https://dx.doi.org/10.4314/jeca.v20i1.7

Original Article

Effects of Aqueous Extract of *Glycine max* on Testis and Epididymis of Adult Male Wistar Rats

[1]D.E. Odiase; [1]O.D. Anigboro; [1]F.O. Tobalu

[1]*Department of Human Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Edo State, Nigeria.*

ABSTRACT

Glycine max has been a source of nutrients to humans and animals for decades. This study was aimed at investigating the effects of aqueous seed extract of *Glycine max* on the testes and epididymis of Wistar rats. Twenty adult male Wistar rats were randomized into four groups of five rats each. Group A served as control, groups B, C and D were administered 100; 200 and 400 mg/kg body weight of aqueous extract of *Glycine max* respectively for 56 days. Thereafter, the testes and epididymis harvested, weighed and fixed in Bouin's fluid for histopathological analysis. Semen was aspirated and analyzed for sperm motility, morphology and total count. Result from the study showed no significant difference ($P<0.05$) in the final body weight and testicular weight across the studied groups when compared to control. Also, there was no significant difference ($p>0.05$) in motility in all the groups administered with the extract. However, there was a significant decrease ($p<0.05$) in total sperm count in the group administered with 400mg/kg of the extract when compared to control. Histologically, the testis of rats treated with 400 mg/kg of *G. max* showed spermatogenic arrest. The epididymis was filled with spermatozoa in the groups administered 100 and 200mg/kg body weight of the extract showing features similar to the control group unlike group treated with 400mg/kg body weight of aqueous seed extract of *Glycine max*. In conclusion, *G. max* was found to have a dose-dependent antifertility effects on the treated animals.

Keywords: Antifertility, Hyperplasia, Morphology, Glycine max

INTRODUCTION

Some plants are natural medicinal herbs because they include a variety of phytochemical and antioxidant elements that contribute to their therapeutic capabilities (Gilani *et al.,* 2010). In this regard, a plethora of plant products have been synthesized and used for the treatment of a variety of disorders as well as the regulation of reproductive factors that affect the reproductive system in general (Sharifi-Rad *et al,* 2014).

One of such medicinal plants is *Glycine max*. It is commonly called Soya bean in various parts of the world. This plant has economical, ecological, medicinal and agricultural uses (Purcell *et al.,* 2014). Like most plants, soybeans grow in distinct morphological stages as they develop from seeds into fully mature plant (Purcell *et al.,* 2014). Soybean seeds come in a wide variety of sizes and hull colors such as black, brown, yellow, and green. Variegated and bicolored seed coats are also common (Purcell *et al.,* 2000). The use of soy in infant food is increasing and this is due to the publicity about the health promoting properties of soy. Concerns have recently been raised that soy isoflavone exposure may offer a developmental risk to newborns, particularly the reproductive system (Purcell *et al.,* 2000). Accordingly, this study is aimed at investigating the effects of *Glycine max* on the testes and epididymis of adult Wistar rats.

Address for Correspondence:

D.E. Odiase
Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Edo State, Nigeria
Email: daniel.odiase@uniben,edu;
+2348067106187

MATERIALS AND METHODS

Plant Material

Fresh seeds of Glycine max were obtained from the Faculty of Agriculture, University of Benin and were authenticated by the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. They were air dried at room temperature and then pulverized with a mechanical grinder into powder form. The powered form of the extract was soaked with distilled water for 24 hours with constant stirring. After 24 hours, it was filtered, the residues were discarded while the filtrates were turned into a container and concentrated in water bath of 45℃. Thereafter, the concentrated filtrates were preserved in the refrigerator.

Experimental Design

Twenty (20) adult male Wistar rats weighing between 168 to 200 g were used for this study. The animals were purchased and kept in the Animal House of the Department of Anatomy, University of Benin. The animals were allowed to acclimatize to the laboratory condition (temperature 24-28°C and 12-hours light-dark cycle) for fourteen days before commencement of the experiment with free access to rat chow (Top feeds Nigeria) and water *ad libitum* throughout the study period. The animals were randomized into a control group (A) and three treatment groups (B, C and D) with each group containing five rats. Rats in Group A served as control and received 1 ml of distilled water daily. Rats in groups B, C and D were administered 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of aqueous seed extract of *Glycine max* respectively. Oral administration of the extract was carried out daily throughout the experimental period which lasted for fifty-six (56) days. At the end of the experimental period, the animals were weighed and euthanized by chloroform anaesthesia.

