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EFFECT OF ISOLATED ALLICIN FROM *ALLIUM SATIVUM* LINN BULB FOR EFFECTIVE ANTI FERTILITY ACTIVITY IN FEMALE WISTAR RATS

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ABSTRACT

The present study was undertaken to evaluate antifertility activity of isolated allicin from garlic cloves and the raw juice of garlic (*Allium sativum*) (AS) on experimental female rats. Control of fertilization in female rats was investigated with estrogenic activity, evaluated using ethinyl estradiol as standard in immature female rats. Anti-implantation and early abortifacient activity was also investigated in female Wistar rats with the number of implantations and implantation resorptions estimations. Acute toxicity studies were carried out for the extracts and the test drug doses for screening were selected depending upon LD_{50} values. Fresh AS juice as well as isolated allicin (dose of 2.5 ml & 5.0 ml/kg b.w, and 10 mg/kg b.w respectively) was administered by oral route after confirmation of acute toxicity. In estrogenic activity evaluation, the juice of AS and isolated allicin (10 mg/kg p.o) showed significant estrogen-like activity at 5.0 ml/kg p.o. respectively by increasing the uterine weight compared to vehicle control group. Further the same treatment significantly decreased the number of implants and increased the number of resorptions compared to vehicle control group which were more significant for isolated compound rather extracts. Finally, the results revealed that the allicin isolated from AS ameliorates the scientific evidence of the anti-fertility activity as traditionally claimed.

Keywords: Antifertility, plant extracts, allicin, fertility regulation

INTRODUCTION

India has the second largest population in the world, with just under 1.4 billion inhabitants of China, in 2021. This increase in population is alarming to India in the need for effective birth control measures to control severe environmental imbalance and also to overcome many disadvantages especially the scarcity of food, shelters and other natural resources **[1, 2]**. Therefore, antifertility agents play immense role in capable of preventing ovulation or fertilization as well as also able to induce termination of pregnancy **[3]**. An ample number of FDA approved synthetic contraceptives are available in market but all are associated with severe side effects namely, hormonal imbalance, hypertension, weight gain and also increased risk of cancer **[4]**. Therefore, the recent trend focused towards the natural herbalism to control fertilization with very low or negligible adverse effects. Traditionally, various plant extracts were applied to inhibit male and female fertility and based on the scientific evidences currently many isolated plant bioactive components are used for effective treatment as anti fertility drug and may be developed into non-steroidal contraceptives with the advanced molecular mechanism studies **[5]**.

The global search involves investigating isolates of single plant extracts or fractions thereof or mixtures of fractions extracts from different plants, which have been carefully standardized for their safety and efficacy **[6]**. *Of late, Allium sativum* (AS) is belongs to the family Liliaceae. It is a very common tradition plant having wide range of therapeutic efficacy and hence is one of the most researched and best-selling herbal products in world market **[7]**. Commonly it is well known as garlic and due to presence of dialkyl thiosulfinates viz. alliin and allicin content in its cloves it has high commercial value **[8]**. Recently, allicin was established as anti viral agent that inhibits SARS-CoV-2 main protease enzyme and useful in treating COVID-19 infection **[9, 10]**. Garlic cloves are excellent cardio vascular protectant by reduced total cholesterol, blood pressure, low-density lipoprotein etc. **[11]**. Garlic essential oil is significantly inhibits epinephrine, collagen and ADP-induced platelet aggregation **[12]**. The methanol extract of garlic showed potent anti-fertility efficacy on male mice and also no teratogenicity on female mice **[13]** but still there are scanty reports on garlic and its isolates allicin as anti fertility agent. Therefore, it was worthwhile to study the comparative antifertility activity of the allicin and the garlic juice in the female Wistar rats.

MATERIALS AND METHODS

Preparation of raw garlic juice:

Marketed garlic were procured and authenticated from Indian Institute of Horticultural Research, Bangalore by Principal Scientist, Dr. Rajsekharan, Department of medicinal biotechnology and kept some garlic cloves in the department as herbarium (No: KCP/2019-2020/AS-508). About 500 g of fresh peeled garlic cloves were crushed by mortar and pestle and then strained through fine filter. The juice that came out was collected (14.8 ml) and utilized for the further experimentation. Various chemical tests were performed to understand the content of phytochemicals present in the juice.

