

The Effects of Aqueous Extract of *Allium sativum* (Garlic) on Some Aspects of Reproduction in the Female Albino Rat (Wistar Strain)

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Abstract: *Allium sativum* (Garlic) is one of the plants used as a therapeutic agent in many cultures. The current study was to investigate the effect of its extract on some aspects of reproduction in the female albino rat (Wistar strain). Seventy-five female albino rats which were in proestrus divided into five groups fifteen each. Group A was given distilled water as control while groups B, C, D and E were given oral daily dose, 200, 400, 600 and 800 mgs/kg body weights of aqueous garlic extract respectively, for twenty-eight days (phase 1) and throughout the gestation periods of 28-30 days (phase 2). Five rats in each group were sacrificed at day fourteen and day twenty-eight for gross and histological examinations during the first phase. The remaining five rats (in each group) were mated at the second phase. Results revealed significant reduction in weights of uterine body and cervix in group E ($P < 0.05$) as compared to groups A, B, C and D while the ovaries did not show any significant change. Significant increase in length of right uterine horn and decrease in length of left uterine horn ($P < 0.05$) were observed in groups B, C, D and E compared with group A. Histological findings revealed no pathological lesions in the genitalia of all the rats. Mated rats all conceived without abortion. There were no significant differences ($p > 0.05$) in litter size, live birth weight and neonatal mortality across the groups. These findings suggest that aqueous garlic extracts have no deleterious effect on the reproductive performance of female rat.

Key words:

INTRODUCTION

In recent years, research work threw 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 [1]. Garlic is one of such plants used as biennial medicinal plant that has been described in the folklore of many cultures as a therapeutic agent, [2]. The plant extract has been reported to have antifungal, antibacterial, antiviral and anticancer activities [3]. Some studies suggest that garlic has potential antioxidant, hypoglycemic, hypotensive and hypothrombotic properties [4]. It has been used to treat cardiac diseases successfully [5] and its gender effect in chronic heart diseases has been reported in favor of women [6]. 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 [3, 7]. The effects of garlic on male reproductive performance have been extensively studied. Garlic was found to be effective in the recovery of testicular functions and spermatogenesis in rats [8]. Morakinyo *et al.*, reported 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 [9]. Garlic extract inhibited the growth of transplanted tumor cells and enhanced killer cell activities in experimental rats [10]. Reports on the effect of garlic on female reproductive performance are yet to be established. This study was undertaken to investigate the possible effects of the aqueous extracts of garlic on some aspects of female fertility using rats as a model.

MATERIALS AND METHODS

Animal: The study was carried out on adult female albino rats (*Rattus Norvegicus*) 120 gram body weight obtained from the animal house breeding unit of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan Nigeria. Animals were housed in the experimental animal unit of under standard environmental conditions (12 hr light and 12 hr dark cycle) and hygiene, fed on commercial rat pellets and water *ad libitum*. The oestrus cycles of the animals were assessed by daily vaginal smears for three consecutive four-day oestrus cycles before the start of administration.

Collection and Preparation of Aqueous Garlic Extracts:

Fresh garlic bulbs were purchased and authenticated at the herbarium of the Botany department of the University of Ibadan, then washed in distilled water and allowed to drain for an hour at room temperature (27°C) before peeling with a clean knife. 50 g of garlic was measured using a weighing balance (Lark^R digital weighing balance LP502A, 500G/0.01g) and then sliced into small pieces and ground in a clean mortar pestle to produce a fine paste. 100 mls of distilled water was then added to the garlic paste and filtered with a filter paper into a conical flask to obtain aqueous garlic extract (stock solution). This was stored at 4°C till use. A fresh preparation was made as needed. Aqueous extract was administered to the rats with the aid of a modified Tuberculin syringe.

Experimental Design: Seventy-five female rats were divided into five groups of fifteen rats each the experiment was carried out in two phases.

