Advances in Biological Research 9 (2): 128-132, 2015
ISSN 1992-0067

DOI: 10.5829/idosi.abr.2015.9.2.949

Evaluation of Antifertility Effect of Methanolic Bulb Extract of *Allium cepa* on Swiss Albino Male and Teratogenic Effect on Female Mice

Rashaduz Zaman, Mohammad Parvez, Md. Sekendar Ali and Mohammed Abu Sayeed

Department of Pharmacy, International Islamic University Chittagong, Chitagong-4203, Bangladesh

Abstract: As an anti-fertility agent *Allium cepa* has been used in traditional medicine. However, there is no scientific report concerning the anti-fertility effect of this plant on male. This study investigated the antifertility effect of *Allium cepa* on Swiss albino male mice and its teratogenecity on female mice. The antifertility activity was evaluated by cohabitation method. The experiment was carried out for two months.The male Swiss albino mice were treated with methanolic bulb extract of *Allium cepa* at doses of 400 and 800 mg/kg body weight of the mice. The lower concentrations (400 mg/kg body weight) of methanolic bulb extract of *Allium cepa* gave temporarily antifertility activity. However, the higher dose (800mg/kg body weight) gave relatively more permanent antifertility activity on Swiss albino mice in comparison with the control. No teratogenic effect was found in pregnant female mice and the delivered litters. The results of this study demonstrate that methanolic bulb extract of *Allium cepa* possess antifertility effect on male mice and have no teratogenic or unthrifty effect on female mice.

Key words: Antifertility · Teratogenecity · Cohabitation · Unthrifty · *Allium cepa*

INTRODUCTION

Population explosion has become a burning issue throughout the world.Itis 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 one 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 contraceptive drug because plants are easily available, economic and devoid of harmful side effects [1].

Synthetic hormonal contraceptives cannot be used continuously because of their health related effects, like increase in blood transminase 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 [2]. Therefore, scientists are on the hunt for newer alternatives, with lesser side effects, self-administrable, less expensive and with complete reversibility.

Several medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases including for inducing infertility without proper knowledge of their function. Although several herbal plants possess different types of antifertility activities such as anti implantation, Abortification, Ecobolic, Oestrogenic and Spermicidal, a large number of medicinal plants posses some degree of toxicity [3].

Though considerable progress has been made in the development of highly effective, acceptable and reversible methods of contraception among females, progress possibilities on males are still slow and limited. With recent progress towards a better understanding of male reproductive physiology there is needed to develop new contraceptive modalities for male [4].

Plant Species: *Allium cepa* L. (Family: Liliaceae) is used as a vegetable and is the most widely cultivated species of the genus Allium. It is cultivated all over the world. It is most frequently a biennial or a perennial plant, but is usually treated as an annual and harvested in its first growing season. A type of onion, Shallot has been selected for this study. Indian names for shallotare *kanda* or *gandana* or *pyaaz*, *gundhun* (Bengali). This plant has been collected from local market of Bohoddarhut, Chittagong, Bangladesh. Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331,

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

identified the plant and a voucher specimen of the plant was deposited in the herbarium of Department of Botany, University of Chittagong.

Therapeutic Uses: The bulb is used in Abscess, Ache (Ear), Ache (Stomach), Albuminuria, Alopecia, Anasarca, Antiseptic, Apertif, Aphrodisiac, Arteriosclerosis, Bactericide, Bite (Snake), Bright's Disease, Bronchitis, Cancer, Cancer (Liver), Carminative, Cataract, Cold, Cough, Diaphoretic, Digestive, Diphtheria, Diuretic, Dropsy, Drowning, Dysentery, Edema, Emmenagogue, Expectorant, Fever, Flatulence, Flu, Fracture, Diabetes [5], Inflammation, Piles, Repellant, Rheumatism, Scarlatina, Scurvy, Sedative, Soporific, Sore(Throat), Stimulant, Stomachic, Suppurative, Tuberculosis, Venereal, Vermifuge, Wart, Wound, used as a treatment for respiratory problems [6], anticlotting [7,8], antihypertensive [9], antihyperlipidemic [10].

