

Histological Effect of Aqueous Garlic (*Allium Sativum*) Extract on the Testis and Ovary of an Adult Wistar Rat

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Abstract

Introduction: Garlic is one of the historical age-long spice used as food especially as condiments and therapeutic purposes. However, its effect on the reproductive health of man is sometimes not considered by man. This study is aimed at investigating the possible effect caused by consumption of aqueous garlic extract on the histology of the testis and ovary using adult albino wistar rat as a model.

Materials and Methods: A total of twenty four (24) adult wistar rats were used, comprising of twelve (12) males and twelve (12) females. They were randomly divided into four (4) groups, A, B, C, & D. Group A served as control while groups B, C & D were administered with aqueous garlic extract of 100, 200, & 300mg/kgs body weight for twenty one (21) days. Twenty four (24) hours after last administration of the extract, the animals were sacrificed for histological examination of the testes and ovaries.

Results: The body weight of the treated animals which significantly increased were compared to the control animals. Histological findings revealed dose dependent congestion of interstitial vessels of testes and ovaries administered with aqueous garlic extract, although other architectures appears essentially normal.

Conclusion: Garlic extract has a negative effect on interstitial vessels of both the testis and ovary, but has no effect on other components of the studied organs.

I. Introduction

In recent years, studies have shown the effect of several kinds of plants on the reproductive health of man and animals, where it is commonly used for therapeutic purposes or as condiments for cooking purposes (Ibe and Nwofu, 2005). *Allium sativum* commonly known as garlic is one of those plants used as biennial medicinal plant described in folklore of many cultures as a therapeutic agent (Amagase et al., 2001).

Allium sativum also known as garlic is a specie in the onion genus, *Allium* (Magaret et al., 1988). It's a bulbous plant that grows up to 1.2m (4ft) in height, it produces hermaphrodite flowers (Belman, 1983). The plant extract have been reported to have antifungal, antibacterial, anticancer and antiviral activities (Anderson, 2006). Studies carried out by El-Demerdash (2008), suggested that garlic has potential antioxidant, hypoglycemic, hypotensive and hypo-thrombotic properties. *Allium sativum* is widely used around the world for its pungent flavor as condiments or seasonings (Simonetti et al., 1990). Various formulation of garlic exist, which include raw garlic homogenate (Isaachsohn et al., 1998), garlic powder (Lawson et al., 1998), aged garlic extract (Banerjee et al., 2002), etc. Aqueous extract of garlic is a form of raw garlic homogenate (Aqel et al., 1991). Garlic contains some active composition of chemicals which include sulfur composition and non-sulfur compositions. The sulfur compositions are; allicin, diallyldisulphide (DADs) and diallytrisulphide (DATs) which are responsible for most of garlic therapeutic properties, while the non-sulfur composition include alixin, flavonoids, saponin and fructans (Reuter et al., 1996; Silvam, 2001).

The testes are the primary reproductive organ or gonad in the male. They are ovoid reproductive and endocrine organs responsible for sperm production. An average testicular dimension are 4-5cm in length, 2.5cm in breadth, and 3cm in thickness. Their weight varies from 10.5-14g and the left testis usually lies lower than the right testis (Gray, 2005). Histologically, the capsule is surrounded by a thick capsule of dense connective tissue, the tunica albuginea. The tunica albuginea is thickened on the posterior surface of the testis to form the mediastinum testis from which fibrous septa penetrate the gland, dividing it into about 250-300 pyramidal compartments, called the testicular lobules. Each lobule is occupied by one to four seminiferous tubules where sperm are produced and it's lined with a complex, specialized stratified epithelium (Anthony, 2010). The primary function of the testis is to produce sperm (spermatogenesis) and to produce androgens, primarily testosterone.

Research work on the effect of garlic extract on the testis have been extensively studied. Garlic was found to be effective in the recovery of testicular functions and spermatogenesis in rats (Ksasuga et al., 2001). Another study was carried out by Hammami et al., (2013) and he observed that the seminiferous tubules of rats treated with garlic fractions showed an increased number of seminiferous tubules deprived of spermatozoa which is at variance c to Kasuga's findings.

