Global Journal of Pharmacology 9 (3): 272-277, 2015
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2015.9.3.95142

Antifertility Activity of Methanol Bulb Extract of *Allium sativum* on Swiss Albino Male Mice and Teratogenic Effect on Neonates of female Mice

[1]Mohammad Parvez, [1]Rashaduz Zaman, [1]Mohammed Abu Sayeed,
[2]Md. Areeful Haque and [3]Mohammad Abdur Rashid

[1]Department of Pharmacy, Faculty of Science and Engineering,
International Islamic University Chittagong, Chittagong-4203, Bangladesh
[2]Drug and Herbal Research Centre, Faculty of Pharmacy, Universiti Kebangsaan Malaysia,
Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh

Abstract: *Allium sativum* (Garlic) is one of the plants used as a therapeutic agent in many cultures. The current study was intended to investigate the anti-fertility activity of methanol bulb extract of *Allium sativum* on Swiss albino male mice. Swiss albino mice were divided into three groups. Each of the groups contained five sub-groups. Each of the sub-groups consisted of 1 male and 1 female mouse. The first group was given 1% of Tween 80 solution as control while the second group and third groups were given extract orally as test group at dose of 500 mg/kg per body weights and 1000 mg/kg per body weights respectively. The lower concentration (500 mg/kg/BW) of methanol bulb extract of *Allium sativum* showed significant result and gave temporarily anti-fertility activity. However, the higher dose (1000mg/kg/BW) gave relatively more permanent antifertility activity on Swiss albino mice in comparison with the control. The duration of gestation was considered for statistical significance. The duration of gestation at dose 500 mg/kg was 33±0.37 which was significantly different ($P<0.05$) from the control group in which the duration of gestation was 18±0.37. However, no neonate was produced at dose of 1000mg/kg. No teratogenic effect was found in pregnant female mice and the delivered litters. The results of this study demonstrate that methanol bulb extract of *Allium sativum* possess anti-fertility effect on male mice and have no teratogenic or unthrifty effect on female mice.

Key words: Antifertility · Co-habitation · *Allium sativum* · Teratogenicity · Male contraceptive

INTRODUCTION

Population explosion has become a burning issue throughout the world. It is an imminent hurdle for a country's development as the natural resources are limited. The population of Bangladesh is multiplying at an alarming rate and has crossed 180 million. Fertility regulation has therefore become the major concern of people of all walks of life. In recent years, plants are pursued over synthetic antifertility drug because plants are easily available, economic and devoid of harmful side effects [1]. In recent years, research work through light on the effect of several kinds of plants on the reproductive health of man and animals where it is commonly used as medicine for treatment of certain ailments or as condiments for cooking purposes [2]. *Allium sativum* is one of such plants used as biennial medicinal plant that has been described in the folklore of many cultures as a therapeutic agent [3]. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being [4].

Various methods of contraception were used for fertility control. There are a variety of methods available and are in use for female contraception [5]. In contrast, except for the barrier method and vasectomy, there are no methods available for male anti-fertility. Thus, there is a need to develop multiple male anti-fertility methods.

Corresponding Author: Rashaduz Zaman, Department of Pharmacy, International Islamic University Chittagong, Chittagong-4203, Bangladesh. Cell: +8801822323331.

The risk obtained by the drugs has triggered the need to develop newer molecules from medicinal plants. Hence there is need to search suitable products from indigenous medicinal plants that could be effectively used. Many local plants have been identified and tested for their antifertility effect in male rats and mice [6,7].

Synthetic hormonal contraceptives cannot be used continuously because of their health related effects, like increase in blood transaminase and cholesterol levels, dyspepsia, headache, depression, tiredness, weight gain, hyper menorrhea and inter-menorrheal hemorrhage and also disturb the metabolism of lipid, protein, carbohydrate, enzymes and vitamins. Therefore, scientists are on the hunt for newer alternatives, with lesser side effects, self-administrable, less expensive and with complete reversibility [8].

In our country as well as in the world, there are several medicinal plants associated with antifertility properties. Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous system of medicine. A large number of plant species with antifertility effects have been screened in China and India, since last 50 years and were subsequently fortified by national and international agencies [9,10]. However, the search for an orally active, safe and effective plant preparation or its compound is yet to be fulfilled for fertility regulation as many of them have incomplete inhibition of fertility or side effects.