Phase 1: Group A (control) were given distilled water while groups B, C, D and E received 200, 400, 600 and 800 mg/kg body weights of aqueous garlic extracts respectively for 28 days. Five rats from each group were sacrificed by cephalic dislocation for gross and histological details at day 14 and day 28. The morphological changes, weights, diameters, lengths of the ovaries, uterine horns, uterine bodies and cervixes were measured, recorded and subjected to statistical analysis.

Histology: The ovaries, uterine horns, uterine bodies and cervixes and vaginae were dissected and processed for histopathology Briefly the tissues were fixed in Bouin's fluid and later dehydrated in different concentrations of ethanol (70%, 75%, 100%), cleared in three changes of xylene for one hour and infiltrated in paraffin wax using L-mould, then cooled to make blocks. Sectioning and

trimming were done, de-waxed, for fifteen minutes, processed, stained with Haematoxylin and Eosin and viewed under microscope.

Phase II: During the second phase, the remaining five rats in each group were mated with fertile males (one male for each group). Doses of aqueous garlic extracts as done in phase 1 were given to the rats in each group throughout the gestation period of 28-30 days. The rats were observed for conception rates, early embryonic deaths, abortion rate, live birth weights, litter size and neonatal mortality.

Mating: Confirmation of mating was done by putting a dark paper under the breeding cage 12-24 hours post coital, where the white discharges was observed on the dark paper as well as the waxy copulatory plug was found in the vagina of the rats.

Conception Rate: Conception was confirmed by the presence of sperm cells in fresh vaginal smear made on a clean slide and observed under the x10 magnification of the wide angle eyepiece of the light microscope and/or the presence of a copulatory (vaginal) plug. This also marked the day one of pregnancy.

Pregnancy and Early Embryonic Death: The mammary glands of all the mated rats were observed to be swollen and protruded fifteen days after mating. This confirmed pregnancy and that there was no early embryonic death [12]. The live birth weights of all the pups were measured using digital weighing balance. The litter sizes and neonatal mortalities of the rats were adequately recorded and subjected to statistical analysis.

Data Analysis: Data obtained were analyzed using the Analysis of Variance (ANOVA) and the means were separated by Duncan multiple range test to obtain the significance of the effects of garlic treated rats of the experimental group compared with the control rats given distilled water.

RESULTS AND DISCUSSIONS

The results of the parameters investigated in this study are presented in two phases.

Phase I: The Mean \pm SD for female rat genitalia parameters obtained during the first phase of this study are presented in Table 1. Mean with the same superscript on the same column are not statistically different ($P > 0.05$).

Table 1: The effects of aqueous garlic extract on female rats genitalia

Treatment groups(n=5)	Weight of ovaries(gms)	Weight of uterus(gms)	Weight of cervix(gms)	Length of RUH(cm)	Length of LUH(cm)
A	0.028±0.01	0.030±0.01 ^a	0.025±0.01 ^a	2.65±0.31 ^a	3.05±0.33 ^a
B	0.018±0.01	0.028±0.01 ^a	0.020±0.01 ^b	2.83±0.34 ^b	2.83±0.29 ^b
C	0.023±0.01	0.023±0.01 ^a	0.018±0.01 ^b	2.93±0.38 ^b	2.73±0.45 ^b
D	0.025±0.01	0.020±0.01 ^a	0.018±0.01 ^b	3.18±0.33 ^c	2.38±0.48 ^c
E	0.025±0.01	0.012±0.01 ^b	0.015±0.01 ^c	3.23±0.33 ^c	2.35±0.21 ^c

RUH-right uterine horn, LUH-left uterine horn

Table 2: The effects of aqueous garlic extracts on reproductive parameters measured

Treatment groups(n=5)	Litter size	Live birth weight	Neonatal mortality
A	5.75±0.48	5.25±0.32	0.50±0.29
B	6.00±0.41	5.38±0.31	0.50±0.29
C	5.75±0.48	5.50±0.35	0.50±0.29
D	5.75±0.48	5.13±0.31	0.50±0.29
E	6.00±0.41	5.25±0.32	0.25±0.25

