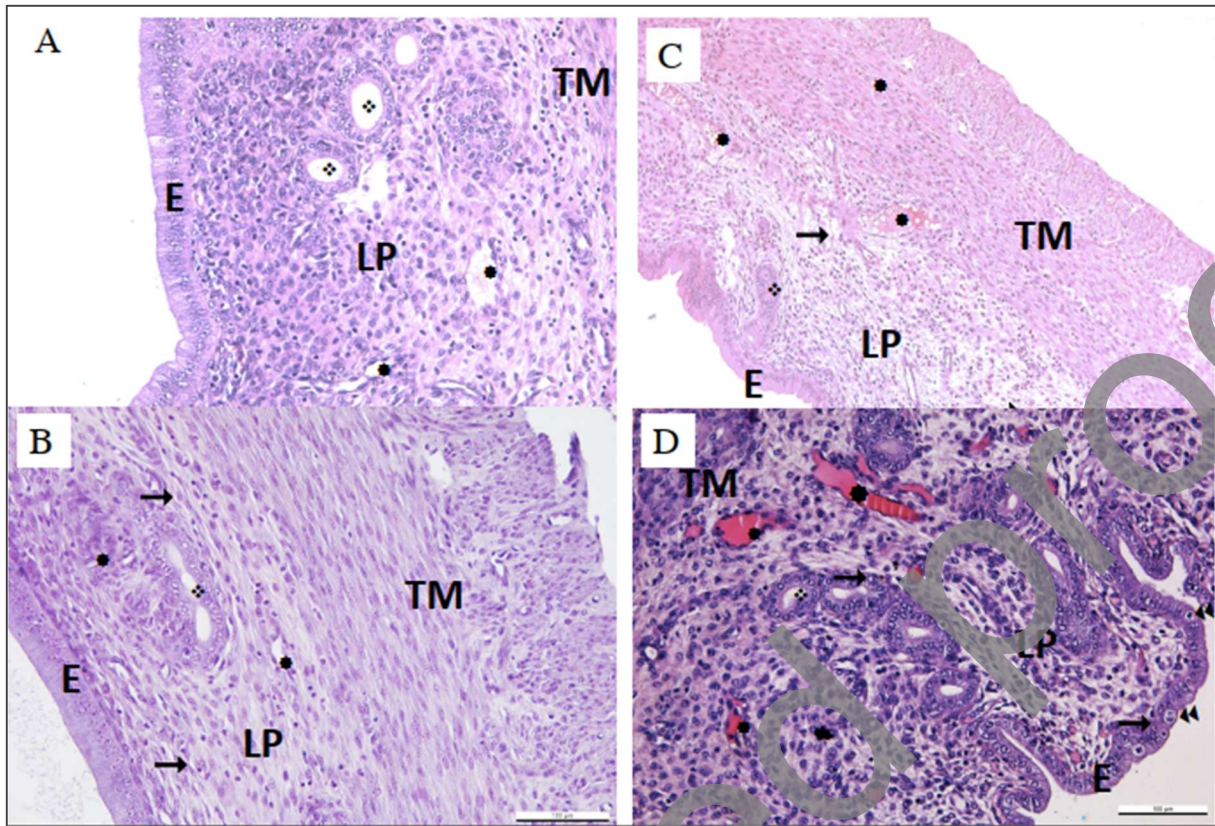


Figure 4. Histological Findings of Uterus

A- Control: Epithelium (E) lamina propria (LP), uterine glands (◇), vascular structures (★) and muscle layer (Tunica muscularis) (TM) observed with normal histological structures (H-E X 100).

B- Carob-150: Epithelium E, lamina propria (LP), uterine glands (◇), vascular structures (★) and muscle layer (Tunica muscularis) (TM) were observed with normal histological structures, minimal edema in the lamina propria (→) (H-E X 100).

C- Carob-300: Vasodilatation and congestion in the vessels in the lamina propria and muscle layer (★) More prominent edema in the lamina propria (→) (◇): normal uterine glands (H-E X 100).

D- Carob-600: Relatively increased mitosis activation in epithelial cells (E) (↔). Increased edema in the lamina propria, more prominent in the juxta epithelial region. (→). Corrugated mucosa towards the lumen. Vasodilatation is very prominent in this group. (★). (◇): normal uterine glands (H-E X 100).