Sperm Analysis

As the animals were being sacrificed, semen were collected from the vas deferens by ligating the vas deferens extremities. 6 µl of normal saline, previously heated to 37+2°C, were introduced to a sterile petri plate containing the ligated extremities. The extremities of the vas deferens were cut and placed on the sterile petri dish. The vas deferens was then teased to allow the sperm cells to swim into the Petri dish. The specimen was viewed under the microscope for the evaluation of semen parameters: sperm motility, sperm viability, sperm count and sperm morphology (Saalu *et al.,* 2008).

Sperm Motility

The motility was evaluated with regards to three variables: Progressively motile (PM), Non-progressive motility (NPM) and Immotile (IM) spermatozoa and it is usually expressed in percent. A drop of the semen was taken from the petri dish and dispensed on a clean grease-free slide and further covered with a transparent cover slip. The slide was placed on the microscope and viewed with the ×20 and ×40 objective lens (Atessahin *et al.,* 2006).

Sperm Morphology

The sperm cell morphology was assessed by staining the slide with the Improved Eosin and Leishman stain (Ibeh e*t al.,* 2018) and in accordance with the method used by Besley *et al.,* (1980). A drop of the sperm cells was dispensed on a grease-free clean slides and a smear was made. The slide was left to air dry. The slide was flooded with the Improved Eosin and Leishman stain for 15 minutes and rinsed and the back was blotted dry with cotton wool and left to air dry. The slide was examined under a microscope with ×100 objective lenses. The slide was viewed with at least 30 magnification fields, the normal and abnormal sperm cells were spotted and scored in percentage. Normal sperm shows a normal sperm characteristic of head, axoneme, middle piece and a tail. Abnormal sperm cells were characterized by large heads; headless, tailless, bulgy mid-piece, curved tail and joined head.

Sperm Count

The sperm count was determined as described earlier by Narayana *et al.,* (2005) with minor modifications, by first making a 1:20 dilution of the spermatozoa with 10% formal saline in test tube. Few drops of mixed solution were added into the chamber and viewed with the microscope using the ×10 objective lens. The sperm cells were counted and are scored in $10^6/mm^3$.

Statistical Analysis

The results obtained were statistically analyzed using descriptive and inferential statistics and reported as Mean ± Standard Error of Means (S.E.M) in tables. The statistical analysis was performed using Graphpad Prism version 7, manufactured by Dotmatic, California, USA

Histological Analysis

The testes and epididymis from the control and experimental groups were dissected out and fixed in Bouin's fluid. The tissues were processed using the routine methods for histological examination. Paraffin sections were stained with hematoxylin and eosin and qualitative microscopic examination was made.

RESULTS

Table 1 shows the comparison of initial and final body weights of animals in control group (A) and treatment groups (B, C and D). Analysis of data showed that there was an increase in the final body weight across the groups, although this increase was not statistically significant. Table 2 shows the testicular and epididymal weight changes across the treatment groups (B, C and D) when compared with control group. Analysis of data showed that there was no significant difference (p>0.05) in testicular and epididymal weights in the groups treated with 100 mg/kg, 200 mg/kg and 400 mg/kg *Glycine max* extract when compared to the control group. Table 3 shows the total sperm count, progressive motility, non-progressive motility, immotile, normal morphology and abnormal morphology of sperm in all the experimental groups. Analysis of data shows a significant decrease in total sperm count in rats treated with 200 mg/kg and 400 mg/kg extract of *Glycine max* when compared to control.

Histological Findings

Photomicrograph of histological sections of the testes of rats in Control (Plate 1; upper left) show the following features; tubules lined by spermatogenic series, interstitial cells of Leydig and Sertoli cells. Plate 1, upper right is the photomicrograph of the testes of rats treated with 100 mg/kg extract of *Glycine max* showing spermatogenic series and interstitial congestion. Plates 1, lower left is photomicrograph of rats treated with 200 mg/kg *Glycine max* extract showing relatively normal spermatocytes, while plate 1, lower left is the photomicrograph of rats treated with 400 mg/kg *G. max* extract showing spermatogenic cells and spermatogenic arrest. Plate 2, upper left is the photomicrograph of histological section of the epididymis of rats in control group displaying the following features; ducts lined by columnar epithelial cells and filled with spermatozoa. Plate 2, upper right is the photomicrograph of the epididymis of rats treated with 100 mg/kg *Glycine max* extract displaying features similar to histological findings in the control group. However, photomicrographs of the epididymes of rats treated with 200 mg/kg (plate 2, lower left) and 400mg/kg (plate 2, lower right) of *Glycine max* extract (Plates 7 and 8) respectively, show intact epididymal epithelial ducts lining with moderate degree of spermatozoa depletion in the lumen when compare to the control group.