Litter size, live birth weight and Neonatal mortality were not significant (P > 0.05)

The differences in mean values obtained in groups A-E for ovarian weights were not significant (p>0.05). The mean values obtained in group E for uterine body weights (0.012±0.01) were significantly different (p<0.05) from the mean values obtained in groups A-D respectively. The difference in the mean values obtained in group E for weight of cervix, (0.015 ± 0.01) was highly significant (p<0.05) from the mean values obtained in group A (0.025 ± 0.01) and was also significantly different from the mean values obtained in groups B-D respectively (0.020 ± 0.01, 0.018 ± 0.01, 0.018 ± 0.01). The mean values obtained in group D and E respectively for the length of the right uterine horn (3.23 ± 0.33, 3.18 ± 0.33) were significantly higher (p<0.05) from the mean values obtained from the group B and C respectively (2.83 ± 0.34, 2.93 ± 0.38) and

also significantly different (p<0.05) from the mean values of group A (2.65 ± 0.31). The mean values obtained from groups D and E respectively for the left uterine horn (2.38 ± 0.41, 2.35 ± 0.21) were significantly different (p<0.05) from the mean values obtained from groups B and C (2.83 ± 0.29, 2.73 ± 0.45).

Phase II: Table 2: Presents the Mean ± SD of the effect of garlic extract on reproductive parameters measured during second phase of this study (p>0.05).

The differences in the mean values obtained from the reproductive parameters measured during the second phase of this study in groups A-E for litter size, live birth weight and neonatal mortality were similar and not significant (P >0.05).

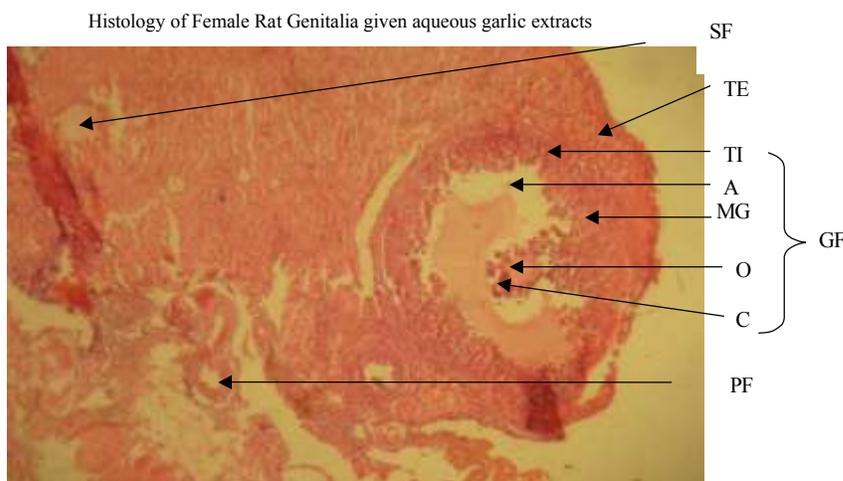


Fig. 1: Photomicrograph of ovary of rat administered with aqueous garlic extract H and E (x100) Containing different follicles at various stages of development; primary, secondary and matured (Graffian) follicles. There were no pathological lesions.

TE-Theca externa, TI-Theca interna, A-Antrum, MG-Membrana granulosa, O-Oocyte cytoplasm, C-Corona radiata, SF-Secondary follicle, PF-Primary follicle, GF-Graffian follicle..