Experimental Animals:

Female albino Wistar rats weighing between 200-250 gm were used. Institutional Animal Ethics Committee approved the experimental protocol (REG.No.378/PO/ReBi/S/01/CPCSEA) and animals were maintained under standard conditions in an animal house (22 ± 2°C), humidity (50 ± 5%) and 12-h light-dark cycles in Krupanidhi College of Pharmacy animal house. All the studies conducted were according to the prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Extraction of allicin

500 g fresh garlic cloves were crushed in a mortar and pestle and transferred the whole parts including oil into a beaker. Then, soak the crushed materials in 20 ml of ethanol and kept aside by covered the lid for half an hour in room temperature. Allicin got separated and further purified by flash chromatography using hexane and isopropanol mobile phase (3: 1).

Pharmacological Evaluation:

Determination of acute Toxicity [14]:

Acute toxicity study was carried out using female Albino mice (20-30g) by up and down/staircase method. The MP Linn leaves extracts were orally administered to mice at the doses of 50, 300, 1000 and 2000 mg/ kg b. w respectively. Animals were observed for 48 h to study the general behavior of animals, sign of discomfort and nervous manifestation. No mortality was found upto a dose of 2000 mg/kg body weight which was taken as end point dose till completion of 24 hours. The doses 200 mg/kg, and 400 mg/kg, *p.o*. were selected for further activity.

Experimental Models

Anti-implantation activity [15]:

Wistar female albino rats (150-200 gm) of proven fertility and regular estrous cycle were caged with males of proven fertility in a ratio of (2:1); the females were examined the following morning for evidence of copulation. The animals, which showed thick clumps of spermatozoa in the vaginal smears, were separated and that day was designated as day 1 of pregnancy. The garlic juice was administered from day 1 to

7 day of pregnancy at 2 and 5 ml/kg body weight to different groups of pregnant rats. Control animals received saline. The animals were laparotomised under ketamine (anesthetic) and xylazine (skeletal muscle relaxant) and semi-sterile conditions on day 10 of pregnancy. Both horns of the uterus were observed for the number of implants. The rats were allowed to recover and deliver after full term. Each fetus was weighed and examined for gross defects. The litters were allowed to grow to check their post natal growth and monitor any congenital abnormalities.

Estrogenic/anti-estrogenic activity [16]:

The uterine histological study was employed for this assay. Colony-bred immature ovariectomised female albino rats (wistar strain), 21 to 23 days old and weighing between 30 and 40 gm were used. They were divided into 4 groups' viz. control, raw garlic juice (high and low doses) and isolated allicin groups, consisting of 6 animals in each group. The two different doses of (2 ml/kg, 5 ml/kg) were given to animals of low dose and high dose group respectively for 7 days where as control receives only saline and isolated allicin (10 mg/kg p.o).

On the 8th day of the experiment, all the animals were killed by decapitation under ether anesthesia and the uteri were dissected out, cleared off their surrounding tissue and the uterine tissues from the control group and treated group were fixed in Bouin's fluid for 24h, dehydrated in alcohol and then embedded in paraffin. The paraffin blocks were sectioned at 6 µm intervals and stained with hematoxylin-eosin for histological examination **(Figure 1, 2, 3 and 4)**.

Ponderal changes in genital organs [17]:

Colony bred matured female rats of wistar strain (150-200 g) were bilaterally ovariectomised. Sixteen days after the ovariectomy, the rats were divided into three groups of 6 each and were treated in the following way:

Gr-I: Control group received only saline orally for 5 days.

Gr-II: Low dose received raw garlic juice of 2 ml/kg bodyweight basis for 5 days by oral route.

Gr-III: High dose received raw garlic juice of 5 ml/kg bodyweight basis for 5 days by oral route.

Gr-IV: Isolated allicin (10 mg/kg p.o) for 5 days

The animals were killed 24 hrs after the last treatment. The genital organs; uterus, cervix and vagina of each animal was dissected out, blotted, dry and weighed.

Effect on estrous cycle [18]:

Colony bred virgin female rats showing normal estrous cycle of Wistar strain (150-250 g) were selected and divided into 3 groups of six each.

Treatment was same as the pattern mentioned above; however duration of treatment was for 30 days. The vaginal smears were observed every morning during the treatment period in both the treated groups and control group of animals to check any variation in the estrous (sexual) cycle which includes proestrous, frankestrous, metaestrous and diestrous phases.