Plant Species: *Allium sativum* L. (Family: Amaryllidaceae) commonly known as garlic, is a species in the onion genus, Allium. Usually it is used as spice and widely cultivated. It is a bulbous plant. It grows up to 1.2 m (4 feet) in height. It produces hermaphrodite flowers. Pollination occurs by bees and other insects. Proper identification of the plant was carried out by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331 and was also confirmed by standard book. A voucher specimen of the plant was deposited in the herbarium of Department of Botany, University of Chittagong.

Chemical Constituents: *Allium sativum* (garlic) contains at least 33 sulfur compounds, several enzymes, 17 amino acids and minerals such as selenium, germanium. It contains a higher concentration of sulfur compounds than any other Allium species.The sulfur compounds are responsible both for garlic's pungent odor and many of its medicinal effects. Dried, powdered garlic contains approximately 1% alliin (S-allyl cysteine sulfoxide). One of the most biologically active compounds, allicin (diallylthiosulfinate or diallyl disulfide) does not exist in garlic until it is crushed or cut. It is also contains enzymes such as allinase, peroxidases, myrosinase and amino acids and their glycosides such as arginine [11].

Therapeutic Activities: Scientific reports revealed that the bulb extracts of *Allium sativum* has abortifacient, antilipemic, antihhypertensive, anti-atherosclerotic, spasmolytic, hepatoprotective, antiviral, antibacterial, antifungal, antiparasitic, antioxidant, immunostimulant activities [11]. It inhibits platelet aggregation and enhances fibrinolytic activity, reducing clots on damaged endothelium. Another important use of *Allium sativum* is as antidiabetic. It controls the blood sugar level by different types of mechanisms [12]. Due to its antiseptic properties, garlic is a good remedy for the prevention and treatment of colitis, dysentery and diarrhea. Claims also have been made for its use in the treatment of whooping cough and tuberculosis and its effect in treating ring worm as well as an anti-asthmatic and anti-epileptic and an immune system booster [13, 14].

MATERIALS AND METHODS

The main objective of performing experiment is to find out the anti-fertility activity of orally administered bulb extract of *Allium sativum* on Swiss albino male mice. The present study is designed to evaluate the antifertility activity of methanol bulb extract of *Allium sativum* regarding daily sperm production with abnormality of male sperm and weight change. Besides these teratogenicity in female mice and neonates was also determined.

Test Materials Used for the Study:

- The methanol crude extract for investigation of anti-fertility activity.
- Solvent (methanol) was used for dissolving the compounds.
- Adult Swiss albino Mice (15 males and 15 females) of 30-35 days of age for test.

Extraction of Plant Material: The bulb of *Allium sativum* L. was cut into small pieces, soaked 500 gm of chopped *Allium sativum* bulb in 1000 ml of methanol and placed in rotary shaker machine for 7 days with constant shaking. After filtration, the filtrate was placed in water bath at temperature of 60-65 °C.

Ethical Consideration for Animals Used in the Study: Adult Swiss albino mice (n = 22) of 30 - 35 days age were used. The mice were collected from the Faculty of Pharmacy, Jahangirnagar University, Savar, Dhaka. The mice weighing about 30-40 grams, was house in colony cages (4 mice per cage) at an ambient temperature of 25 to 27°C with 12 hours light and dark cycles and having proper ventilation in the room. The mice were kept male and female in separate cages. They were fed normal diets purchased commercially from the vendors and water *ad libitum*. The animal was allowed to acclimatize to the laboratory environment for one week and then divided into groups for experiments. Guidelines of Institutional Animal Ethics Committee were followed to carry out this study [15].

Preparation of the Doses: The extract showed no toxicity even at dose of 2000 mg/kg. Hence 1/2th (1000 mg/kg) and 1/4th (500 mg/kg) of this dose were selected for this study. The samples were prepared at doses of 500 and 1000 mg/kg per body weight of the male mice by dissolving 200 mg of extract in 4 ml and 400 mg of extract in 4 ml of 80% tween solution respectively.

