IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 17, Issue 4 Ser. II (April 2023), PP 36-43
www.iosrjournals.org

Effect Of *Allium Sativum* On Testicular Functions Of Male Swiss Albino Mice

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Abstract
Male infertility is on continuous rise worldwide. People from different communities across the globe are more inclined to use medicinal plants for treating various diseases including male infertility. Allium sativum (garlic) is a common medicinal plant used to treat a variety of diseases. Though it is known to increase virility of male, yet some studies on animal model have shown its adverse effect on male reproductive system which has raised questions about its usage as a potent agent to cure male reproductive disorder. Hence, this study was conducted to find the effect of different doses of garlic on sperm quality and serum testosterone level in male Swiss Albino mice

Keywords : *Male infertility, Male reproduction, Medicinal plant, Allium sativum (garlic), Sperm, Testosterone*

Date of Submission: 16-04-2023 Date of Acceptance: 29-04-2023

I. Introduction

Scientific studies in recent time have focussed much on various health benefits of medicinal plants. People prefer to consume natural antioxidant compounds compared to the synthetic ones. These naturally derived products are safe to consume because of the least side effect. Also, they are cost effective compared to modern medicine. *Allium sativum* (Garlic) is one of the most common food material consumed worldwide. The edible portion of garlic is the bulb which is composed of a bunch of cloves (1, 2, 3). It contains many useful substances like minerals, carbohydrates, proteins and vitamins. Vitamins A, B, C and E along with riboflavin, thiamine, nicotinic acid forms the constituent of garlic. Various studies conducted on garlic have found that the sulphur compounds present in them are mainly responsible for its pharmacological effect (4, 5, 6).

Garlic is well recognised for its medicinal attributes since ancient times. (7).Garlic has a wide range of benefits and is used as antimicrobial, hypolipidemic, antidiabetic and anticarinogenic agent. Its antioxidant properties help to eliminate free radicals from the body (8.9,10,11,12,13)

There are many studies which prove the beneficial effect of garlic on male reproductive system. However, these studies were conducted as curative agent against chemical toxicity or any disease induced in animal model. There are few studies conducted to study different doses of garlic on animal model. Among them, some reported positive impact and few have shown negative impact of garlic on male reproductive system. The studies which showed negative effect of garlic on male reproductive system have used chronic exposure of garlic with comparatively higher doses per animal (14,15).

Male fertility has declined in the world in the last few decades as per the studies reported in different countries .These studies have been supported in major by decline in the sperm quality. Assisted Reproductive Technology (ART) has gained importance in the recent times among couples seeking children to overcome the fertility problems diagnosed in them. However, these procedures are costly and sometimes positive results are not achieved**.** Herbal remedies are preferred by people for treatment of infertility (16,17).

The aim of the present study was to find the impact of different amount of garlic on the sperm quality and serum testosterone level in male Swiss albino mice.

II. Methodology

Plant extract preparation

Allium sativum used in this experiment was purchased from the vegetable market of Patna, Bihar. Each day fresh clove of garlic was peeled and crushed using distilled water with the help of motor pestle. The extract was transferred in glass bottle and was administered to the experimental animals within 30 minutes.

Animal experiment

The animal work was conducted in accordance to the norms provided by CPCSEA, New Delhi, India. The ethical committee of Mahavir Cancer Sansthaan and Research Centre, Phulwarisharif, Patna has approved this study (1129/PO/ReBi/S/07/CPCSEA). Care was taken while handling the animals to minimize the stress and pain. Twelve male Swiss albino mice, three months old, weighing 28gm to 32 gm were obtained from breeding sections and kept in propylene cages for acclamatization for a week. The room where mice were placed maintained at 22°C to 34°C. Mice were randomly divided in 3 groups of 4 mice in each cage. They were provided fresh drinking water and laboratory prepared chow as per recommended diet. There was free access to food and water.

The four groups of mice were divided as Control, AS 200 and AS 400. The control received 0.5 ml distilled water while AS200, AS-400 received 200mg and 400mg/kg body weight of aqueous *Allium sativum* orally for 45 days.

Body weight and testicular weight

Each animal was weighed each day before the administration of dose. The testicular weight was taken on the completion of experiment after 45 days. After 45 days, the animals were euthanized and testes were removed and weighed.