Effect on uterus and ovary [17]:

The female albino rats of wistar strain (150-250 g) were treated with raw garlic juice for a period of one month. At the end of one month, animals were sacrificed and their uterus and ovary were dissected out. Histological studies were carried out to determine the following parameters **(Figure 1, 2 and 3)**:

Uterus

i Diameter of the uterus

ii Thickness of endometrium

iii Endometrial epithelial height

Ovary

i Number of Primary follicles

ii Number of Secondary follicles

iii Number of Tertiary follicles (maturing or graafian follicles)

iv Number of Corpus luteum

The total number of cells was counted using a reticule of 1 cm. Total number of follicles especially Graafian or tertiary follicles indicate fertility of the animal and the number of corpus luteum indicates the number of ova released by that particular ovary.

Effect on serum estrogen and progesterone [19]:

Female albino rats of wistar strain (150-200 g) were divided into three groups and treatment continued for 30 days in the same manner as mentioned above. On the 31st day, both the control and treated groups were anesthetized and blood was drawn from retro orbital plexus. The blood was allowed to coagulate for an hour. The separation of the serum from other cellular components of the blood was done by centrifuging the coagulated blood at 3800 rpm for 10 minutes. The sera were collected and analyzed for estrogen and progesterone by the methods of electrochemical luminescence immunoassay (by ELISA method), using human kits.

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test. The values are expressed as mean ± SEM and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION:

The preliminary phytochemical investigation of the raw juice of the bulb of *Allium sativum* showed that it contains carbohydrates, proteins, steroids and saponins. Thereafter, allicin was isolated

and was confirmed with TCL, HPLC, IR, NMR and MASS spectroscopy analysis (data not shown).

Antifertility activity:

Anti-implantation activity: The anti-implantation activity was expressed as the decrease in the number of implants and the percentage of animals showing absence of implantations in uteri when laparotomised on day 10 of pregnancy. The drug in both the low dose and high dose produced a significant reduction in the number of implants ($P<0.01$) when compared to control. However, the percentage of anti-implantation activity observed in animals of low dose was only 33.33%, whereas in animals of high dose, it was 66.66 % with complete absence of any implants whereas isolated allicin showed 100% result **(Table 1)**. The litters born to the experimental and control animals showed no morphological defects and no appreciable changes in their weights. Hence, the treatments do not exhibit any teratogenic effects.

Estrogenic/anti-estrogenic activity:

Administration of raw garlic juice to ovariectomized immature rats for 7 days produced a significant increase in the diameter of uterus and the thickness of the endometrium. The low dose (2 ml/kg) produced more increase in diameter of the uterus ($p<0.01$) than the high dose ($p<0.05$). There was no significant change in the height of endometrial epithelium after treatment with either low dose or high dose of the garlic juice **(Table 2)** but isolated allicin showed significant change in the height of endometrial epithelium.

Ponderal changes in genital organ:

The administration of raw juice as well as isolated allicin from *Allium sativum* to matured ovariectomized rats produced a significant increase in the weight of genital organs ($P<0.01$). The higher dose (5 ml/kg) was more effective than the lower dose **(Table 3)** but was lesser than isolated allicin compound.

Effect on estrous cycle:

Administration of raw garlic juice prolonged the duration of proestrous phase of estrous cycle and reduced the duration of metaestrous phase ($P<0.01$) when compared to control. The same trend followed by the isolated sample allicin and resulted more significant activity than raw juice of garlic **(Figure 1)**.

Effect on uterus and ovary:

Administration of raw garlic juice for a period of one month to non-ovariectomized animals produced opposite effect on the uterus compared to those seen in ovariectomized animals. The diameter of the uterus and the height of endometrial epithelium was significantly reduced by isolated allicin and high dose of raw garlic juice when compared to control ($P<0.01$). The low dose did not show any significant

effect **(Table 5)**. Histological examination of the ovary showed that there was a significant reduction in number of corpus luteum in both the isolated allicin low dose and the high dose treated animals. The decrease was more in the allicin group followed by high dose of garlic juice treated group ($P<0.01$) than the low dose treated group ($P<0.05$). There was no significant effect on the number of primary, secondary and tertiary (Graafian) follicles in both the low dose and high dose treated animals when compared to control **(Table 6)**.

Effect on serum estrogen and progesterone:

The raw garlic juice treatment produced a significant decrease in serum estrogen level after high dose treatment while an increase in serum progesterone level in high dose treated group and a decrease in low dose treated group was seen when compared to control. However, the values were lesser than standard allicin **(Table 7)**.