Treatment Protocol: For this study we followed the procedure of Abu *et al*., 2012 [16] with slight modification. Total of 15 groups of mice (1 male and 1female in each sub-group) were taken and assigned as sub-groups I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV and XV. Only the male mice of proven fertility were treated with extract. Sub-groups-I, II, III, IV and V were assumed as control groups and treated with 1% of tween 80solution. The sub-groups-VI, VII, VIII, IX and X were treated with 500mg/kg dose while sub-groups-XI, XII, XIII, XIV and XV were treated with 1000 mg/kg dose. The experimental procedure was as follows:

- The weights of both male and female mice (fresh) were taken and ensured non pregnant female mice for the study.
- Male mice were separated after taking weight before starting the treatment.
- A feeding tube was kept ready for oral administration and washed with DMSO.
- The appropriate dose of sample solution was taken according to the body weight of the test male mice by means of feeding tube.
- The measured amounts (500 mg/kg/BW and 1000 mg/kg/BW) of extract were fed orally into gavages of the test male mice.
- Before given the next dose, the male mice were kept separately in colony cages with standard laboratory diet and water for two days.
- The 2nd doses were given after two days of 1st doses, the 3rd doses were given after two days of 2nd doses, the 4th doses were given after two days of 3rd doses and the 5th doses were given after two days of 4th doses at the same time interval with standard laboratory diet and water
- Again kept separately for two days with full living support.
- The treatment period of male mice with doses was total of ten days.
- After completion of the doses male mice were kept with female mice for intercourse.
- The mice were kept in the following order (Table 1)

Table 1: Order of grouping of mice

Groups		Weight of male (gm)	Weight of female (gm)	Doses for male (mg/kg/BW)
Control	I	32	32	--
	II	33.1	32.7	--
	III	32.3	33	--
	IV	33.7	32.9	--
	V	34	33.5	--
MBEAS 500 mg/kg/BW	VI	32.7	30	500
	VII	33	34.4	500
	VIII	33.3	33	500
	IX	33.2	32.6	500
	X	32.7	31.8	500
MBEAS 1000 mg/kg/BW	XI	38	33.2	1000
	XII	38.5	34.4	1000
	XIII	37.9	34	1000
	XIV	36.4	33.8	1000
	XV	37	34.2	1000

MBEAS: Methanol Bulb Extract of *Allium Sativum*

- All groups of mice were kept under observation for forty days in colony cages with standard laboratory diet and water and after ten days interval every group were checked for pregnancy of female mice.
- After withdrawal of the treatment again body weight were taken for both control and test mice.
- The reversibility in fertility action was observed in all treated animals for 13 days of withdrawal of treatment.

To evaluate the teratogenic effect we considered the

- Weight variation in neonates
- Rate of mortality
- Gross malformation

Statistical Analysis: The duration of gestation in each groups was considered for statistical significance. The statistical analysis was carried out by GraphPad Prism (ver. 6.05, GraphPad software, Inc). The level of significance was set at $P<0.05$ and the values were expressed as Average± S.E.M.

RESULT AND DISCUSSION

The antifertility effect of methanol bulb extract of *Allium sativum L.* was found at different concentrations in Swiss albino mice. The treatment duration was total two months. After 17 days of treatment, Control sub-groups-I and III reproduced 10 and 9 pups (neonates of mice) respectively, after 18 days of treatment, Control sub-group-II reproduced 9 pups and after 19 days of treatment, Control sub-groups-IV and V reproduced 8 and 7 pups i.e., female mice of control groups got pregnant which was the evidence of fertility. On the other hand the mice of test groups did not reproduce any fertile action with this treatment procedure. Thus, it was assumed that methanol bulb extract of *Allium sativum* induced a highly significant decrease in testicular sperm count and daily sperm production in all the test groups compared to Control.

The levels recovered to almost normal for 15 days of withdrawal of treatment in all treated groups. After 30 days of withdrawal of treatment, the female mice of the sub-groups-VI, VII, VIII, IX, and X which treated with 500mg/kg/BW got pregnant. However until then the female mice of sub-groups-XI, XII, XIII, XIV and XV which treated with 1000mg/kg/BW were not pregnant. After that their weight were noted and significant weight change has been observed (Table 2 and 3). Then they were allowed to deliver their pups naturally.

On the gestation of 32nd day sub-groups-IX and X, 33rd day sub-groups-VI and VIII and 34th days sub-group-VII dams (pregnant mice) were delivered their litters. At birth, the number of pups (litters) were counted (Table 4 and Figure 1) and examined for gross malformations. However, until then the mice of sub-groups-XI, XII, XIII, XIV and XV did not give birth to any pup within the treatment period.

Above results shows that the lower concentrations (500 mg/kg/BW) of methanol bulb extract of *Allium sativum* gave temporarily anti-fertility activity. But the higher dose (1000 mg/kg/BW) gave relatively more permanent anti-fertility activity.

