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Assessment of the antifertility effects of some plants in male albino mice

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Some medicinal plants contain natural compounds such as isoflavonoids and polyphenols that produce sterility in male animals. The aim of the present study is to investigate the effects of the chloroform extract of the seeds of Sea Island cotton, *Gossypium barbadense*; the ethanolic extract of seeds of soybean, *Glycine max*; and the ethanolic extract of the leaves of rosemary, *Rosmarinus officinalis*, on the fertility of male albino mice as a prelude for their usage in the control of house mice. The extraction of cottonseeds was performed by soaking and shaking, and the extraction of soybeans seeds and rosemary leaves was performed using the Soxhlet apparatus. Sperm parameters, histology of testes, and biochemical parameters of treated mice were compared with the corresponding ones of untreated mice. The oral administration of all extracts caused a significant reduction in sperm count and motility, and had severe histopathological effects on the testes. The plant extracts also caused significant decreases in the concentration of free testosterone, significant increases in the activities of alanine aminotransferase and aspartate aminotransferase, and a significant decrease in the concentration of urea in serum (except the extract of soybeans). The plant extracts tested in the present study proved to have anti-fertility effects on male albino mice, but it caused significant perturbations in the kidney and liver. These extracts could be used in the control of house mice.

Key words: Cotton seeds, plant extracts, Rosemary, semen analysis, soybeans, testosterone.

INTRODUCTION

Traditional poisonous methods used for the control of vertebrate pests have proved to be inefficient and inhumane. Application of such methods is also restricted in many places such as food stores, hospitals, and human habitations. All of these limitations have forced pest managers to look for non-lethal alternatives. One of these alternatives is the use of antifertility compounds. This approach aims at reducing the population growth of nuisance animals through the reduction of natality rather than the increase of mortality (Miller, 2002).

In their review of wildlife fertility control, Fagerstone et al. (2002) listed many compounds that proved to induce sterility in vertebrate pests such as synthetic steroids, estrogens, progestins, androgens, and natural plant extracts. Studies carried out on natural plants have indicated that many medicinal herbs have successfully induced sterility in experimental animals including rodents (Oderinde et al., 2002; McNeil et al., 2003; Olabiyi et al.,

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2006). Some of these plants target the fertility of females by inhibiting ovulation, preventing implantation, or inducing abortion. Some others target the fertility of males by disrupting spermatogenesis, coagulating semen, or destroying sperms (Trishna et al., 2010; Kumar et al., 2013).

The aim of the present study is to evaluate the effects of the extracts of some locally available plants in Egypt on the fertility of male albino mice. The botanical materials tested are the seeds of Sea Island cotton, *Gossypium barbadense*; the seeds of soybean, *Glycine max*; and the leaves of rosemary, *Rosmarinus officinalis*. The results would be greatly helpful in choosing the proper antifertility plant extract(s) to be used in the control of the commensal house mouse, *Mus musculus*.

MATERIALS AND METHODS

Compliance with ethical standards

The albino mice were humanely treated in accordance with the principles of laboratory animal care of the National Institute of Health (NIH publication No. 86-23, revised 1985). The study design including treatment of experimental animals was approved by the Research Ethics Committee of the Faculty of Science, Ain Shams University (9/2018) prior to the commencement of the study.

Plant material and extraction techniques

The extraction of cotton seeds was carried out by soaking and shaking. One hundred (100) grams of cotton seeds was soaked in 300 ml chloroform in a conical flask with continuous shaking for 36 h at room temperature using SK-0330-pro shaker. The solvent containing the plant extract was separated from the solid residue by filtration through Whatman No. 1 filter paper. The solvent was removed under vacuum using R-215 vacuum rotary evaporator; thereafter, the crude concentrated extract was weighed and kept at 4°C until used. The extraction of soybeans and rosemary leaves was carried out by Soxhlet apparatus according to the method of Freedman et al. (1979). One hundred (100) grams sample of the plant material was chopped into small pieces. Ethanol (75%) was used as a solvent at a ratio 3 ml solvent/g plant material for 36 h extraction period. The alcohol extract of each sample was evaporated to dryness under vacuum using R-215 vacuum rotary evaporator. The crude residue was then weighed and kept at 4_oC until used.