In people eating onion, 88% reduced risk for esophageal cancer, 56% reduced risk for colorectal cancer, 83% reduced risk for laryngeal cancer, 25% reduced risk for breast cancer, 73% reduced risk for ovarian cancer, 71% reduced risk for prostate cancer, and 38% reduced risk for renal cell cancer [11]. *Allium cepa* has been used as a contraceptive agent among the people of Tripura state in India [12].

Antifertility Activity: Scientific reports revealed the presence of the following chemical entities in the of *Allium cepa*: Oleanolic acid which acts as abortifacient, Melatonin as anaphrodisiac, β-sitosterol, Diallyl-trisulfate, Kaemeperol, Oleanolic acid, P-coumaric acid as antifertility agent, Kaemeperol also acts as antiimplant agent, Arginine, Zinc as antiimpotence, α-tocopherol, Ascorbic acid, Arginine, Zinc also act as antiinfertility, Stigmasterol as ovulant, Acetic acid, β-sitosterol, Diallyltrisulfate as spermicide. Arginine and Zinc also act as spermigenic agents [13]. Bulb extract of *Allium cepa* showed ecobolic effect in mice and rats [14]. Ethanolic bulb extract showed antiimplantation activity in female rats [15]. *Allium cepa*showed less significant sperm immobilization activity [16].

MATERIALS AND METHODS

The main objective of performing the antifertility screening is to determine the contraceptive activity of orally administered *Allium cepa* bulb extract on Swiss albino mice, in turn it is used for the selection of the extract as therapeutic agent. The present study was designed to evaluate the antifertility activity of methanolic bulb extract regarding daily sperm production with abnormality of male sperm and weight changes as well as to determine the extract as effective contraceptive. Besides, teratogenecity in female mice and neonates was also evaluated.

Extraction of Plant Material: Extraction of plant material was done as described in the early report [4]. 750 gm of chopped *Allium cepa* bulb was emerged in 700 ml of methanol for 7 days with constant shaking in rotary shaker machine, then filtered by muslin cloth and by no.1 Whatman filter paper successively. Extract sample was concentrated by evaporating the solvent using a water bath at a temperature of 60-70ºC.

Ethical Consideration for Animals Used in the Study: Adult Swiss albino mice of 30 - 35 days age (n=10) were used. The mice were collected from the ICDDRB; Mohakhali, Dhaka. The mice weighing about 30-40 grams, were housed in colony cages (4 mice per cage) at an ambient temperature of 25 to 27ºC with 12 hours light and dark cycles 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 the one week and then divided into groups for experiments. The study was conducted by the permission of P & D committee, Department of Pharmacy, International Islamic University Chittagong for two months.

Dose Preparation: The samples were prepared at doses of 400 and 800mg/kg per body weight of the male mice by dissolving 200 mg of extract in 5 ml and 400 mg of extract in 5 ml of 80% tween solution respectively.

Treatment Protocol: For this study, the cohabitation method [4] was adopted with minor modification. 5 groups of mice (1 male and 1female in each group) were taken (n=10) and assigned as groups I, II, III, IV and V. Group I was assumed as control group and treated with 80% tween solution. Only the male mice of proven fertility were treated with extract. The groups II and III were treated with 400mg/kg dose and groups IV and V were treated with 800mg/kg dose. The experimental procedure was as follows:

- The weights of both male and female mice (fresh) were taken and ensured non-pregnant mice.
- Male mice were separated from the female after taking the weight before starting the treatment.

- A mice feeding tube was prepared to feed the extract orally through the mice gavages.
- The appropriate dose of sample solution was taken according to the body weight of mice by means of feeding tube.
- The measured amounts (400 and 800mg/kg/ body weight) of extract were fed orally into gavages of the test male mice.
- Before the next dose is given, 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 first doses, the 3rd doses were given after two days of 2nd doses, the 4th doses were given after two days of 3rd doses.
- Again, the mice were kept separately for two days with full living support.
- The treatment period of male mice with doses was total of eight days.
- After completion of the doses male mice were kept with female mice for intercourse.
- Female mice of each group were observed for vaginal smear to ensure the successful intercourse.
- The mice were kept in the following order: (Table 1)
- All groups of mice were kept under observation for forty days in colony cages with standard laboratory diet and water.
- After ten days interval female mice of each group were checked for pregnancy.
- After withdrawal of the treatment, again body weights of both control and test mice were taken.
- The reversibility in fertility action was checked in all treated mice for 15 days of withdrawal of treatment.