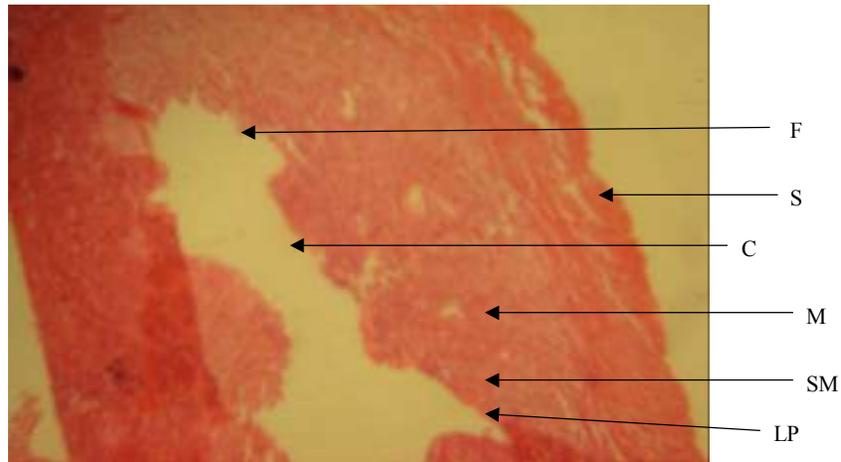


Fig. 2: Photomicrograph of uterine tube of rat administered with aqueous garlic extract H and E (x100). There were no pathological lesions.

F-Folds, S-Serosa, C-Cilia, M-Muscularis, SM-Submucosa, LP-Lamina propria

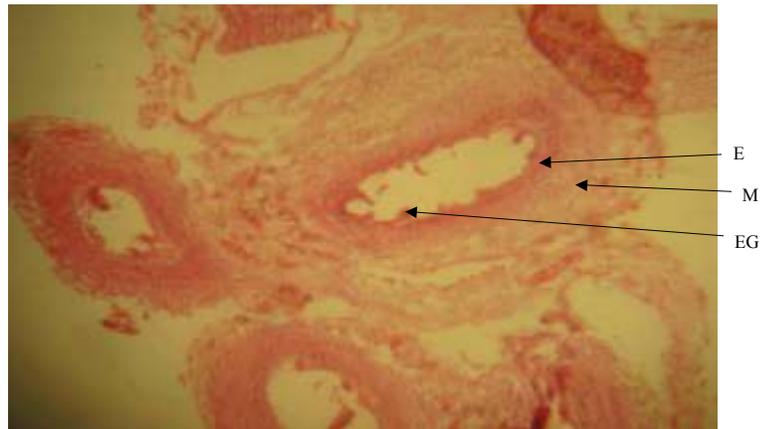


Fig. 3: Photomicrograph of uterus of rat administered with aqueous garlic extract H and E (x100).

E-Endometrium, M-Myometrium, EG-Endometrial gland. There were no pathological lesions.

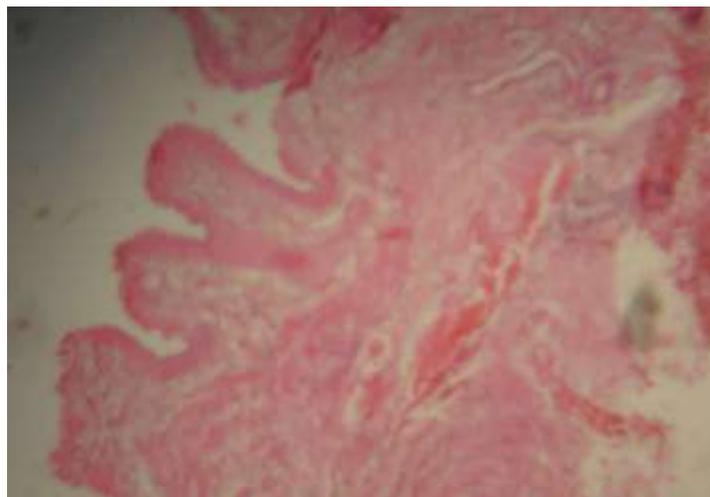


Fig. 4: Photomicrograph of cervix of rat administered with aqueous garlic extract H and E (x100).

The epithelial lining is thrown into deep furrows giving the appearance of branched tubular gland. There were no pathological lesions.