Table 2: Weight variation of mice before and after the treatment

		Weight of male (gm)				Weight of female (gm)			
Group		Before	Avg	After	Avg	Before	Avg	After	Avg
MBEAS									
500 mg/kg/BW	VI	32.7		34		30		36.3	
	VII	33		33.9		34.4		39.8	
	VIII	33.3	32.98	35.1	34.46	33	32.36	38.2	38.32
	IX	33.2		34.7		32.6		39	
	X	32.7		34.6		31.8		38.3	
MBEAS									
1000 mg/kg/BW	XI	38		39.2		33.2		36.6	
	XII	38.5		40		34.4		36.9	
	XIII	37.9	37.56	39.6	39.4	34	33.92	36.2	36.5
	XIV	36.4		38.7		33.8		37	
	XV	37		39.5		34.2		35.8	

Table 4: Effect of methanol bulb extract of *Allium sativum* on the duration of gestation and number of neonatal produced

Groups		No. of pups produced in 40 days		Duration of gestation (in days)	Average ±S.E.M
		No. of neonatal	Average		
control	I	9	8.2	18	18±0.37
	II	7		17	
	III	8		17	
	IV	8		19	
	V	9		18	
500mg/kg/BW	VI	1	2.4	33	33±0.37*
	VII	3		34	
	VIII	3		33	
	IX	2		32	
	X	3		32	
1000mg/kg/BW	XI	--	--	--	
	XII	--	--		
	XIII	--	--		
	XIV	--	--		
	XV	--	--		

Note: (--) means no delivery; *Significant at $P<0.05$ in comparison to control (Dunnett's Multiple Comparison Test)

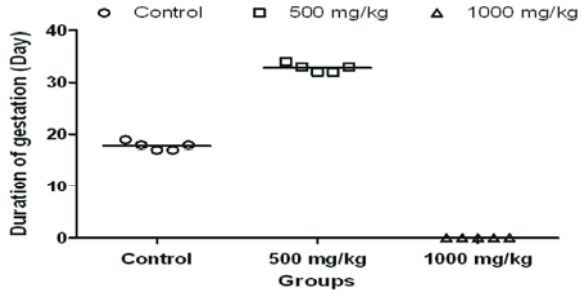

Fig. 1: Duration of gestation (Day) in control and *Allium sativum* treated mice

The result also shows that at lower concentration the female mice of sub-groups-VI, VII, VIII, IX and X gave birth to one, three, three, two and three pups respectively that were significantly lower than the control groups. It is the evidence of lower sperm production in male mice of sub-groups-VI, VII, VIII, IX and X (500mg/kg/BW) (Table 4 and Figure 1).

Similar results were reported that *Allium sativum* was found to be effective in the recovery of testicular functions and spermatogenesis in rats [17] and decrease in sperm count, motility, percentage normal morphology and epididymal volume when the aqueous extract of garlic was investigated for testicular functions in the rat [18]. Reports found to have decrease in the number of implantations and the number of viable fetuses in the similar treatment [19,20]. Oyedeji *et al*. [21] reported similar effects that could be due to reductions in sperm motility, sperm counts, sperm viability and increase in the percentage of abnormal sperm cells induced by the crude extracts.

No significant weight variation was found in the litters produced by mice (sub-groups-VI, VII, VIII, IX and X) in comparison to Control groups. Morphological formation was normal and no significant gross malformation (morphological change) was found among the litters produced. Moreover, no record of neonatal mortality had been found in this study.

CONCLUSION

Thepresent study indicates that the methanol bulb extract of *Allium sativum* exhibits antifertility effect on Swiss albino male mice. This extract does not cause teratogenic and deleterious effects on the fertility of female albino mice and in the produced neonates. However, the exact mode of action was not a concern of this study and requires further investigation. This study may be helpful for further investigation in the formulation of male contraceptives and specific chemical entity elucidation responsible for antifertility activity.

ACKNOWLEDGEMENT

The authors are grateful to the authority of Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong for the grant of this research work.