Sperm count, Sperm motility and sperm morphology

Collection of sperm

Testes were kept in 0.9% Normal Saline (NS) and punctured in cauda epididymis. The sperms oozed out and formed a suspension. The suspension was transferred in tube. The suspension was made in 2ml NS. Further dilutions were done as per the requirement for observation of sperm quality.

Collection of blood and obtaining serum

The blood collected was kept at room temperature for 30 minutes. Thereafter, the blood was centrifuged at 3000 rpm for 15 minutes. The serum obtained after this was stored in the refrigerator at -20°C for testosterone and biochemical assay.

Sperm count

For sperm count, hemocytometer was used to count the sperms under light microscope. Sperms were calculated as sperms in million/ ml.

Sperm motility

Sperm suspension was kept on hemocytometre and observed under microscope to find the motile sperms. 200 sperms were observed for each animal and motility was counted as percentage.

Sperm morphology

Sperms stained with Eosin Yellow (Eosin Y) only dissolved in NS were observed under light microscope to find the normal morphology of sperm. A good sperm was categorized by intact head with proper acrosome hook and uncoiled tail and unbent mid-piece. Abnormal sperms were categorised as headless, bent mid-piece, hooked tail, coiling of whole tail, damaged acrosome, narrow head.

The procedure for sperm parameters was applied as mentioned in previous studies (18,19, 20, 21).

Serum Testosterone

Testosterone in serum was estimated through ELISA method using a kit manufactured by Abia and procured from Angel Scientific, Patna. Testosterone level was calculated as ng/ml.

Total cholesterol level in serum

Total cholesterol in serum was estimated through kits manufactured by Coral and result was obtained as mg/dl.

Statistical Application*:*

All the results were analysed using Graph pad Prism software. Mean of the four samples in each individual test were represented as ±SD. Tukey's Test was applied to get the result analysed through Analysis of Variance (ANOVA). $P<0.05$ was considered as significant.

III. Result

Weight of Body

The timely observation of the body weight of experimental animals did not showed any drastic decrease in weight. However, when the difference in body weight between those of the first day of the dose administration and the last day of experiment was calculated, there was lesser gain of body weight in AS400.(Table-1).

	Initial body weight(gm)	Final body weight(gm)	Difference in body weight(gm)
CONTROL	31.0±.0.8	35±1.5	4
AS200	30.75±1.5	33.75±0.95	3
AS400	31.75±1.2	33. ±.25	2.75

Table1: Initial and Final body weight (gm) represented as ± S.D of three groups Control, AS200, AS400 (n=4)

Weight of Organs

The weight of reproductive organs, testes, seminal vesicles and prostate glands are represented in Table-2. There was non significant decrease in weight of testes and prostate gland after 200mg and 400mg of *Allium sativum* treatment for 45 days. However, the weight of seminal vesicle increased significantly in AS-200 ($p<0.01$) and non significantly in AS-400.

	Testes(gm)	Seminal vesicles(gm)	Prostate (gm)
CONTROL	211±6	345±4.5	51±2.5
GARLIC200	208±8.1	356±5.1*	50±3.6
GARLIC400	207±5.4	350±4.5	49±3.7

Table2: Weight of testes, seminal vesicles and prostate of groups Control, AS-200 and AS-400 depicted in milligram ±SD

Sperm parameters:

Sperm count

The number of sperms obtained from epididymis increased in AS-200 but decreased in AS-400. However, both the reductions were non significant ($p<0.06$). There was a non significant increase in sperm motility of AS200 and AS-400($p<0.06$).

Sperm motility

There was a gradual increase in sperm motility in AS-200 and AS-400 but non significantly.

Sperm morphology

There was a non significant increase in percentage of normal sperm morphology in AS-200. The percentage of normal sperm morphology decreased in AS-400 significantly with respect to AS-200 ($p<0.01$) but non significantly with respect to control.