Table 1: showing the initial and final body weights of rats in control and treatment groups.

	Initial body weight	Final body weight
Control	176.33±2.02	257.33±16.16
100 mg/kg Glycine max	187.00±3.60	242.33±9.20
200 mg/kg Glycine max	200.66±11.85	241.00±17.38
400 mg/kg Glycine max	168.33±10.17	215.66±13.93

Table 2: showing Testicular and Epididymal weights of rats in control and treatment groups

	Right testis weight	Left testis weight	Epididymis weight
CONTROL	1.2000±0.10	1.2333±0 .08	0.5333±0.06
100 mg/kg Glycine max	1.2000± 0.15	1.2000± 0.20	0.4667±0.03
200 mg/kg Glycine max	1.1667± 0.23	1.1333±0.22	0.6000±0.00
400 mg/kg Glycine max	1.2667± 0.03	1.2667± 0.03	0.4667± 0.03
P-value	0.970	0.937	0.139

Table 3: showing the total sperm count, progressive motility, non-progressive motility, immotile, normal morphology and abnormal morphology of rats in control and treatment groups.

GROUP	Total Sperm Count	Progressive Motility	Non-Progressive Motility	Immotile	Normal Morphology	Abnormal Morphology
Control	470.0 ± 10.00	56.7 ± 3.33	16.7 ± 3.33	26.7 ± 3.33	80.0 ± 0.00	20.0 ± 0.00
100 mg/kg *Glycine max*	463.3 ± 38.44	43.3 ± 12.01	16.7 ± 3.33	40.0 ±11.54	86.7 ± 3.33	13.3 ± 3.33
200 mg/kg *Glycine max*	280.0 ± 11.54*	46.7 ± 13.33	20.0 ± 5.77	33.3 ±14.52	80.0 ± 0.00	20.0 ± 0.00
400 mg/kg *Glycine max*	283.3 ± 20.28*	70.0 ± 0.00	13.3 ± 3.33	16.7 ± 3.33	80.0 ± 0.00	20.0 ± 0.00
P-VALUE	<0.001	0.239	0.728	0.411	0.052	0.052