Table 1: Anti implantation activity of garlic

Treatment	Dose	No. of implantation sites	No. of rats having no Implantation sites on day 10/Total number of rats used	Anti-implantation Activity%	No. of Litters born
Control	4ml/kg	7.16±0.600	0/6	0	7.00 ±0.57
Garlic juice	2ml/kg	2.50 +0.957*	2/6	33.33	2.00±0.63
Garlic juice	5ml/kg	1.00±0.000**	4/6	66.66	1.00 ±0.21
Allicin	10 mg/kg	0.00±0.000**	6/6	100.0	0.00 ± 0.00

All values expressed as Mean ± S.E.M. (n=6 in each group). *p <0.05= significant, **p<0.01 = High significant as compared to vehicle control (saline) by one-way ANOVA followed by Dunnett multiple comparison test

Table 2: Estrogenic / anti-estrogenic activity in immature ovariectomized rats

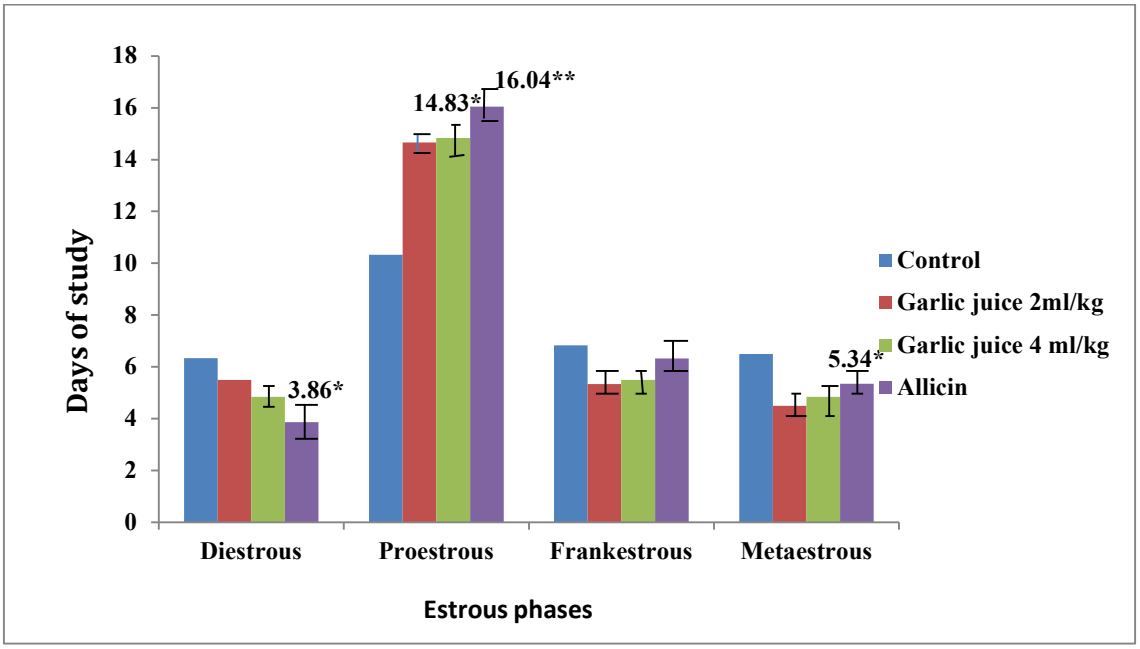
Treatment	Dose	Diameter of uterus (µM)	Thickness of endometrium (µM)	Height of endometrical epithelium (µM)
Control	4ml/kg	2226.18± 35.22	596.97 ± 34.01	27.32 ± 0.80
Garlic juice	2ml/kg	2772.18± 58.58	788.62 ±27.08**	26.38 ± 0.56
Garlic juice	5ml/kg	2485.26± 86.53	810.07 ± 36.23**	29.90 ± 2.07
Allicin	10 mg/kg	2589.11	913.43 ± 23.40**	36.12 ± 0.22*

All values expressed as Mean ± S.E.M. (n=6 in each group). *p <0.05= significant, **p<0.01 = High significant as compared to vehicle control (saline) by one-way ANOVA followed by Dunnett multiple comparison test

Table 3: Effect of raw garlic juice and isolated allicin on the weight of uterus, cervix and vagina of ovariactomized rats

Treatment	Dose	Weight in mg/100g		
		Uterus	Cervix	Vagina
Control	4ml/kg	41.16±0.49	15.00 ± 0.40	47.25 +1.04
Garlic juice	2ml/kg	50.53±0.86	17.68 ±0.815	57.85 ± 0.47*
Garlic juice	5ml/kg	59.48± 0.68**	21.07 +0.510*	69.41 ± 1.11**
Allicin	10 mg/kg	67.22 ± 1.03**	26.04 ± 0.21**	87.23 ± 1.60**