	Sperm count($\times 10^6$)	Sperm motility(%)	Normal Sperm morphology(%)
Control	12.5±1.2	64±8	61±6.2
AS-200	14.3±1.7	73±5	68±5.8
As-400	11.5±61.3	77±6	53±6.1*

Table 3: Sperm count, Sperm motility and sperm morphology of three groups represented in million/ ml sperm suspension, Percentage of Motile sperms and percentage of sperms with normal morphology. (* = $p<0.01$)

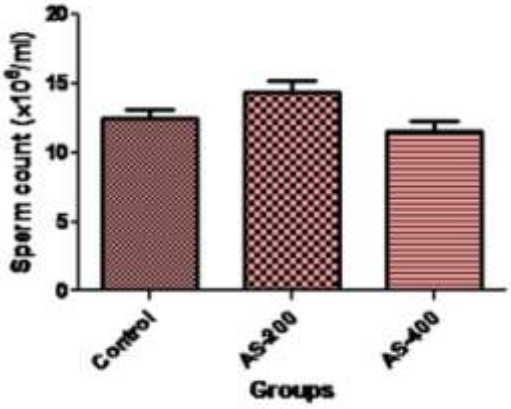

Figure1: Graphical representation of sperm count in million per ml of sperm suspension.

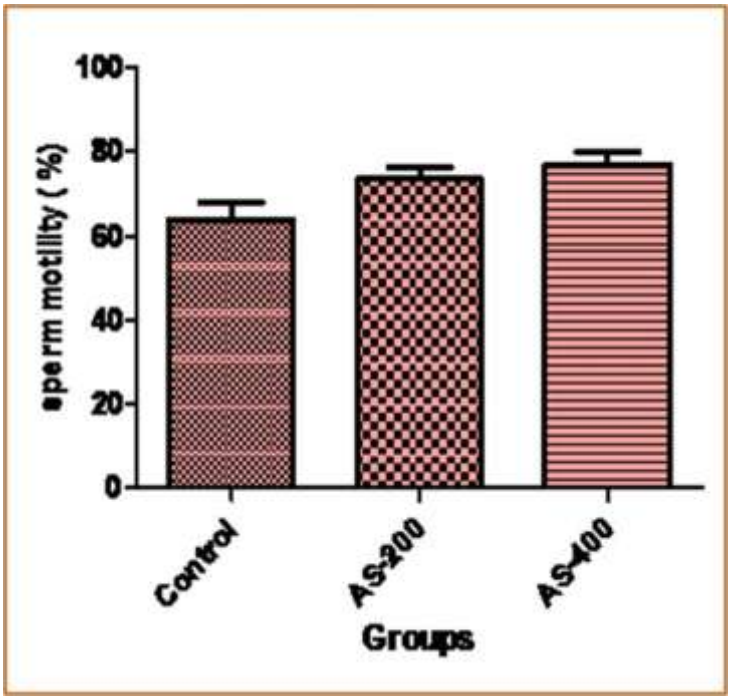

Figure2: Graphical representation of Sperm Motility in percentage

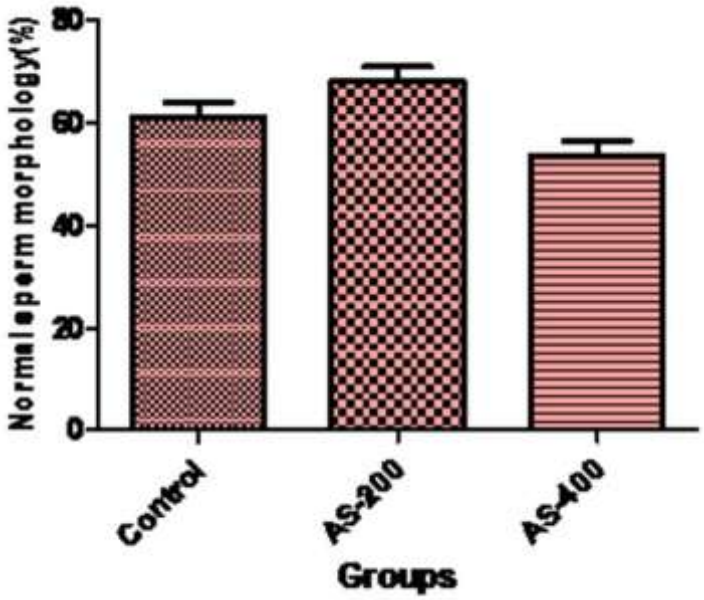

Figure3: Graphical representation of normal sperm morphology in percentage.