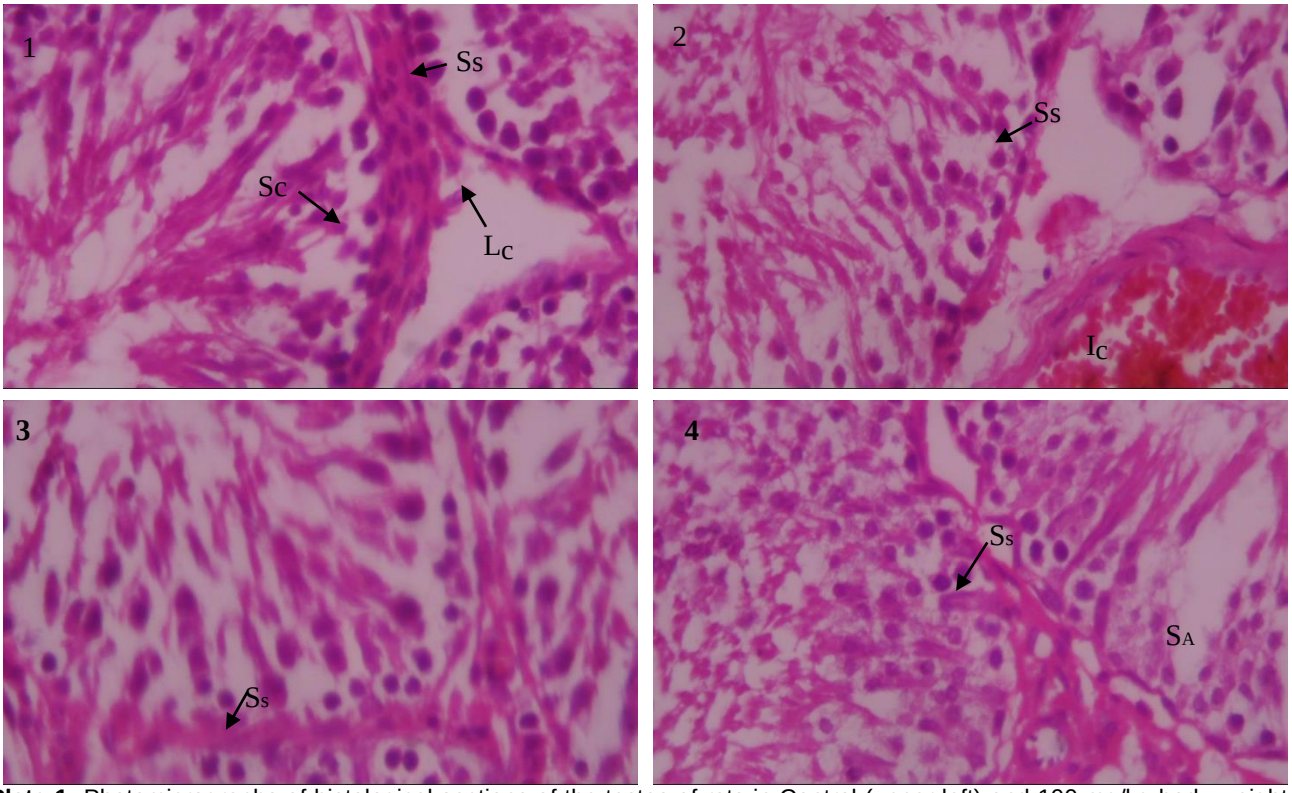

Plate 1: Photomicrographs of histological sections of the testes of rats in Control (upper left) and 100 mg/kg body weight (upper right), 200 mg/kg body weight (lower left) and 400 mg/kg body weight (lower right) of *Glycine max* extract*lycine max* extract, respectively. Features; tubules lined by spermatogenic cells (Ss), interstitial cells of leydig (Lc), Sertoli cells (Sc) and prominent blood vessel (Ic) and spermatogenic arrest (SA). H&E 400x