The ovary is an ovum reproductive organ that is analogous to the male testes. Each ovary is whitish in color with an average diameter of 3cm in length, 2cm in breadth, and 1cm in thickness (Kinsky, 1971). There are two extremities to the ovary; the end to which the fallopian tube attaches is called the tubal extremities and the ovary is connected to it by the infundipulopelvic ligament (Darftary et al., 2011). The other extremity is called the uterine extremity. It points downward and it's attached to the uterus via the ovarian ligament (Darftary et al., 2011). Histologically, each ovary is covered by a simple cuboidal epithelium (Anthony, 2010). The tunica albuginea like that of the testis is responsible for the whitish color of the ovary (Hansen et al., 2008). The function of the ovary is analogous to that of the testis; produces ovum and secretes androgens (Oktay, 2008). Rajiet al., (2012) reported that garlic extract have no deleterious effects on the reproductive performance of female albino rats. Animals treated with garlic extract got pregnant and there were no abortion of the pregnancy. This study was done to investigate and ascertain the possible effect of aqueous extract of *Allium sativum* (garlic) on the histology of the testis and ovary using adult wistar rat as model.

II. Materials And Methods

Experimental Animals

This experiment was carried out on twenty-four (24) adult albino wistar rats weighing between 150g-200g. The animals were obtained from the animal house breeding unit of the college of Basic Medical Sciences, Delta State University, Abraka, Delta State. The animals were randomly assigned into four (4) groups. The animal's room was adequately ventilated and kept at room temperature, with a 12hours natural light-dark cycle. Animals were fed ad libitum with commercially prepared rat feed and water. Good hygiene was maintained by constant cleaning and removal of feaces and spilled water/feed from the cages daily. Ethical approval was sought and obtained from Ethics and Research Committee of the Department of Human Anatomy and Cell Biology, Delta State University, Abraka.

Collections and Preparation of Aqueous Garlic Extract

Fresh garlic bulbs were purchased from Abraka central market, Abraka, Delta State and was taken to Botany Department of Delta State University, Abraka for identification and authentication of the plant. The identified garlic was taken to the Department of Pharmaceutical Chemistry Laboratory of the faculty of Pharmacy, Delta State University, Abraka for extraction. Cold maceration (soaking) method was used to prepare the extract (Tatara, et al., 2005). The garlic was peeled off, washed and weighed using a digital weighing balance. The weighed garlic was pounded in a clean mortar with pestle into a paste. The pounded garlic was soaked in distilled water for 48hours (steering at intervals for proper mixing). After 48hours, it was sieved into a conical flask using a clean sieve and a filter paper. The filtrate was taken to the rotary evaporator to remove excess amount of water, at a temperature of 60-80°C leaving behind the crude active in the flask. The crude active was further concentrated using the oven at a temperature of 30-40°C. A stock solution of 100, 200, 300mgs/kg was prepared from the concentrated paste-like garlic extract. (Uvieghara, 2014). The stock solution was kept in the refrigerator at a temperature of 4°C till use.

Experimental Design

Twenty-four (24) adult wistar rats of twelve (12) males and females each, with an average weight of about 150g-200g. They were randomly grouped into four (4) groups (1, 2, 3 & 4) of six (6) rats (comprising 3 males & 3 females) per group as follows;

Group 1 ----- control group given distilled water and rat chow.

Group 2 ----- Received 100mg/kg body weight of aqueous garlic Extract, rat chow and water.

Group 3 ----- Received 200mg/kg body weight of aqueous garlic Extract, rat chow and water.

Group 4 ----- Received 300mg/kg body weight of aqueous garlic Extract, rat chow and water.

Distilled water and feed were given to all groups but only groups 2, 3 & 4 were administered with the extract for 21days.

Extract Administration

The extract was administered orally using an orogastric cannula. The extract was administered once daily between the hours of 10:00 am and 12:00 pm for a period of 21days.

Animal Sacrifices and Sample Collection.

Twenty-four (24) hours after the last day of administration, the animals were sacrificed by cervical dislocation. The animal was placed on a flat board in a supine position and dissected open using a scalpel and blade. The testes and ovaries were harvested and fixed in 10% formal saline fixative.