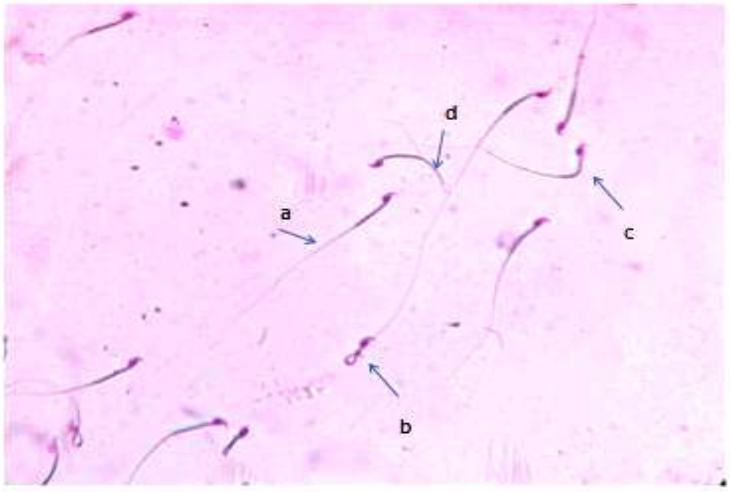

Figure4: Figure showing sperm morphology of sperms stained with Eosin Y (a) Normal (b) coiled mid piece (c) bent mid piece (d)coiled tail

Serum Testosterone:

There was a significant decrease in serum Testosterone level in AS-400($p<0.01$) compared to Control. There was no change AS-400.

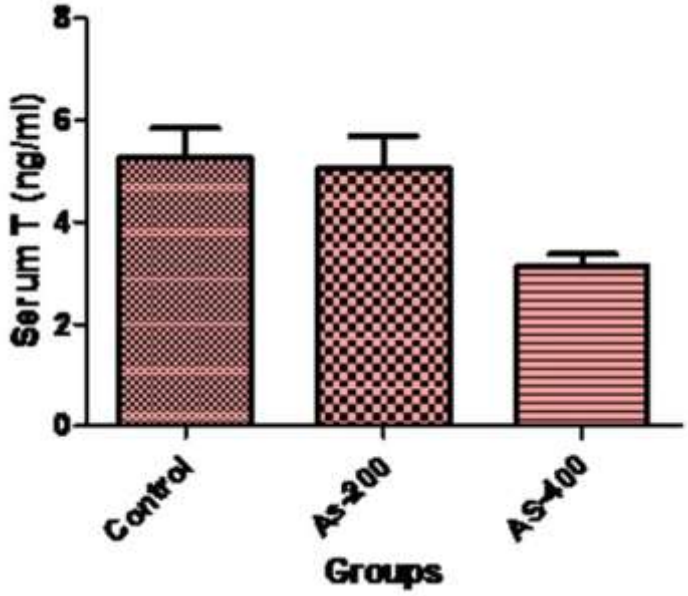

Figure 5: Graphical representation of Serum Testosterone (T) in ng/ml. of Control, AS-200 and AS-400. Testosterone level declined in AS-400 significantly ($p<0.01$) compared to Control and AS-200.

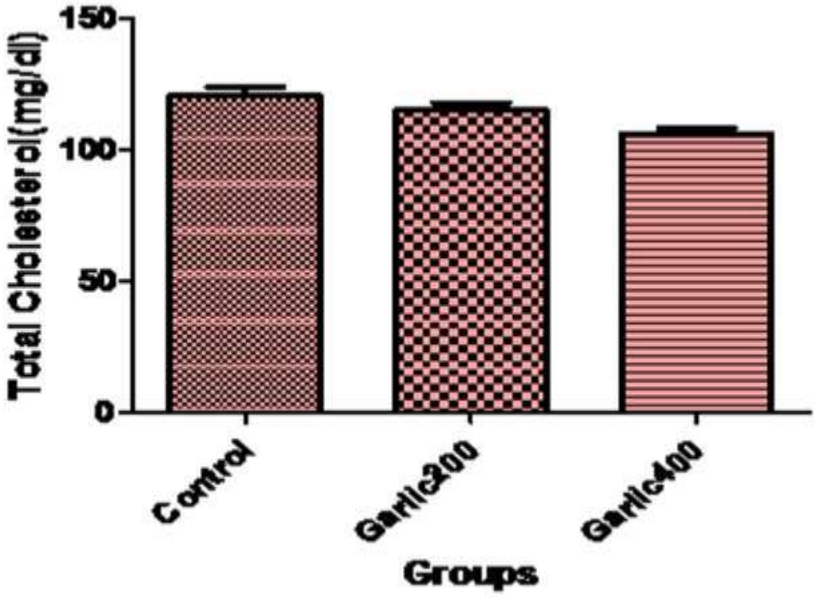

Figure 6: Graphical representation of total cholesterol. A significant decline in AS-400 ($p<0.01$) compared to Control was observed.