All values expressed as Mean ± S.E.M. (n=6 in each group). *p <0.05= significant, **p<0.01 = High significant as compared to vehicle control (saline) by one-way ANOVA followed by Dunnett multiple comparison test



All values expressed as Mean ± S.E.M. (n=6 in each group). *p <0.05= significant, **p<0.01 = High significant as compared to vehicle control (saline) by one-way ANOVA followed by Dunnett multiple comparison test

Table 5: Effect on uterus in normal female rats

Treatment	Dose	Diameter of uterus (µM)	Thickness of endometrium (µM)	Height of endometrical epithelium (µM)
Control	4ml/kg	1587.84 ± 43.30	424.62 ± 32.61	15.71 ± 0.63
Garlic juice	2ml/kg	1655.80 ± 63.94	416.34 ± 29.94	16.21 ± 0.59
Garlic juice	5ml/kg	1417.22± 34.17*	398.30 ± 0.32	12.62 ± 0.80*
Allicin	10 mg/kg	1348.18 17.95**	355.11 ± 15.29	9.02 ± 0.52**

All values expressed as Mean ± S.E.M. (n=6 in each group). *p <0.05= significant, **p<0.01 = High significant as compared to vehicle control (saline) by one-way ANOVA followed by Dunnett multiple comparison test

Table 6: Effect on ovary in normal female rats

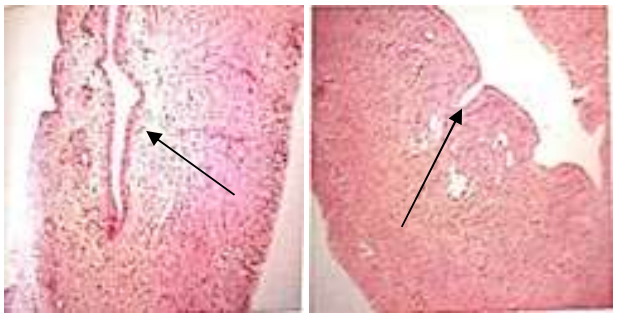
Treatment	Dose	No.of primary Follicles/cm³	No.of secondary Follicles/cm³	No.of tertiary Follicles/cm³	No.of corpus Luteum/cm³
Control	4ml/kg	2.83 ± 0.49	1.52 ± 0.22	1.28 ± 0.28	2.81 ± 0.15
Garlic juice	2ml/kg	3.37± 0.81	1.57 ± 0.52	0.73 ± 0.17	1.71 ± 0.40
Garlic juice	5ml/kg	3.68±0.47	1.68 ± 0.37	1.05 ± 0.25	1.33 ± 0.13*
Allicin	10 mg/kg	3.89±0.41*	2.62±0.33*	1.79 ± 0.82*	1.94 ± 0.10*

All values expressed as Mean ± S.E.M. (n=6 in each group). *p <0.05= significant, **p<0.01 = High significant as compared to vehicle control (saline) by one-way ANOVA followed by Dunnett multiple comparison test

Table 7: Effect of raw garlic juice in serum estrogen and progesterone in rats

Treatment	Dose	Estrogen (ng/ml)	Progesterone (ng/ml)
Control	4ml/kg	2.91 ± 1.09	14.52 ± 1.18
Garlic juice	2ml/kg	0.93 ± 0.37	10.63 ± 0.77
Garlic juice	5ml/kg	0.62 ± 0.43*	13.44 ± 0.32
Allicin	10 mg/kg	0.40 ± 0.25**	28.80 ± 0.83**

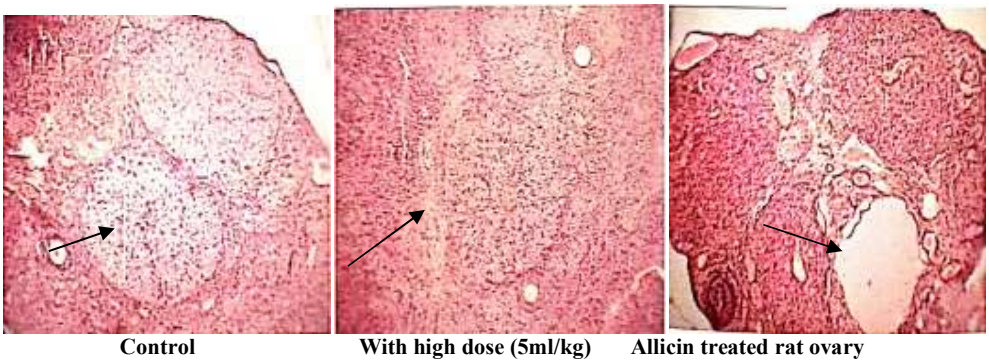
All values expressed as Mean ± S.E.M. (n=6 in each group). *p <0.05= significant, **p<0.01 = High significant as compared to vehicle control (saline) by one-way ANOVA followed by Dunnett multiple comparison test