Histological Technique (Tissue Processing)

The testes and ovary were fixed in 10% formal saline, blocks embedded in paraffin and sections cut at 5 micron which was then stained with H&E and mounted with DPX . Microscopic examination of the sections was then carried out under a light microscope.

III. Results

Body Weight

Table 1 Changes in body weight of wistar rats treated with aqueous extract of garlic.

	GROUP 1 (CONTROL)	GROUP 2 (100mg/kg)	GROUP 3 (200mg/kg)	GROUP 4 (300mg/kg)
DAY 1	152.00 ± 2.280	175.67 ± 24.40	139.67 ± 24.40	176.67 ± 26.88
DAY 7	169.50 ^a ± 8.09	172.17 ^a ± 23.46	138.33 ^a ± 26.54	153.50 ^a ± 33.61
DAY 14	175.50 ^b ± 12.03	190.83 ^b ± 27.46	160.00 ^b ± 45.49	161.67 ^b ± 33.71
DAY 21	180.00 ^c ± 12.62	203.00 ^c ± 32.25	160.00 ^b ± 45.49	161.67 ^b ± 33.71

Values are expressed as Mean ± SD. Values on the same column with different superscripts, differ significantly (P<0.05).

From table 1, the final body weight of the control animals (group 1) significantly increased (p<0.05), when compared with the initial body weight. There was an observed reduction in weight of 300mg/kg of the extract treated animals (group 4), not significant (p<0.05), when compared with the initial and final body weight. The body weight of the 100mg/kg of extract treated rats (group 2) significantly increased (p<0.05) when initial and final body weights were compared. Also, there as an increase in the body weight of the animals treated with 200mg/kg of extract (group 3), but not significant (p<0.05).

Photomicrographs Of The Testes

Figure 1: Group 1 (control).

Sections of the testis shows an essentially normal general architecture with numerous seminiferous tubules with spermatogenic series at different level of maturation (H&E X100).

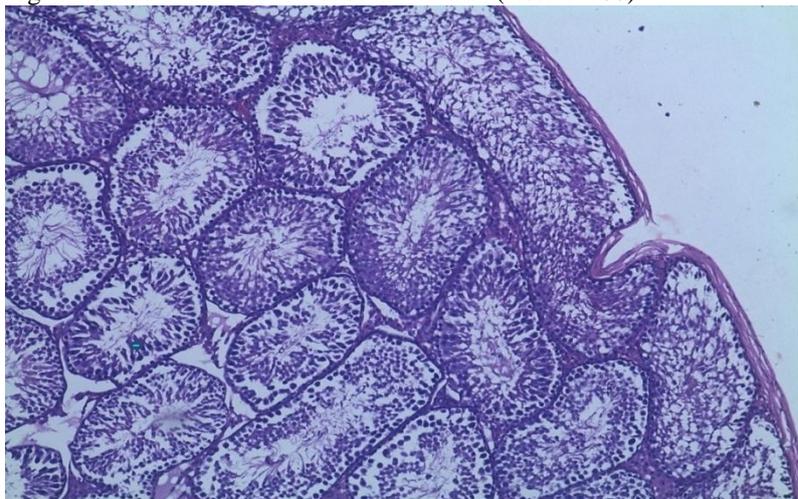


Figure 2 Group 2 (received 100mg/kg body weight of garlic extract)

Section of the testis shows numerous seminiferous tubules at various stages of maturation with a normal germ cell and leydig cells and congestion of interstitial vessels seen. (H&E X100).



Figure 3 Group 3 (received 200mg/kg body weight of garlic extract)

Section shows numerous seminiferous tubules with spermatogenic series at different level of maturation. The germ cell, leydig cells appear normal. Presence of congested interstitial vessels. (H&E X100).