Experimental animals

Twelve-week-old Swiss albino male mice were purchased from the National Research Centre, Cairo, Egypt. Mice were provided with the standard diet and water *ad libitum*. The animals were left for one week before treatments for acclimatization at 22±2°C and 40-60% humidity with natural light and dark cycle.

Experimental design

Thirty-two adult male mice with body weights ~25 g were randomly divided into four groups, viz;

Group 1 (Control): Mice that orally received distilled water.

Group 2: Animals that received the chloroform extract of cotton seeds at a daily oral dose of 210 mg/kg for 21 days (Gadelha et al., 2014).

Group 3: Animals that received the ethanolic extract of soy beans at a daily oral dose 300 mg/kg for 21 days (Guan et al., 2008).

Group 4: Animals that received the ethanolic extract of rosemary at a daily oral dose of 500 mg/kg for 21 days (Nusier et al., 2007).

Blood sampling

At the end of treatments, mice were anesthetized by isoflurane. The blood was collected into clean non-heparinized test tubes, left to coagulate at room temperature and then centrifuged at 4000 rpm for 20 min at 4°C. The serum was separated at once, divided into aliquots, and stored at -80°C until used for biochemical assays.

Body weight, testes weight, and sperm count and motility

The body weight of each mouse was recorded before and after treatment. The testes were dissected out and weighed after removing the blood and adhering tissues. The epididymis was removed, perfused in 2 ml of isotonic saline solution, and cut with scissors to release sperms. The sperm suspension was incubated for 15 min at 38°C, followed by a determination of the sperm number using a hemocytometer and expressed as 10_6/ml. The same sperm suspension was used for assessing the sperm motility by light microscope (Oliveria et al., 2014).

Biochemical determinations

The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated colorimetrically using Biodiagnostic kit (Cairo/Egypt) following the method of Reitman and Frankel (1957). The serum testosterone level was assayed by ELISA kit (Diametra, Italy) and the serum urea was estimated using a commercial colorimetric assay kit (Spectrum, Egypt) as described by Fawcett and Scott (1960).

Histopathological examination

Small pieces of the testes were fixed in Bouin's solution for 24 h. The tissues were dehydrated in ascending series of ethyl alcohol and then cleared in terpineol for three days. Pieces of the testes were embedded in blocks of paraffin wax after three changes in the wax, one hour each. The paraffin blocks were sectioned at 5 µm and mounted on clean slides. The sections were stained with Harris haematoxylin and eosin.

Statistical analyses

Statistical analysis was carried out using Instat Program GraphPad (San Diego, USA) version 3.6. Results were expressed as mean ± SEM. The data distribution was tested by the Kolmogorov-Smirnov test. The statistical analysis was performed using one-way ANOVA followed by Tukey's test for multiple comparisons between the various groups; thereafter, *P* values less than 0.05 were considered significant.

Table 1. Effect of chloroform extract of cotton seed, and ethanolic extracts of soy beans and rosemary on body weight, testes weight, sperm count and motility.

Group	Body weight (g)	Testes weight (g)	Sperm count x 10^6	Sperm motility%
Control	32.2±0.6 (30-33.5)	0.09±0.003 (0.08-0.1)	10.0±0.7 (8-12)	70.0±3.5 (60-80)
Chloroform extract of cotton seeds	26.0±0.3 (25-27)	0.08±0.003 (0.08-0.1)	2.2±0.4** (0-4)	18.0±1.7** (10-20)
Ethanolic extract of soy beans	28.0±1.1 (25-28)	0.08±0.003 (0.08-0.1)	1.8±0.3** (1-3)	54.0±1.6* (50-60)
Ethanolic extract of rosemary	26.0±0.5 (25-28)	0.09±0.002 (0.08-0.1)	3.0±0.3** (2-4)	46.0±2.7* (40-55)

Data are expressed as Mean ± SEM followed by range in (parentheses), n=8. * Significant difference (p≤0.05); ** Highly significant (p≤0.01).