IV. Discussion

In the present work, garlic showed changes in few parameters of male reproductive system while other parameters remained unaffected.

The body weight of experimental animals after 45 days of treatment with 400mg/kg body weight declined more compared to the control and AS-400. Garlic is known to reduce obesity (22) which can be related with the reduction in body weight.

The weight of testes did not showed any marked change. However, seminal vesicles of mice fed with 400mg/kg b/w garlic showed a significant increase in weight($p<0.01$). Seminal vesicles are the accessory gland of male reproductive system. Its secretion plays key role in formation of good quality of semen. The fructose in the secretion of seminal vesicles provides the major nutrient for maintaining appropriate quality and quantity of sperms in semen (23). Potassium, bicarbonate and prostaglandin and prolactin secreted by seminal vesicles

directly influence sperm motility as these substances provides the energy for sperm movement. There is a positive relation between seminal vesicle secretion and sperm motility (24). The polysaccharides present in garlic contain three monosaccharides, fructose, glucose and galactose (25). Among polysaccharide, 85% is fructose (26). In present study, the weight of seminal vesicles increased significantly ($p< 0.01$) after 45 days of treatment with crude aqueous garlic extract at the dose of 200mg/ kg body weight. The weight of prostate gland did not varied significantly after garlic feeding. This suggests a positive impact of garlic on proper function of the male accessory sexual gland.

A non significant change in sperm count in lower and higher does not shows any adverse effect of garlic on production of sperms. The motility of sperms increased in both the groups of garlic fed mice. The normal sperm morphology declined markedly in AS-400. The serum Testosterone level decreased significantly in AS-400. These two results indicate an adverse effect of garlic on testosterone production and normal architecture of sperms at higher dose. The reduction in cholesterol due to garlic can be the reason behind the low level of testosterone as cholesterol is required during testosterone synthesis. This result is in consistence with previous work of Hammami where high dose of crude garlic extract had lowered the serum testosterone level in rat (14, 15).

There are a good number of studies giving evidences that semen of infertile men have lower concentration of zinc (27, 28). Zinc is required for synthesis of hormones, sperm production and for maintaining sperm DNA integrity. As the zinc level of seminal plasma increased, mannose receptor expression level increased as found in a study on infertile men. Mannose receptor expression has a positive association with capacitation of sperms. Mannose receptor expression reduced in sperms with low cholesterol (29). The bioavailabity of zinc from grains and other food increase with simultaneous uptake of garlic (30). The increase in sperm motility in Garlic fed mice in present study can be correlated with increase in bioavailable zinc through garlic.

The antioxidant properties of garlic help to scavenge the reactive oxygen species (ROS) from different tissues including testes. However, adequate amount of ROS formation is necessary within the testes for providing energy to sperms to maintain their architecture and acquiring capacitation for fertilizing the ovum (31). The experimental mice group AS-200 exhibited good male reproductive status with respect to sperm quality and serum testosterone level. This study shows that aqueous garlic extract in distilled water at dose of 200 mg per kg body weight for 45 days has a beneficial effect on testicular function of normal Swiss Albino mice compared to 400mg per kg body weight.

V. Conclusion

Though *Allium sativum* has enormous medicinal benefits yet its overdose or prolonged use may harm the testicular function and hence optimization of the mode and duration of use of this plant is essential to reap its maximum benefit.

Acknowledgement: The authors are thankful to Mahavir Cancer Sansthaan and Research Centre, Phulwarisharif, Bihar for providing the facility of laboratory during the entire study. The authors are thankful to Magadh University, Bodh Gaya and A.N College, Patna for providing the infrastructure facility.

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"Effect Of Allium Sativum On Testicular Functions Of Male Swiss Albino Mice."*IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT),17(4),* (2023): pp 36-43.