Uterus in normal rats **Rat uterus with high dose (5ml/kg)**

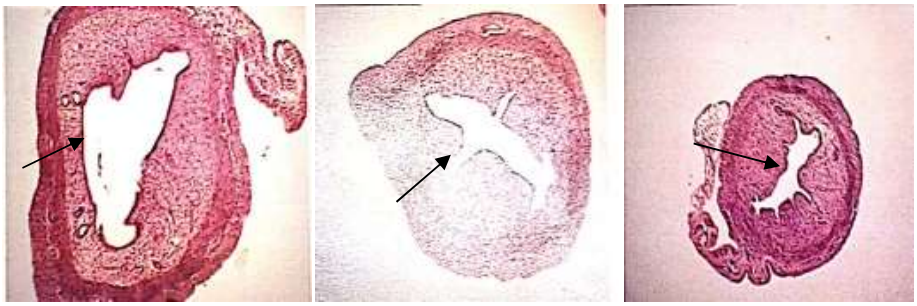
Allicin treated rat uterus

Figure 1: Effect of Allicin and garlic juices on uterus in immature ovariectomized rats (magnification at 25 µm)



Control **With high dose (5ml/kg)** **Allicin treated rat ovary**

Figure 2: Effect on ovary in matured non-ovariectomized rats (magnification at 25 µm)



Control **With high dose (5ml/kg)** **Allicin treated rat Uterus**

Figure 3: Effect on Uterus in matured non-ovariectomized rats (magnification at 25 µm)

The present study was carried out to evaluate the anti-fertility activity of *Allium sativum* (garlic) in female rats. The results indicate that raw juice of *Allium sativum* and also isolated allicin possesses good anti-fertility effect. The raw garlic juice and allicin were effective in reducing the number of implants and the number of litters delivered. The juice was also effective in increasing the diameter of the uterus and the endometrial thickness in immature ovariectomized rats but was lesser than isolated allicin. The juice also produced an increase in the weight of genital organs in matured ovarietomized rats. The proestrous phase was increased and metaestrous phase was reduced by raw garlic juice. The juice produced opposite effect on the uterus when administered to normal female rats when compared to effect on ovariectomized rats. The uterine diameter and the height of endometrial epithelium were reduced. The similar results were also reported by the earlier literatures **[20-22]**.

Thereafter, the anti-implantation and abortifacient effect is usually produced due to an increase in uterine contractions. The contractions of the uterus may be increased either due to reduction in progesterone secretion or due to progesterone receptor blockade. Progesterone is known as the 'hormone of pregnancy' and any change in the progesterone level will lead to inhibition of implantation of fertilized ova and if the ova is already implanted, it leads to detachment of the ova from the uterine wall **[23]**. Uterine contraction can also be induced by the direct action of the drug. Some of the agents that induce contraction of the uterus and resultant abortion are the prostaglandins, ergot alkaloids and oxytocin **[24]**. Apart from induction of uterine contractions, failure of fertilized ova implantation may also occur due to changes in the uterine structure that occurs due to imbalance in the estrogen/progesterone ratio **[25]**. The estrogenic / anti-estrogenic effect was carried out in ovariectomised immature female rats. The raw garlic juice as well as isolated allicin increased the uterine diameter and the endometrial thickness indicating that the drug possesses estrogenic effect. The estrogenic effect of the drug was confirmed by the ponderal changes induced in mature ovariectomized rats. The juice produced an increase in weight of genital organs namely, the vagina, cervix and the uterus. Study on the effect on estrous cycle revealed that garlic juice increased proestrous and decreased the metaestrous phase of estrous cycle with no significant effect on diestrous and frankestrous phases **[26]**. Despite showing an estrogenic effect in immature and mature ovariectomized rats, the drug failed