To evaluate the teratogenic effect we considered the following factors

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

Table 1: Order of grouping of mice

Group	Weight of male (gm)	Weight of female (gm)
Group-I (Control)	31.5	32.2
Group-II	39	32.5
Group-III	38	33.4
Group-IV	32.5	28.3
Group-V	33.8	33.4

RESULTS AND DISCUSSION

The antifertility effect of methanolic bulb extract of *Allium cepa* was found at different concentrations in Swiss albino mice. The treatment duration was total of two months. After 20 days of treatment, Group-I (control) reproduced eight mice, i.e., the female mouse of control group got pregnant which was the evidence of fertility. On the other hand, mice of Groups-II, III, IV and V mice did not reproduce any fertile action till then with this treatment procedure. Thus, it was assumed that methanolic bulb extract of *Allium cepa*induced a highly significant decrease in testicular sperm count and daily sperm production in all the treatment groups compared to Control.

The levels recovered to almost normal for 15 days of withdrawal of treatment in all treated groups. After 29 days of withdrawal of treatment the female mouse of Group-II got pregnant and so happened in case of female mouse of Group- III in 31 days after withdrawal of treatment. Thus notable weight variations were observed and we noted them (Table 2,3).

On the 20th day of gestation, the dams (pregnant rats) (groups I) is allowed to deliver their litters naturally. At birth, the number of pups (litters) were counted, weighed and examined for gross malformations. After 29 days of gestation, the female mice of Group- II gave birth to only one pup (litter). After 31 days, the female mice of Group-III gave birth to three pups (litters). However, until then the mice of Groups- IV and V did not give birth to any pup.

Table 2: Weight of the mice before the treatment with methanolic bulb extract along with the doses.

Group	Weight of male (gm)	Weight of female (gm)	Doses (for male)
Group-I (control)	31.5	32.2	-
Group-II	39	32.5	400 mg/kg/BW
Group-III	38	33.4	400 mg/kg/BW
Group-IV	32.5	28.3	800 mg/kg/BW
Group-V	33.8	33.4	800 mg/kg/BW

Table 3: Weight of the mice after the treatment with methanolic bulb extract along with the doses.

Group	Weight of male (gm)	Weight of female (gm)	Doses (for male)
Group-I (control)	34.5	37	-
Group-II	40	37.8	400 mg/kg/BW
Group-III	36.5	36.5	400 mg/kg/BW
Group-IV	33	30	800 mg/kg/BW
Group-V	36	39.5	800 mg/kg/BW

Table 4: Effect of methanolic bulb extract of *Allium cepa* on duration of gestation and number of pup produced.

Group	No. of litter produced in 40 days	Duration of gestation (in days)	Dose
Group-I (control)	8 Pups	21 days	-
Group-II	1 Pups	29 days	400mg/kg/BW
Group-III	3 Pups	31 days	400mg/kg/BW
Group-IV	--	--	800mg/kg/BW
Group-V	--	--	800mg/kg/BW

Note: (--) means no delivery

From our observation we found that, the lower concentration (400mg/kg/BW) of methanolic bulb extract of *Allium cepa* induced temporary antifertility activity whereas the higher concentration (800mg/kg/BW) gave relatively more permanent anti-fertility activity.

The result also shows that at lower concentration the female mouse gave only one (Group-II) and three (Group-III) pups respectively that were significantly lower than the control group, which gave eight pups. It is the evidence of lower sperm production in male mice of Groups- II & III (400mg/kg/BW) (Table. 4).

There was no significant weight variation in the litters produced by mice (Groups-II & III) in comparison to Control group. No significant gross malformation (morphological change) was found among the litters produced. Moreover, no record of neonate mortality had been found in this study.

The results presented in this paper show that the ingestion of methanol extract of bulb of *Allium cepa* (400, 400, 800 and 800mg/kg body weight) by adult male mice decreased the number of impregnated females. The number of implantations and the number of viable fetuses were decreased. This effect may be due to decrease in sperm motility and sperm density.

Similar results were reported in rats treated with *Barleria prionitis* extracts [17] and *Carica papaya* extract [18]. This observation 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 as reported by Oyedeji *et al.* [19].