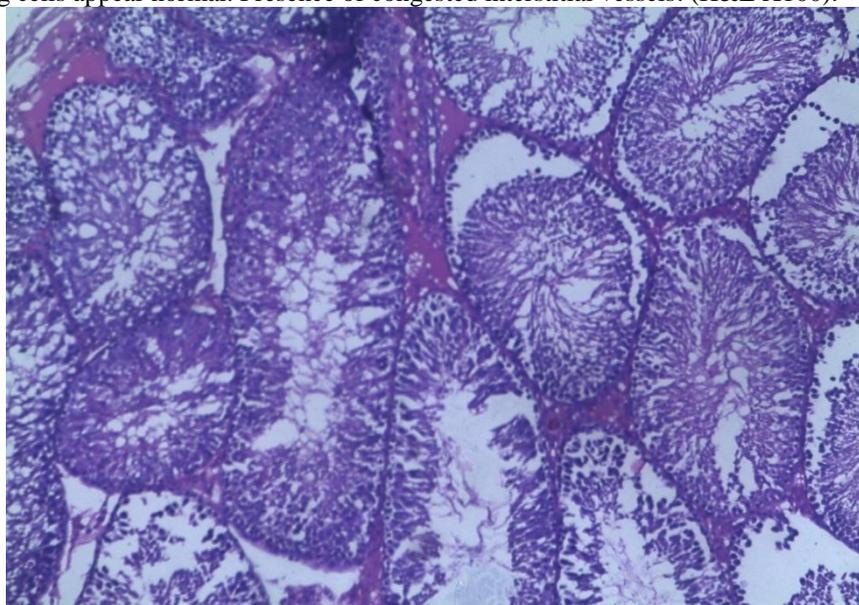
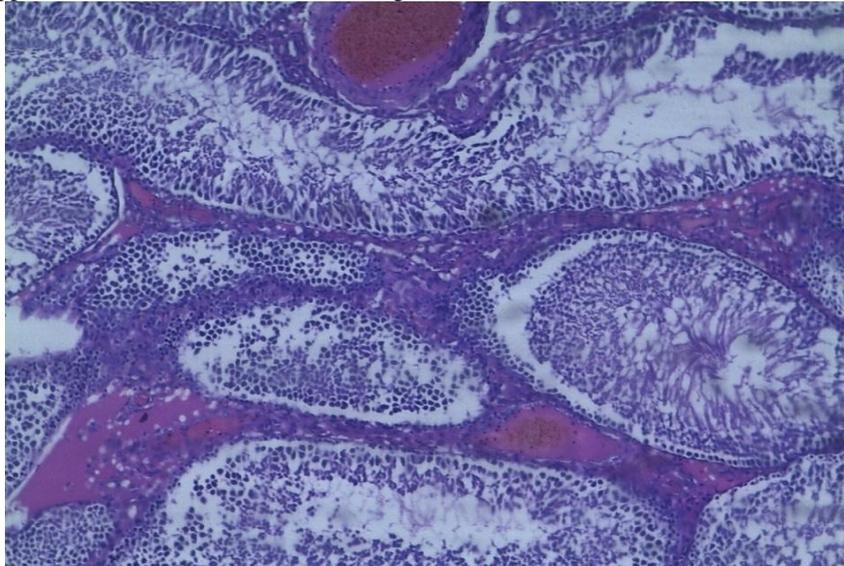


FIGURE 4 Group 4 (received 300mg/kg body weight of garlic extract).section Shows numerous seminiferous tubules with spermatogenic series at different level of maturation. The germ cell, leydig cells appear normal. There is increased congestion of interstitial vessels. (H&E X100).



Photomicrograph of the Ovaries

FIGURE 5 Group1 (control).

Micrograph shows an ovarian tissue with follicle at different stages of maturation around the cortical region. The medullary region is composed of blood vessels, nerves and lymphatic. Tissue is supported by dense connective tissues. General architecture essentially normal. (H&E X100)