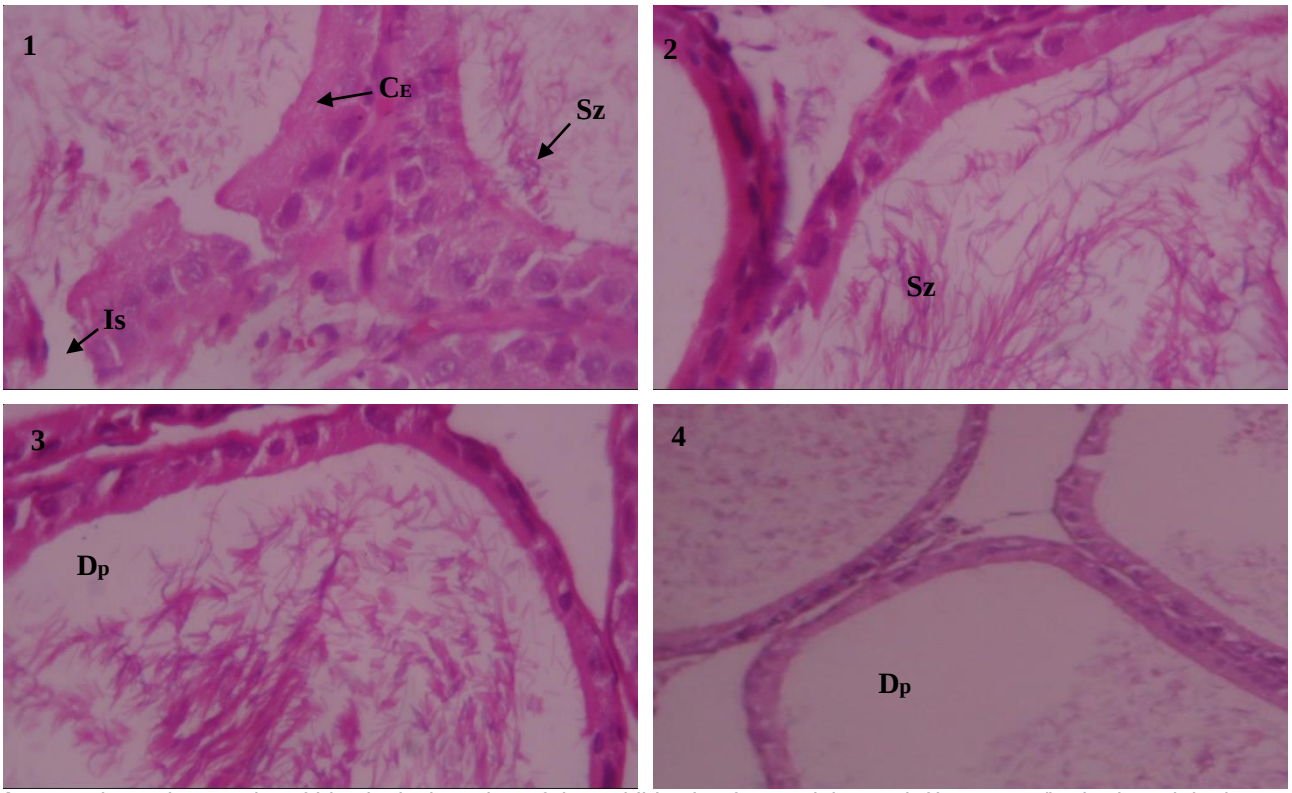

Plate 2: Photomicrographs of histological section of the epididymis of control (upper left), 100 mg/kg body weight (upper right), 200 mg/kg body weight (lower left) and 400 mg/kg body weight (lower right) of *Glycine max* extract respectively. Features; ducts lined by columnar epithelial cells (CE) filled with spermatozoa (Sz), interstitial space (Is) and region of partially depleted spermatozoa (Dp). H&E; 400x

DISCUSSION

The male reproductive system is a complex system which comprises the hypothalamus, anterior pituitary gland, the testes and epididymis. These structures work together to maintain potency, fertility and male secondary sexual characteristics (Sharpe, 1994). There is a surge in the world population and in a bid to keep the world's population under control, researchers have resorted to screening various botanicals for anti-fertility effects.

The current investigation found that aqueous seed extract of *Glycine max* had no effect on the body weight of rats treated with 100, 200 and 400 mg/kg body weight of the extract when compared to control. This observation is in contrast to the previous work done by Aryani *et al.,* (2019), who reported a significant increase in body weight of animals treated with *G. max* extract. Findings from this study showed that there was also no significant difference in the testicular weight across the groups treated with the aqueous seed extract of *Glycine max* when compared to the control group. Our finding is consistent with previous study by Ekaluo, (2013) who reported indifference in the testicular weights of rats treated with *G. max* extract. However, this is in contrast to the previous work done by Aryani *et al.,* (2019), who reported decrease in testicular weights of rats treated with *Glycine max.* They attributed this decrease in testicular weight to aberrant spermatogenic activity caused by the active ingredient in the extract. Testicular weight is influenced by the quantity of spermatogenic cells (Kianifard *et al.,* 2013), potentially as a result of fewer germ cells, suppression of spermatogenesis, and decreased activity of spermatogenic enzymes (Sakr and Al-Amoudi, 2012). The epididymal weights of rats treated with 100, 200 and 400 mg/kg of the extract were also indifferent when compared to control.

Findings from this study indicated that there was a significant decrease in sperm count in groups administered with 200mg/kg and 400mg/kg body weight of *G. max* when compared to the control group. Reports have indicated that treatment with plants which have shown anti-fertility properties will negatively affect some or all of the sperm parameters (Chinoy *et al.,* 1995). It has been proposed that extracts from these plants affect sperm physiological maturation by depleting androgen at the target level, especially in the cauda epididymis (Chinoy *et al.,* 1995). However, there was no significant difference in the sperm motility, sperm viability and sperm morphology of the rats treated with the extract when compared to control.

Histological analysis revealed that the testes of rats treat with 100 mg/kg of the extract showed normal histological features and the epididymal sperm reserve of the rats in this group were not different from the control. Also, the lumen of the epididymis of rats treated with the medium dose showed that the ducts were still filled with spermatozoa. This suggests that the medium dose of the extract did not directly affect the epididymal histoarchitecture. At high dose, the epididymis sections of rats revealed only a relative patent lumen with substantial depletion of developed spermatozoa. This may be related to the toxic effects of the extract on the spermatozoa content of the epididymis at high dose as unprocessed soya bean used in this study may have anti-nutritive properties which must have negatively impacted the luminal content of the epididymis. This concurs with earlier research done by Odum *et al*. (2001) and Atia *et al.,* (2023) who also reported spermatogenic arrest and depletion of mature spermatozoa in the lumen of the epididymis.