to increase the diestrous phase. When the drug was administered to mature non-ovariectomized rats, the diameter of the uterus and height of the endometrial epithelium was significantly reduced. This is just opposite to those observed in ovariectomized animals. Apart from this, the number of corpus lutea was reduced. It is presumed that the drug may act as partial agonist and may produce an anti-estrogenic effect in the presence of endogenous estrogen thereby producing a decrease in the weight of the uterus and the endometrial epithelial height. This is supported by the fact that high dose of garlic showed a decrease in uterine diameter while the low dose showed a non-significant increase in the uterine diameter and endometrial epithelial height was observed. Such effects have been observed with other plants **[27, 28]**. Estrogen is known to play important role in the maturation of follicles. The decrease in the number of corpus lutea may also be due to partial agonistic activity. Although the drug did not show any significant reduction in the number of secondary and matured follicles, the number of corpus lutea were significantly reduced. The garlic juice produced a significant decrease in serum estrogen levels and the effect on progesterone levels was not clear with low dose showing significant decrease and the high dose showing a significant increase. The effect on estrogen level may be due to partial agonistic activity **[29]**.

Finally, the results of the present study supports the traditional belief that garlic possess anti-fertility and abortifacient activity as is used in Unani medicine and traditionally in different parts of the world that includes India, Indonesia, South Korea, Mexico to name few. Further studies on the effect of isolated components from garlic should be carried out to determine the exact constituent(s) responsible for anti-fertility effect.

CONCLUSION

The raw garlic juice showed dose-dependent anti-fertility effect but was lesser than isolated allicin from garlic. It reduced the number of implants and the number of litters delivered when administered for seven days to pregnant rats. The juice produced estrogenic effect in both immature and mature ovariectomized rats as indicated by an increase in the diameter of the uterus and the endometrial thickness in immature ovariectomized rats and an increase in the weight of genital organs in matured ovarietomized rats. It produced an increase in the proestrus phase and a decrease in metaestrus phase in estrous cycle study. In non-ovariectomized rats, the raw garlic juice produced opposite effect on the uterus when compared to effect on ovariectomized rats, the uterine diameter and the height of endometrial epithelium

were reduced. Finally, it was concluded that garlic in high doses and isolated compound allicin possess anti-fertility effect in female rats.

REFERENCES

[1] Khushalani H, Tatke P, Singh KK. Antifertility activity of dried flowers of *Woodfordia fruticosa* kurz. Indian J Pharm Sci. 68(4), 2006, 528-29.

[2] Shibeshi W, Makonne E, Zerihum L, Debella A. Effect of *Achyranthes aspera* L. on fetal abortion, uterine and pituitary weights, serum lipids and hormones. Afr Health Sci. 6(2), 2006, 108-112.

[3] Shaik A, Kanhere RS, Cuddapah R, Nelson KS, Vara PR, Sibyala S. Antifertility activity of *Artemisia vulgaris* leaves on female wistar rats. Chin J Nat Med. 12(3), 2014, 180-185.

[4] Mani M, Rathore DS. Pharmacological evaluation of anti fertility effect of stem bark of *Ailanthus altissima* in Wistar albino rats. Research J Pharm. And Tech. 9(5), 2016, 497-500.

[5] Montaserti A, Pourheydar M, Khazaei M, Ghorbani R. Anti-fertility effects of *Physalis alkekengi* alcoholic extract in female rat. Iran J Reprod Med. 5(1), 2007, 13-16.

[6] Izzo AA. Interactions between Herbs and Conventional Drugs: Overview of the Clinical Data. Med Princ Pract. 21, 2012, 404-428.

[7] Majewski M. *Allium sativum*: facts and myths regarding human health. Rocz Panstw Zakl Hig. 65(1), 2014, 1-8.

[8] Rahman MS. Allicin and Other Functional Active Components in Garlic: Health Benefits and Bioavailability, International Journal of Food Properties. 10(2), 2007, 245-68.

[9] Khubber S , Hashemifesharaki R, Mohammadi M, Mohammad S, Gharibzahedi T. Garlic (*Allium sativum* L.): a potential unique therapeutic food rich in organosulfur and flavonoid compounds to fight with COVID. Nutr. J. 19, 2020, 20-22.

[10] Shekh S, Reddy KKA, Gowd KH. In silico allicin induced S - thioallylation of SARS-CoV-2 main protease**.** J. Sulfur Chem. 2020, 1-12.

[11] Kwak JS , Kim JY , Paek JE , Lee YJ , Kim HR, Park DS, Kwon O. Garlic powder intake and cardiovascular risk factors: a meta-analysis of randomized controlled clinical

trials. Nutr. Res. Pract. 8, 2014, 644-654.