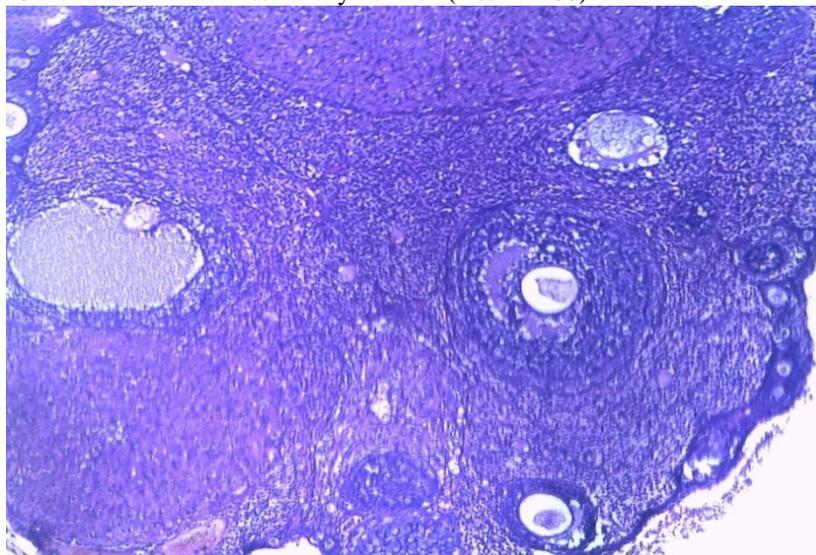


FIGURE 6 Group2 (received 100mg/kg body weight of garlic extract) Micrograph shows an ovarian tissue with follicle at different stages of maturation around the cortical region. The medullary region is composed of blood vessels, nerves and lymphatics. Tissue is supported by dense connective tissues. No congestion seen. (H&E X100)

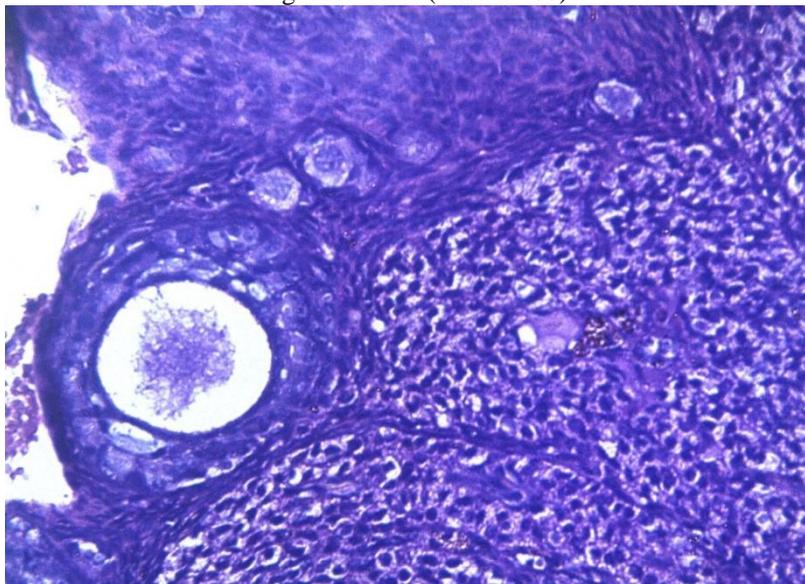


FIGURE 7 Group3 (received 200mg/kg body weight of garlic extract) Micrograph shows an ovarian tissue with follicle at different stages of maturation around the cortical region. The medullary region is composed of blood vessels, nerves and lymphatic. Tissue is supported by dense connective tissues. However, foci of congestion was seen around the cortical region. (H&E X100)