Table 2. Effect of chloroform extract of cottonseed, ethanolic extracts of soy beans and rosemary on some biochemical parameters.

Group	Free testosterone (pg/ml)	ALT (U/l)	AST (U/l)	Urea (mg/dl)
Control	591±36 (480-580)	16.0±0.9 (14-19)	20.0±0.9 (18-23)	55.0±1.7 (50.4-60)
Chloroform extract of cotton seed	0.61±0.002** (0.61-0.63)	23.0±0.7* (22-25)	50.0±0.8** (48-53)	41.0±2.7* (37-44.7)
Ethanolic extract of soy beans	0.17±0.003** (0.17-0.18)	17.0±0.4 (16-18.5)	17.0±0.9 (15-20)	126.0±1.8** (121-132)
Ethanolic extract of rosemary	0.65±0.01** (0.59-0.68)	30.0±0.8** (28-33)	16.0±0.6* (15-18.5)	30.0±0.5* (29.5-32)

Data are expressed as Mean ± SEM followed by range in (parentheses), n=8. * Significant difference (p≤0.05); ** Highly significant (p≤0.01).

RESULTS AND DISCUSSION

Body weight, relative testes weight, and semen analysis

The three plant extracts in the present study insignificantly decreased the mean body weight and relative testes weight of treated mice compared with the control group. The chloroform extract of cotton seeds caused a highly significant decrease in the sperm count and motility (Table 1). The administration of cotton seeds into rats damaged both the mitochondria and flagella of testicular and epididymal spermatozoa and decreased sperm ATP content with concomitant loss of motility (Ke and Tso, 1982). ATP is critical for normal spermatozoan forward progressive movement and a slight decrease in its content would lead to the inhibition of sperm motility (Singla and Garg, 2013). The ethanolic extract of soybeans caused highly significant decrease in sperm count and significant decrease in sperm motility (Table 1). The negative effect of soybeans on the fertility of mice could be attributed to the presence of some isoflavonoids which are almost exclusively produced by members of the bean family; Fabaceae. Many of these natural compounds act as phytoestrogens in mammals (Zeriouh et al., 2014) and are known to decrease the reproductive ability of males (Branham et al., 2002). Such compounds affect the male fertility through decreasing the level of follicle stimulating hormone (FSH) which is essential for spermatogenesis. The decrease in FSH causes a decrease in adenylate cyclase enzyme and a decrease in androgen binding protein. The result is that the testosterone hormone could not be guided to seminiferous tubules (Adeeyo et al., 2011, Modaresi et al., 2011). The ethanolic extract of rosemary also caused a highly significant decrease in sperm count and significant decrease in sperm motility (Table 1). Similar results have been documented by several authors (Heidari-vala et al., 2013; Nusier et al., 2007). The antifertility effects of rosemary could be attributed to the rich contents of diterpenoids such as carnosic acid and carnosol (Mena et al., 2016). These compounds have antiandrogenic activity and reduced the fertility in male dogs (Bhargava, 1989). Rosemary extract also reduced the FSH and testosterone. Decreased androgen production would be reflected by a decrease in the number of mature Leydig cells and their functional status (Nusier et al., 2007). Contrary to the previous findings, the oil of this plant had beneficial effects on the male reproductive system of boars (Superchi et al., 2016).