In conclusion, it may be inferred that *Glycine max* is probably spermicidal at high doses and can be utilized to induce infertility in male animals. However, further research should be conducted to demonstrate its mechanism of action of the seed extract of *Glycine max.*

REFERENCES

1. Aryani R, Manurung H, Moeljopawiro S, Nugroho LH, Astuti P (2019). The effect of methanol extract of soybean (Glycine max L. Merr.) on rat testicular steroid hormones. *J Phys Conf Ser* 1277(1): 012012.
2. Atessahin AI, Karahan G, Turk S, Yilmaz S, Ceribasi AO (2006). Protective role of lycopene on cisplatin induced changes in sperm characteristics, testicular damage and oxidative stress in rats. Reprod Toxicol 21: 42-47.
3. Atia MM, Yahia HM, Abdeltwab HS, Omar HEM (2023). Dietary soybean sauce impact testicular tissue of rat in dose duration dependent. Assiut Univ *J Multidiscip Sci Res* 52(1): 98-122.
4. Belsey MA, Eliasson R, Gallegos AJ, Moghissi KS, Paulsen CA, Prasad MRN (1980). Laboratory manual for the examination of human semen and semen cervical mucus interaction. Singapore: Press Concern.
5. Chinoy NJ, D'souza TM, Padman P (1995). Contraceptive efficacy of Carica papaya seed extract in male mice (Mus musculus). J Physiother 9: 30-36.
6. Ekaluo E (2013). Reproductive toxicity of soybean (Glycine max L.) in rat. IOSR IOSR J Environ Sci Toxicol Food Technol 3, 28-32E
7. Gilani A, Fujii Y, Sugano M, Watanabe KN (2010). Chemotypic variations and phytotoxic studies of essential oils of endemic medicinal plant, Seriphidium kurramense, from Pakistan. J Med Plant Res 4 (2010): 309-315

8. Ibeh NI, Otabor F, Oyedeji JT, Emili A, Omorodion NT (2018). Improvised Eosin and Leishman as Morphological Stain for Sperm Cell Analysis; Adult Male Wister Rats and Rabbit as a Model of Study. Acta Sci Microbiol 1(1): 18-20
9. Kianifard D, Hasanzadeh S, Kianifard L (2013). The study of time dependent administration of methylphenidate on the microscopic indices of spermatogenesis and spermanalysis inadults rats J.Exp. Integr.Med. 3(2): 121-5
10. Narayana K, Prashanthi N, Nayanatara A, Kumar HH, Abhilash K, Bairy KL (2005). Effects of methyl parathion (o,o-dimethyl o-4-nitrophenyl phosphorothioate) on rat sperm morphology and sperm count, but not fertility, are associated with decreased ascorbic acid level in the testis. Mutat Res 588: 28- 34.
11. Odum J, Tinwell H, Jones K, Van Miller JP, Joiner RL, Tobin G, Kawasaki H, Deghenghi R, Ashby J (2001). Effect of rodent diets on the sexual development of the rat. Toxicol Sci 61:115-127.
12. Purcell LC, King CA, Ball RA (2000). Soybean genotype differences in ureides and the relationship to drought-tolerant nitrogen fixation and manganese nutrition. Crop Sci 40:1062–1070.
13. Purcell LC, Salmeron M, Ashlock J (2014). Soybean growth and development. Arkansas Soybean Production Handbook. 1-8.
14. Saalu LC, Udeh R, Oluyemi KA, Jewo PI, Fadeyibi LO (2008). The ameliorative effects of grapefruit seed extract on testicular morphology and function of varicocelized rats. *Int J Morphol.* 26(4): 1059-1064.
15. Sakr SA, Al-Amoudi WM (2012). Effect of Ginger extract on deltamethrin induced histomorphological and immunohistochemical changes in testes of albino rats. J Life Sci 9(1): 771-8
16. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M (2014). *In vitro* assessment of antibacterial activity of *Salicornia herbacea* L. seed extracts against multidrug resistant grampositive and gram-negative bacteria. Int J Biol Sci 4 (6): 217-222.
17. Sharpe R (1994). Regulation of spermatogenesis. In: Knobil E., Neill J.D, editor. The Physiology of Reproduction. Raven Press, New York, 1363-1434
18. WHO (2010). Laboratory Manual for the examination and Processing of Human Semen 5th edition. 33.