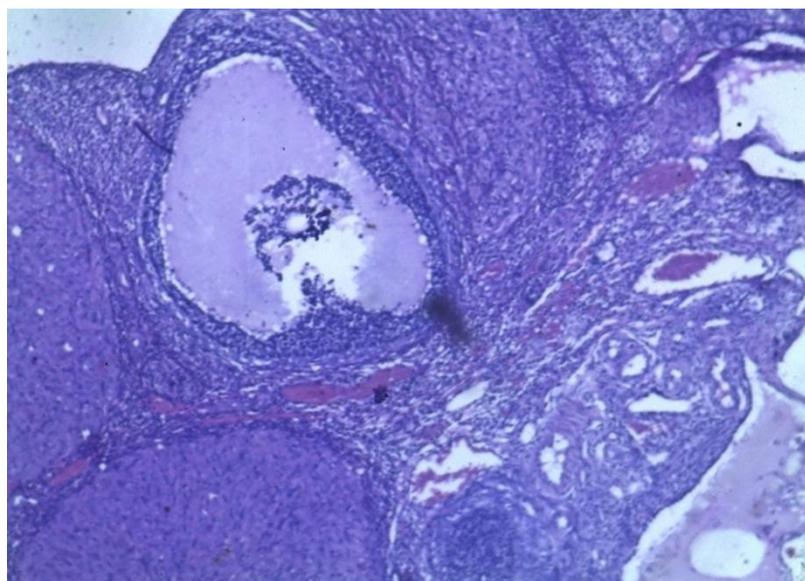
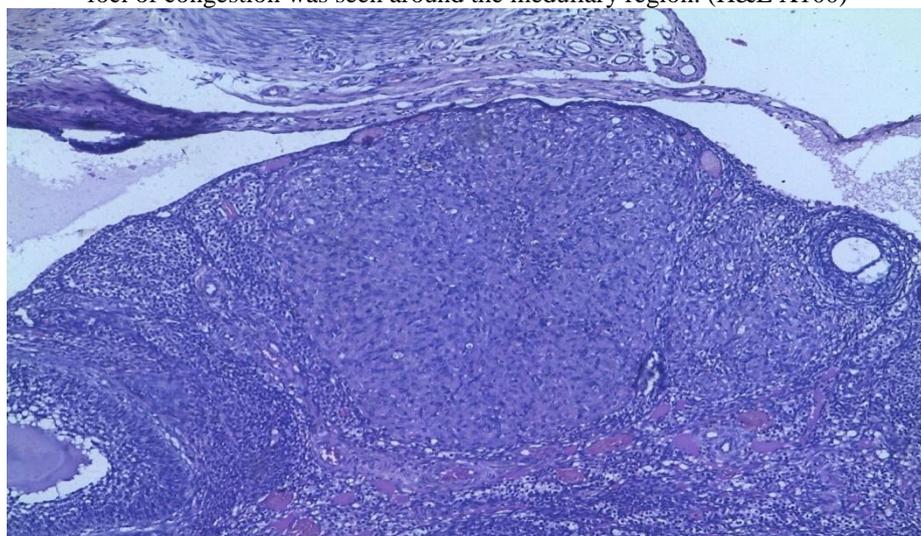


FIGURE 8 Group 4 (received 300mg/kg body weight of garlic extract) Micrograph shows an ovarian tissue with follicle at different stages of maturation around the cortical region. The medullary region is composed of blood vessels, nerves and lymphatic. Tissue is supported by dense connective tissues. However, foci of congestion was seen around the medullary region. (H&E X100)



IV. Discussion

The present study showed that the result obtained from the testis photomicrograph of figure 1 group 1 (control), revealed the testis with numerous seminiferous tubules with spermatogenic series at different level of maturity. The germ cells, leydig cells and general architecture appeared essentially normal. However, the testis micrographs of groups 2 & 3, i.e. figures 2 & 3 administered with 100 & 200mgs/kg body weight of garlic extract revealed a normal histological seminiferous tubules, germ cells and leydig cells. This means that there was no significant histological changes of the seminiferous tubules, which correspond with the findings of Kasuga et al., (2001) who observed that garlic is effective in the recovery of testicular function and spermatogenesis in rats. Since this experiment was carried out on healthy rats the seminiferous tubules were unchanged. However, the photomicrograph of groups 2 & 3 (figures 2 & 3) revealed a significant congestion of interstitial vessels with normal seminiferous tubules. In group 4 (figure 4) dosed with 300mg/kg body weight of garlic extract showed no histological distortion on the seminiferous tubules and leydig cells, except for the interstitial vessels that is highly congested.