Biochemical assay

All extracts caused a highly significant decrease in the free testosterone level (Table 2). In the cotton seeds, this effect was attributed to gossypol (Itodo et al., 2020). Studies on rats and deer have referred to the inhibitory effect of this compound on testosterone production by Leydig cells through affecting pregnenolone, the precursor of testosterone formation (Gizejewski et al., 2008). It has also been shown that gossypol had interfered with key

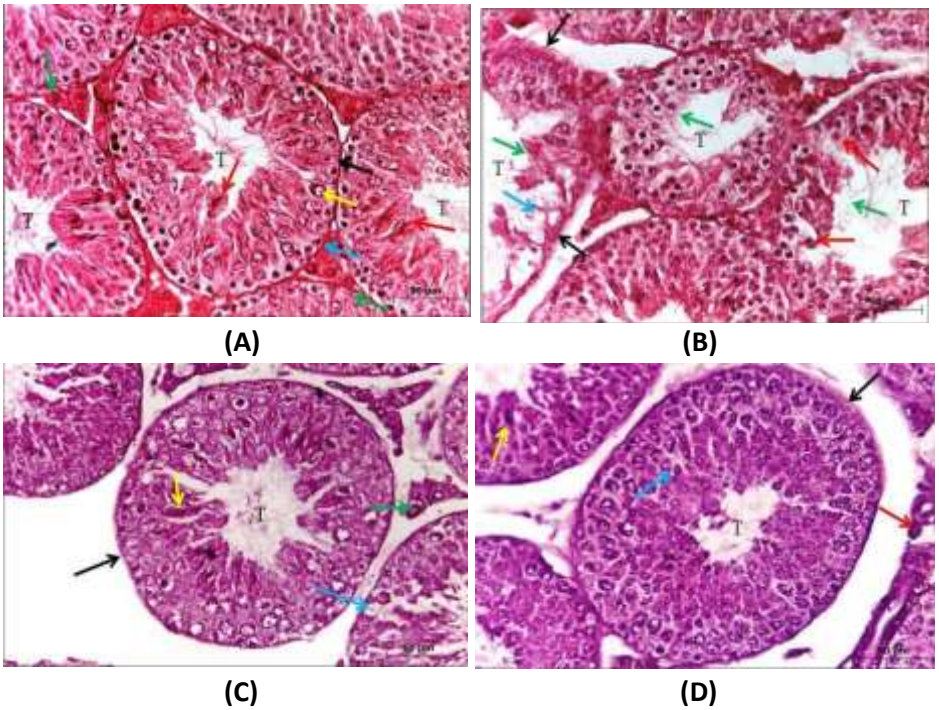

Figure 1. A) A photomicrograph of a transverse section of testis of control mouse showing average-sized seminiferous tubules (T) with basement membranes (black arrow), spermatogonia (blue arrow), primary spermatocyte (yellow arrow), many sperms (red arrow) and average interstitium showing Leydig cells (green arrow). **B)** A photomicrograph of a transverse section of testis of mouse treated with chloroform extract of cotton seeds showing small-sized seminiferous tubules (T) with thickened basement membranes (black arrow), areas of markedly damaged germinal lining (blue arrow) with scattered apoptotic cells (red arrow), and absence of sperms (green arrow). **C)** A photomicrograph of a transverse section of testis of a mouse treated with ethanolic extract of soybeans showing seminiferous tubules (T) with average basement membranes (black arrow), mildly edematous germinal lining (blue arrow), many sperms (yellow arrow), and average interstitium with average Leydig cells (green arrow). **D)** A photomicrograph of a transverse section of a testis of a mouse treated with ethanolic extract of rosemary showing seminiferous tubules (T) with thickened basement membranes (black arrow), germinal lining with scattered apoptotic cells (blue arrow), few sperms (yellow arrow), and average interstitium with average Leydig cell (red arrow). H&E, ×400.

steroidogenic enzymes such as 3α- hydroxysteroid dehydrogenase (which catalyzes the reversible conversion of 3α-androstanediol to dihydrotestosterone) and 5α-reductase (which catalyzes the reversible conversion of dihydrotestosterone to testosterone) in rat testis (Moh et al., 1993). In case of soybeans, this effect was due to isoflavones that inhibit 17-β-hydroxysteroidoxireductase enzyme which is needed for synthesis of testosterone (Margo et al., 2019). In case of rosemary extract, the reduction in testosterone was also attributed to the diterpenoids carnosic acid and carnosol (Mena et al., 2016).