CONCLUSION

Cohabitation of treated male mice with the crude methanolic bulb extract of *Allium cepa* and untreated female mice for 40 days produced positive reproducible antifertility. In conclusion, this study has shown that the crude extracts of *Allium cepa* have antifertility effects in male albino mice. This extracts have no teratogenic and deleterious effects on the fertility of female albino mice and in the produced neonates. However, the exact mode of action requires further studies. This study may be helpful for future investigation in the formulation of male contraceptives and specific chemical entity elucidation responsible for antifertility activity.

ACKNOWLEDGMENT

The authors are grateful to the authority of P&D committee, Faculty of Science and Enigineering, Department of Pharmacy, 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 betlelinn*. (petiole) on female albino rats. Asian Journal of Experimental Sciences, 21(1): 145-150.
2. Noumi, E. and N.Y.C. Tchakonang, 2001. Plants used as abortifacients in the sangmelima region of southern Cameroon. Journal of Ethnopharmacology, 76(3): 263-68.
3. Marles, R. and N. Farnsworth, 1994. Plants assources of antidiabetic agents. Economic and Medinal Plant Research, 6: 149-187.
4. Abu Sayeed, M., A.B.M. Monirul Hossain, A.M. Mondol and M.A. Islam, 2012. Antifertility studies on ethanolic extract of *Abrusprecatorius* onSwiss male albino mice.International Journal of Pharmaceutical Sciences and Research, 3(1): 288-92.
5. Galal, E.E. and M.A. Gawad, 1965. Antidiabetic activity of Egyptian onion "*Allium cepa*" extract. Journal of Egyptian Medical Association, 48: 14-45.
6. Craig, W., 2015. (n.d.). Health benefits of onions. Retrieved 3 May, from http://www.vegetarian-nutrition.info/updates/onions.php
7. Yamada, K., A. Namaura, N. Sawashita, Y. Naguchi and J. Yamamoto, 2004. An onion variety has natural antithrombotic effect as assessed by thrombosis/thrombolysis models in rodents. Thrombosis Research, 114(3): 213-20.
8. Food that Heal, "Onion—Heart Disease," www.indiangyan.com, accessed 01 May 2015.
9. Sakai, Y., T. Murakami and Y. Yamamoto, 2003. Antihypertensive effects of onion on no synthase inhibitor-induced hypertensive rats and spontaneously hypertensive rats. Bioscience, Biotechnology and Biochemistry, 67(6): 1305-11.

10. Lata, S., K.K. Saxena, V. Bhasin, R.S. Saxena, A. Kumar and V.K. Shrivastva, 1991. Beneficial effect of *Allium sativum*, *Allium cepa* and *Comiphoramukul* on experimental hyperlipidemia and arthrosclerosis- A comparive evaluation. Indian Journal of Pharmacology, 37: 132-5.
11. Galeone, C., C. Pelucchi and C. Vecchia, 2012. Added sugar, glycemic index and load in colon cancer risk.Current Opinion in Clinical Nutrition and Metabolic Care, 15(4): 368-73.
12. Das, B., A.D. Talukdar and M.D. Choudhury, 2014. A few traditional medicinal plants used as antifertility agents by ethnic people of Tripura, India.International Journal of Pharmacy and Pharmaceutical Sciences, 6(3): 47-53
13. Specific Queries of the Phytochemical Database-Chemicals in: *Allium cepa* L. (Liliaceae) -- Onion, Shallot,www.ars-grin.gov/duke.
14. Sharaf, A., 1969. Ecoboliceffect of *Allium cepa*in mice and rats. Qalitas Plantarumet Materiae Vegetabiles, 17: 153.
15. Thakare, V., P. Kothavade, V. Dhote and A. Deshpande, 2009. Antifertility activity of ethanolic extract of*Allium cepa* Linn in rats. International Journal of PharmTech Research, 1(1): 73-78.
16. Chakrabarti, K., S. Pal and A.K. Bhattacharyya, 2003. Sperm immobilization activity of *Allium sativum* L. and other plant extracts. Asian Journal of Andrology, 5: 131-135.
17. Gupta, R.S., P. Kumar, V.P. Dixit and M.P. Dobhal, 2000. Antifertiliy studies of the root extract of the *Barleriaprionitis*in male albino rats with special reference to testicular cell population dynamics. Journal ofEthnopharmacology, 70: 111-17.
18. 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.
19. 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.