The statistical analysis (table 1) obtained, showed that the final body weight of the control animals (group 1 i.e. figures 1 & 5) significantly increased ($p < 0.05$), when compared with the initial body weight. Also, there was a significant increase ($p < 0.05$) in the body weight of group 2 (figures 2 & 6) administered with 100mg/kg of the extract. But the mild increase in the body weight of groups 3 & 4 (figures 3, 4, 7 & 8 respectively) were not significant ($p < 0.05$). This results agrees with that of Behnaz (2014), which induced weight loss in experimental rats using radiation and noted a reversal, that is, significant weight gain on administration of garlic extract; showing that garlic was able to prevent the weight loss caused by radiation.

These results obtained revealed that aqueous extract of garlic (*Allium sativum*) have no significant harmful effect on the seminiferous tubules/testicular functions. This is in consonance with Kasuga et al., (2011) whose studies revealed that garlic is effective in the recovery of testicular function and spermatogenesis in rat. It is also in line with Odumosu et al., (2013) who revealed in their study that aqueous extract of garlic, onion and aridan reversed the testicular shrinkage/reduction in the rate of spermatogenesis caused by induced testosterone and estradiol hormones. From this present study, it is suggested that the mechanism behind the reversed effect observed by Kasuga (2001) and Odumosu et al., (2013) might be brought about by the increased supply of nutrient rich blood to the testis as seen in the micrographs of the groups administered with garlic extract. "Figures 2-4".

This research is not in agreement with Hammami et al., (2008) on the inhibitory effect on adult male reproductive function of crude garlic. They stated that testicular histology showed a dose dependent increase in the percentage of empty seminiferous tubules. They also said that crude garlic consumption affect testicular function and altered spermatogenesis. The reason for the difference, could be because of the longer duration and higher concentration of doses administered to the rats by Hammami et al. It is also contradicting the study of Hammami et al., (2013). They treated the rat with garlic fraction precipitated with ethanol. They observed that the seminiferous tubules were deprived of spermatozoa. In addition, they stated it induced apoptosis of testicular

germ cells and decreased seminiferous tubules DNA concentration. The reason for this findings, could be because of the product (ethanol) used for making the extract.

This current study also revealed that administration of garlic extract have no harmful effect on ovary. Histological findings revealed a normal, primary and secondary and graffian follicle in the control and dosed rats. This is also similar to the findings of Raji et al., (2012) stating that aqueous garlic extract have no deleterious effect on the reproductive performance of female rats. The findings of this study did not reveal any significant toxic effect of aqueous garlic extract on the dosed rats. Though congestion of the interstitium that was increased with higher doses of the extract was noticed. These congestions (seen in the testes & ovaries) could be due to increasing capillary hydrostatic pressure pushing fluid into the interstitial space: now since the testes can be divided into two functional units, the interstitium and the seminiferous tubules. The interstitium forms the path way for blood and lymph circulation and any congestion here may eventually cause damage albeit not yet noticeable in this present study, but may be similar to that in the study by Hammami et al., (2008).

V. Conclusion

Conclusively, the evaluation of the possible effect of aqueous extract of garlic (*Allium sativum*) did not record any histological distortion or damages on the seminiferous tubules, germ cells, leydig cells ovarian follicles of testes and ovaries respectively. But there were congestion of interstitial vessels seen in the testis and ovary which are relatively new. Also the significant increase in weight ($p < 0.05$) of the dosed groups as compared with the control suggests that the histological functions of the ovary and testis were unaffected. But, the congestion of the interstitial vessels could also have contributed to the weight gained.

VI. Recommendation

It is recommended that more research on this area should be carried out using longer days and higher doses and electron microscope should be used where the cells of the organ can be studied in detail.