The administration of chloroform extract of cotton seeds and the ethanolic extract of rosemary significantly increased the activities of serum ALT and AST (Table 2), indicating a damage of hepatic parenchyma cells. Whole cotton seed meals have also caused liver toxicity manifested by degenerative changes and increased activities of serum ALT and AST in crossbred cows (Saijpaul et al., 2006). The aqueous extract of cotton seeds has also increased ALT and AST activities in adult Wister male rats (Thomas et al., 1991).

Serum urea was significantly elevated in mice treated with the ethanolic extract of soybeans. However, the extracts of cotton seeds and rosemary significantly decreased the urea (Table 2). This effect might be due to the fact that soybeans contain high-quality proteins (Rafieian-Kapaei et al., 2017). Excess dietary proteins are not stored in body tissues, but are degraded to urea and other waste products (Dickerson, 2016). Urea is produced from the metabolism of protein in liver tissue. The low concentration of urea supports the hepatotoxicity results and elevation of ALT activity seen in the cotton seeds and rosemary groups (Wang et al., 2014).

Histopathology of the testes

The testes from mice treated with the chloroform extract of cotton seeds showed small-sized seminiferous tubules with thickened basement membranes. After the end of treatments, the decrease in the mean diameter of seminiferous tubules was -35.7% of that of the control group (Figure 1A, 115 µm *vs.* 179 µm). They also showed the presence of markedly damaged germinal lining, vacuolations in the cytoplasm of spermatogonia and primary spermatocytes, scattered apoptotic cells, and mildly edematous interstitium. Sperms were completely absent (Figure 1B). Similar results were reported in previous studies (Hahn et al., 1981; Singla and Garg, 2013). Gossypol was reported to cause disruption of testosterone secretion, inhibition of germinal cell differentiation, and breakdown of the blood-testis barrier (Singh and Rath, 1990). Cotton seed oil altered the protein adhesion molecules in the seminiferous tubules leading to disorientation of spermatogenesis and detachment of spermatogenic cells from basal layer (Ozoko et al., 2018). Gossypol was also reported to interfere with the oxidative phosphorylation in the testis of rats resulting in severe deteriorations in the architecture of the testis (Timurkaan et al., 2005). The testes from mice treated with the ethanolic extract of soybeans showed the presence of average-sized seminiferous tubules with average-thickened basement membranes, apoptotic spermatocytes, and round spermatids. The mean diameter of seminiferous tubules was -2.1% of that of the control group (175.2 µm *vs.* 179 µm). They also showed mildly edematous germinal lining and degeneration in most primary spermatocytes (Figure 1C). Similar results were recorded by Serag El-Din et al. (2011) and were attributed to the reduction of FSH and testosterone levels. The testes of mice treated with the ethanolic extract of rosemary showed the presence of average-sized seminiferous tubules. The decrease in the mean diameter of seminiferous tubules was -0.5% of that of the control group (178 µm *vs.* 179 µm). They also showed thickened basement membranes, germinal lining with scattered apoptotic cells and few sperms (Figure 1D). Similar results were documented by Heidari-vala et al. (2013).

Conclusions

The chloroform extract of cotton seeds, the ethanolic extracts of soybeans and rosemary leaves had negatively affected the fertility of treated mice since they decreased sperm count, sperm motility, and the concentration of free testosterone, and caused histopathological changes in the structure of testes, in addition to some perturbations in the liver and kidney functions. The results of the current study are crucial for launching further prospective studies investigating the potential of these botanical extracts in the control of the commensal house mouse.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adeeyo OA, Salawu EO, Ibidun JO, Adeoye SW, Adeleke EG, Adenly OS (2011). Effects of soya beans supplements on fertility in male Wister rats. Macedonian Journal of Medical Sciences 4(1):54-59.

Bhargava SK (1989). Antiandrogenic effects of a flavonoid rich fraction of *Vitex negundo* seeds: a histological and biochemical study in dogs. Journal of Ethnopharmacology 27(3):327-339.