REFERENCES

1. Sharma, J.D., L. Sharma and P. Yadav, 2007. Antifertility efficacy of *Piper betle* linn. (petiole) on female albino rats. Asian Journal of Experimental Sciences, 21(1): 145-150.
2. Ibe, A.E. and M.I. Nwufo, 2005. Identification, collection and domestication of medicinal plants in Southeastern Nigeria. Council for the Development of Social Science Research in Africa, 3: 66-77.
3. Amagase, H., B.L. Petesh and H. Matsuura, 2001. Intake of garlic and its bioactive components. Journal of Nutrition, 131(3): 9555-9625.
4. Dey, S.K., D. Banerjee, S. Chattapadhyay and K.B. Karmakar, 2010. Antimicrobial activities of some medicinal plants of West Bengal. International Journal of Pharma and Bio Sciences, 1(3): 1-10.
5. Thejashwini, M.S., H. Ram Krishna and Shivabasavaiah, 2012. Reversible antifertility effect of *Cyamposispsoralioides* in male Swiss albino mice. International Journal of advanced biological research, 2(4): 657-665.
6. Verma, P.K., A. Sharma, S.C. Joshi, R.S. Gupta and V.P. Dixit, 2005. Effect of isolated fractions *Barleria prionitis* roots methanolic extract on reproductive function of male albino rats. Fitoterapia, 76(5): 428-432.
7. Remya, M., R.C. Sharma, M. Deepali, B. Sakshi, P. Nilesh and S. Tharini, 2009. *In vitro* effects of *Aegle marmelos* on human sperm Vitality. Biomedicine, 29(2): 183-85.
8. Zaman, R., M. Parvez, M.S. Ali and M.A. Sayeed, 2015. Evaluation of Antifertility Effect of Methanolic Bulb Extract of *Allium cepa* on Swiss Albino Male and Teratogenic Effect on Female Mice, Advances in Biological Research, 9(2): 128-132.
9. WHO, 2000. Reproductive health research at WHO: a new beginning Biennial Report Special Programme of Research, Development and Research Training on Human Reproduction, 1998-99. World Health Organization, Geneva.
10. Lohiya, N.K., 2000. Plant products for contraception: How to make it a reality? In: C.P. Puri, (Edn.). Indian society for the study of reproduction and fertility, Mumbai, 5: 9-12.
11. Kemper, K.J., 2000. Garlic (*Allium sativum*). The Longwood Herbal Task Force and the Center for Holistic Pediatric Education and Research, pp: 1-49.
12. Londhe, V.P., A.T. Gavasane, S.S. Nipate, D.D. Bandawane and P.D. Chaudhari, 2011. Role of Garlic (*Allium sativum*) in Various Diseases: An Overview; Journal of Pharmaceutical Research and Opinion, 1(4): 129-134.
13. Anderson, B., 2006. Garlic is life, Osu Campus, 600 North Greenwood, Tulsa, OK. Gourmet Garlic Gardens com, Bangs, Tx. Available from http://www.garlicislife.org/garlife2003.html.
14. Silvam, G.P., 2001. Protection against *Helicobacter pylori* and other Bacterial infections of Garlic and its preparation. Journal of Nutrition, 13: 1106-118.
15. Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 16: 109.
16. Sayeed, M.A., A.B.M. Monirul Hossain, A.M. Mondol and M.A. Islam, 2012. Antifertility studies on ethanolic extract of *Abrus precatorius* on Swiss male albino mice. International Journal of Pharmaceutical Sciences and Research, 3(1): 288-92.
17. Kasuga, S., N. Uda, E. Kyo, M. Ushijima, N. Morihara and Y. Itakura, 2001. Pharmacologic activities of aged garlic extract in comparison with other garlic preparations. Journal of Nutrition, 131: 10805-45.
18. Morakinyo, A.O., A.K. Oloyo, Y. Raji and O.A. Adegoke, 2008. Effects of aqueous extract of garlic (*Allium sativum*) on testicular functions in the rat. Nigerian Journal of Health and Biomedical Science, 7(2): 26-30.
19. Gupta, R.S., P. Kumar, V.P. Dixit and M.P. Dobhal, 2000. Antifertiliy studies of the root extract of the *Barleri aprionitisin*on male albino rats with special reference to testicular cell population dynamics. Journal of Ethnopharmacology, 70: 111-17.
20. Lohiya, N.K., R.B. Goyal, D. Jayaprakash, A.S. Ansari and S. Sharma, 1994. Antifertility effects of aqueous extract of *Carica papaya*seeds in male rats. PlantaMedica, 60: 400-404.
21. Oyedeji, K.O., A.F. Bolarinwa and A.F. Adigun, 2013. Effect of aspirin on reproductive functions in male albino rats. IOSR Journal of Pharmacy and Biological Sciences, 4(6): 49-54.