References

- [1]. Amagase H., Petesh B. L., Matsuura H. (2001). Intake of Garlic and Its Bioactiv Components. *Journal of Nutrition*. 131 (3): 9555-9625.
- [2]. Amagase H. (2006). Significance of Garlic and its Constituents in Cancer and Cardiovascular Disease. *Clarifying the real bioactive constituents of garlic. Journal of Nutrition*. 136: 716 – 725.
- [3]. Anthony L. M., (2010). Junqueira's Basic Histology Text and Atlas, 12th edition: 371-389
- [4]. Aqel M. B., Gharaiabah M. N., Salhab A. S. (1991). Direct relaxant effect of garlic juice on smooth and cardiac muscle. *Journal of Entropharmacol*. 33:13-19.
- [5]. Banerjee S. K., Maulik K. (2002). Effect of garlic on cardiovascular disorders: *Nutritional Journal*: 1-4.
- [6]. BehnazHajjion (2014). Effect of Garlic (*Allium sativum*) Hydroalcoholic Extraction on Estrogen, progesterone and testosterone levels in rats exposed to cell phone radiation. *Zahedan Journal of Research in Medical Sciences*. 16: 1.
- [7]. Belman S. (1983): Onion and garlic oils inhibit tumor promotion. *Carcinogenesis*. 8:1063-1065.
- [8]. DaftaryShirish, ChakravartiSudip. (2011). *Manual of obstetrics*. 3:1-18.
- [9]. EL-Demerdash F.M. (2008). Dietary garlic and onions reduced the incidence of theieogenic diet-induced cholesterol gallstones in experiment mice. *British Journal of Nutrition*. 5:1-9.
- [10]. Hammami I., Nahdi A., Mauduit C., (2008) .The inhibitory effects on adult male reproductive functions of crude garlic (*Allium sativum*) feeding. *Asian Journal of Androl*. 10 (4): 593-601.
- [11]. Ibe A.E and Nwufu M.I. (2005). Identificaion, collection and demonstration of medicinal plants in Southeastern Nigeria. *Council for the Development of Soc. Sci. Res. Africa*. 3: 66-77.
- [12]. Isaachsohn J.L., Moser M., Stein E.A., Dudley K., Davey J.A., Liskov E., Black H.R. (1998). Garlic Powder and Plasma Lipids and Lipoproteins: a multicentered randomized, plactable-controlled trial. *Arch intern. Med*: 176-209.
- [13]. Kasuga S., Uda N., Kyo E., Ushijima M., Morihara N. Itakura Y. (2001). Phamacologic activities of aged garlic extract in comparison with other garlic preparations. *Journal of Nutrition*. 131:10805-10845.
- [14]. Kinsky F.C. (1971). The consistence presence of paired ovaries in the Kiwi (*Apteryx*) with some discussion of this condition in other birds. *Journal of Ornithology*. 112(3). 334-357.
- [15]. Kodera Y., Matuura H., Yoshida S., Sumida T., Hakura Y., Fuwa T., Nishino H. (1989). "Allixin, a stress compound from garlic".
- [16]. Margaret Steentoft. (1988). *Flowering plants in West Africa*; Cambridge University.
- [17]. Odumosu F.M., Olokodana O., Huthman I.O., Akinyemi R.A., Adesanya O.A., Adefule A.K: (2013); Effect of Onion, Garlic and Aridan Aqueous Extract on Hormone Influenced Testis Weight of Adult Wistar Rats. *International Journal of Chemical and Pharmaceutical Research*. 2:9.
- [18]. Oktay K., Oklem O. (2008). Ovarian cryopreservation and transplantation for Fertility preservation. For medical indications; report of an ongoing experience; fertile. *Steril*. 93(3): 762-8.
- [19]. Raji L.O., Fayemi O.E., Ameen S.A., Jagun A.T. (2012). The Effect of Aqueous Extract of *Allium sativum* (Garlic) on Some Aspect of Reproduction in the Female Albino Rat (Wistar Strain). *Global veterinarian*. 8 (4): 414-420.
- [20]. Reuter H.D., Koch H.P., Lawson L.D. (1996). Therapeutic Effect and Applications of Garlic and its Preparation. In: *Garlic*: 135-213.
- [21]. Silvam G.P., (2001). Protection against *Helicobacter pylori* and other bacterial Infections of Garlic and its preparation. *Journal of nutrition* 13:1106-1118.
- [22]. Simonetti G., Schuler S. (1990). *Simon & Schuster's Guide to Herbs and Spices*. Simon & Schuster, Inc. 255.
- [23]. Uvieghara K.E. (2014). Procedures of Extraction of Garlic Extract. Faculty of Pharmacy, Department of Pharmaceutical Chemistry laboratory. Delta State University, Abraka.