Branham WS, Dial SL, Moland CL, Hass BS, Blair RM, Fang H, Shi L, Tong W, Perkins RG (2002). Phytoestrogens and mycoestrogen bind to the rat uterine estrogen receptor. Journal of Nutrition 132(4):658-664.

Dickerson RN (2016). Nitrogen balance and protein requirements for critically III older patients. Nutrients 8(220):1-14.

Fagerstone KA, Coffey MA, Curtis PD, Dolbeer RA, Killian GL, Miller LA, Wilmot LM (2002). Wildlife fertility control, Technical Review 02–2. Bethesda, MD: The Wildlife Society.

Fawcett JK, Scott JE (1960). A rapid and precise method for the determination of urea. Journal of Clinical Pathology 13(2):156-159.

Freedman B, Nowak LJ, Berry EC, Gutnrie WD (1979). A Bioassay for plant derived pest control agents using the European corn borer. Journal of Economic Entomology 72(4):541-545.

Gadelha IN, Fonseca NS, Oloris SS, Melo MM, Soto-Blanco B (2014). Gossypol toxicity from cottonseed products. The Scientific World Journal 2014:1-11.

Gizejewski Z, Szafranska B, Steplewski Z, Panasiewicz G, Ciereszko A, Koprowski, H (2008). Cottonseed feeding delivers sufficient quantities of gossypol as a male deer contraceptive. European Journal of Wildlife Research 54(3):469-477.

Guan L, Huang Y, Chen ZY (2008). Developmental and reproductive toxicity of soybean isoflavones to immature SD rats. Biomedical and Environmental Sciences 21(3):197-204.

Hahn DW, Rusticus C, Probst A, Homm R, Johnson AN (1981). Antifertility and endocrine activities of gossypolin rodents. Contraception 24(1):97-105.

Heidari-Vala H, Reza E, Sedeghi M, Akhondi M, Novin M, Heridari M (2013). Evaluation of an aqueous-ethanolic extract from *Rosmarinus officinalis* (Rosemary) for its activity on the hormonal and cellular function of testes in adult male rats. Iranian Journal of Pharmaceutical Research 12(2):445-451.

Itodo JI, Ibrahim RP, Rwuaan JS, Aluwong TS, Bugau J (2020). The effects of feeding graded levels of whole cotton seed on semen characteristics and testicular profiles of red sokoto bucks. Acta Scientiarum. Animal Science 43:1-9.

Ke YB, Tso WW (1982). Variations of gossypol susceptibility in rat spermatozoa during spermatogenesis. International Journal of Fertility 27(1):42-46.

Kumar S, Singh J, Baghotia A, Mehta V, Thukur V, Choudhary M, Verma S, Kumar D (2013). Antifertility potential of the ethanolic extract of *Caesalpinia pulcherrima* Linn leaves. Asian Pacific Journal of Reproduction 2(2):90-92.

Margo E, Pangkahila W, Aman I (2019). Soy milk formula increase estrogen and reduce testosterone level in male inftant white Wister rats. Molecular and Cellular Biomedical Science 3(1):22-25.

McNeil RT, Noronha CC, Kusemiju JO, Okanlawon AO (2003). The anti-ovulatory effect of a seed extracts of *Ricinus comminus* L. Nigerian Journal of Health and Biomedical Science 2(1):31-34.

Mena P, Cirlini M, Tassotli M, Herrlinger KA, Asta C, Rio D (2016). Phytochemical profiling of flavonoids, phenolic acids, terpenoids and volatile fraction of a rosemary (*Rosmarinus officinalis*) extract. Molecules 21(1576):1-15.

Miller LA (2002). Reproductive control methods. *In*: Pimental, D. (ed.), Encyclopedia of Pest Management, Marcel Dekker, New York pp. 701-707.