[12] Tripathi K. Garlic, the spice of life-(part -1). Asian J. Res. Chem. 2, 2009, 8-13.

[13] Mohammad P, Zaman R, Mohammed AS, Haque MA, Rashid MA. Antifertility Activity of Methanol Bulb Extract of *Allium sativum* on Swiss Albino Male Mice and Teratogenic Effect on Neonates of female Mice. Global Journal of Pharmacology. 9(3), 2015, 272-277.

[14] Ugwah-Oguejiofor CJ, Okoli CO, Ugwah MO, Umaru ML, Ogbulie CS, Mshelia HE, Umar M, Njan AA. Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats. Heliyon. 5(1), 2019, e01179.

[15] Rani S, Manavalan R, Kilimozhi D, Balamurugan K. Preliminary study on the anti - implantation activity of *Leptadenia reticulata* in female rats. International Journal of PharmTech Research. 1(4), 2009, 1403-1405.

[16] Jawaid T, Awasthi A, Kamal M. Estrogenic activity of a hydro-alcoholic extract of *Bambusa arundinaceae* leaves on female wistar rats. J Adv Pharm Technol Res. 6(1), 2015, 19-24.

[17] Rose Mafo A, Wisdom Otaru O , Fatima Ajuma S, Theophilus Boniface M, Precious Adejoh I. Evaluation of the Fertility Activity of Aqueous Leaf Extract of *Desmodium velutinum* in Male Wistar Rats. Int. J. Adv. Res. Biol. Sci. 7(9), 2019, 1-8.

[18] Ngadjui E, Watcho P, Nguelefack TB, Kamanyi A. Effects of *Ficus asperifolia* on normal rat estrus cyclicity. Asian Pac J Trop Biomed. 3(1), 2013, 53-57.

[19] Adewale OO, Oduyemi OI, Ayokunle O. Oral administration of leaf extracts of *Momordica charantia* affect reproductive hormones of adult female Wistar rats. Asian Pac J Trop Biomed. 4(Suppl 1), 2014, S521-S524.

[20] Vijayanarayana K, Rodrigues RS, Chandrashekhar KS, Subrahmanyam EV. Evaluation of estrogenic activity of alcoholic extract of rhizomes of *Curculigo orchioides*. J.Ethnopharmacol. 1(1 14), 2007, 241–245.

[21] Ghasemi A, Jeddi S, Kashfi K. The laboratory rat: Age and body weight matter. EXCLI J. 20, 2021,1431-1445.

[22] Ahirwar D, Ahirwar B, Kharya MD. Influence of *Calotropis procera* roots on biochemistry of reproductive organs of ovariectomized rats. Indian J Pharm Sci. 69(3), 2007, 459-461.

[23] Thiyagarajan DK, Basit H, Jeanmonod R. Physiology, Menstrual Cycle. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK500020/

[24] Liabsuetrakul T, Choobun T, Peeyananjarassri K, Islam QM. Prophylactic use of ergot alkaloids in the third stage of labour. Cochrane Database Syst Rev. 6(6), 2018, CD005456.

[25] Simon A, Laufer N. Assessment and treatment of repeated implantation failure (RIF). J Assist Reprod Genet. 29(11), 2012, 1227-1239.

[26] Ekambaram G, Sampath Kumar SK, Joseph LD. Comparative Study on the Estimation of Estrous Cycle in Mice by Visual and Vaginal Lavage Method. J Clin Diagn Res. 11(1), 2017, AC05-AC07.

[27] Martí A, Serrano A, Pastor J, et al. Endometrial Status in Queens Evaluated by Histopathology Findings and Two Cytological Techniques: Low-Volume Uterine Lavage and Uterine Swabbing. Animals (Basel). 11(1), 2021, 88.

[28] Wang D, Cheng X, Fang H, Ren Y, Li X, Ren W, Xue B, Yang C. Effect of cold stress on ovarian & uterine microcirculation in rats and the role of endothelin system. Reprod Biol Endocrinol. 2020,18, 29.

[29] Xiong R, Patel HK, Gutgesell LM, Zhao J, Delgado-Rivera L, Pham T, Zhao H, Carlson K, Martin T, Katzenellenbogen JA, Moore TW, Tonetti DA, Thatcher G. Selective Human Estrogen Receptor Partial Agonists (ShERPAs) for Tamoxifen-Resistant Breast Cancer. Journal of medicinal chemistry. 59(1), 2016, 219–237.