Modaresi M, Messripour M, Khorami H (2011). Effect of soybean on male reproductive physiology in mice. International Conference on

Life Science and Technology, Singapore 3:15-18.
Moh PP, Chang GCJ, Brueggemeier RW, Lin YC. (1993). Effect of gossypol on 5-alpha-reductase and 3-mu-hydroxysteroid dehydrogenase-activities in adult-rat testes. Research Communications in Chemical Pathology and Pharmacology 82(1):12-26.
Nusier MK, Batanieh HN, Daradkah HM (2007). Adverse effect of rosemary (*Rosmarinus officinalis* L.) on reproductive function in adult male rat. Experimental Biology and Medicine 232(6):809-813.
Oderinde O, Noroha C, Oremosu A, Kusemiju T, Okanlawon, OA (2002). Abortifacient properties of aqueous extract of *Carica papaya* L. seeds on female Sprague-Dawley rats. Nigerian Postgraduate Medical Journal 9(2):95-98.
Olabiyi OA, Oremosu AA, Noronha CC, Okanlawon AA (2006). Effects of cotton seed oil (*Gossypium* spp.) and cottonseed meal on estrous cycle, ovulation and histoarchitecture of female reproductive organs of adult cyclic Sprague-Dawley rats. Nigerian Postgraduate Medical Journal 5(1):21-26.
Oliveria JBA, Petersen CG, Mauri AL, Vagnini LD, Baruff, RLR, Franco JG (2014). The effects of age on sperm quality: An evaluation of 1,500 semen samples. Journal Brasileiro de Reproducao Assistida 18:34-41.
Ozoko LE, Okpe O, Iju JW, Oyem JC (2018). Histomorphological effects of cotton seed oil on testes in adult male wister rats. Galician Medicine Journal 25(4):1-5.
Rafieian-Kapaei M, Beigrezaei SN, Kafeshani M (2017). Soy protein and chronic disease: An updated review. International Journal of preventive Medicine 8(105):1-6.
Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology 28:56-63.
Saijpaul S, Grewal RS, Ahuja CS, Naik PK (2006). Effects of supplementation of whole cotton seed on the performance and blood biochemical profile of cross bred cows in summer. Animal Nutrition and Feed Technology 6(1):95-102.
Serag El-Din, Batta H, Abd El-Azim, Abd El-Fattah N (2011). Effect of soybean on fertility of male and female albino rats. Journal of American Science 7(6):872-883.
Singh SK, Rath SK (1990). Histologic changes in the mouse testis after treatment with gossypol tetra-acetic acid. Arch Histology and Cytology 53(4):393-396.
Singla N, Garg MK (2013). The effect of crude cottonseed oil containing gossypol on fertility and estrous cycle of female *Bandicota bengalensis* grey and Hardwicke. Journal of Applied Animal Research 41(2):156-165.
Superchi P, Talarico L, Beretti V, Bonomi A (2016). Effect of dietary administration of oil extract from rosemary on reproductive efficiency in boars. Italian Journal of Animal Science 4(2):479-481.
Thomas KD, Caxton-Martins AE, Elujoba AA, Oyelola OO (1991). Effects of an aqueous extract of cotton seed (*Gossypium barbadense* Linn.) on adult male rats. Advances in Contraception 7(4):353-362.
Timurkaan N, Yilmaz F, Risvanli A, Timurkan H, Karadas E (2005). Effects of cotton seed flour on testicular structure and sperm motility of rats. Medycyna Wet 6(8):877-880.
Trishna D, Mishra S, Basak M, Shil D (2010). Evaluation of phytochemical screening and antifertility activity of *Curcuma aromatic* salisb. International journal of Pharmacetical science and research 1(1):18-22.
Wang H, Ran J, Jiang T (2014). Urea. Subcellular Biochemistry 73:7-30.
Zeriouh IF, Addou S, Bouferkas Y, Kheroua O, Saidi D (2014). Effect of the consumption of milk of soya on the male fertility of Swiss mice. International Journal of Pharmacy and Pharmaceutical Sciences 6(4):669-675.