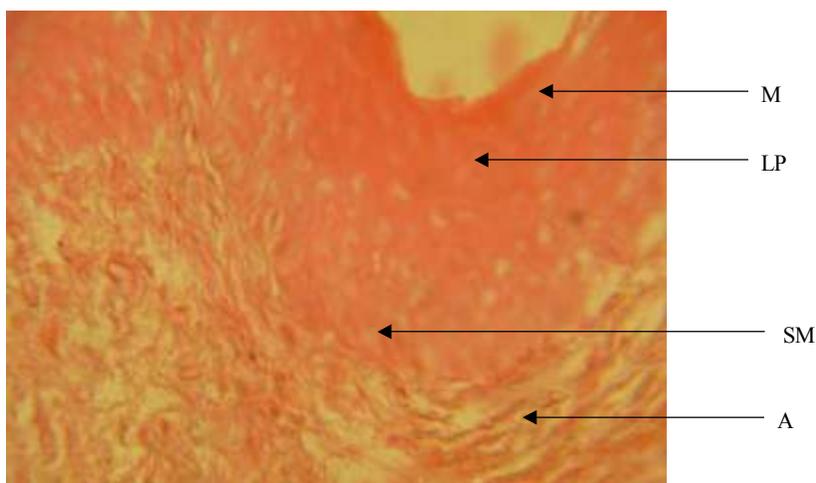


Fig. 5: Photomicrograph of vagina of rat-administered garlic extracts H and E (x100). There were no pathologic lesions. M-Mucosa, LP-Lamina propria, SM-smooth muscle layers, A-Adventitia

Recently it has been found that some of the common plants consumed by man and animals as food and medicine possess deleterious systemic effects, [13]. Garlic has been used as spices in foods and for medicinal purposes as it has antibiotic, antiviral and antifungal properties [14]. The current study revealed that administration of garlic extract has no harmful effect on femal reproductive tract, there were no significant differences in the ovarian weights of the control and experimental rats ($p > 0.05$). other herbal remedies such as *Anethum graveolens* (Dill) showed no harmful effect on ovaries [15]. On the other side there some plants have deleterious effect on ovaries such as seed extracts of *Momordica charantia* [16] while Shweta and Bahava reported significant increase ($p < 0.05$) in weights of ovaries of rats treated with extracts of *Carum carvi* and *Curcuma longa* [17]. The uterine body weights of the rats given the 800 mg/kg were significantly lower than the rats given water and those given 200 mg/kg, 400 mg/kg and 600 mg/kg body weights aqueous extracts of *Allium sativum* ($p < 0.05$). This implied decrease in uterine body weights as a result of the garlic extracts. This is similar to the findings on the aqueous extracts of *Aloe buettneri*, *Dicliptera verticillata*, *Justicia insularia*, *Hibiscus macranthus* which also resulted in significant decrease in weights of uterine body of dosed rats [18]. However, the extract of *Carum carvi* and *Curcuma longa* showed significant increase in uterine body weights of treated rats [17]. Result from this study also showed that there were significant reduction in weights of the cervixes of the rats dosed with 800 mg/kg aqueous garlic extract when compared to the control and the other groups ($p < 0.05$). Kuniyoshi observed significant increase in weights of

cervix in rats dosed with ethanol extract of *Ganoderma lucidum* [19]. There were significant increases in the lengths of the right uterine horns and significant decreases in the lengths of the left uterine horns of the dosed rats especially at the 800 mg/kg and 600 mg/kg body weights when compared to the control, 200 mg/kg and 400 mg/kg body weights groups ($p < 0.05$). This requires further investigation. However, Chukwuka and Thomas, [20] reported reduction in size of uterine horns in ovariectomized rats dosed with aqueous extracts of *Spondias mombin* (Anacardiaceae). The conception rate was 100% for the control and experimental rats. This suggests that the garlic extract did not have any anti-conception effect on the treated rats. This is similar to the findings of Dileep and Oomen, [21] that successful mating and conception rates were observed in Swiss albino mice dosed with aqueous extract of *Stevia rebaudiana bertani*. Ezumi *et al.*, [22] also reported successful mating and conception rates in Sprague Dawley rats dosed with aqueous extract of *Labisia pumila* var. *alata*. There was neither early embryonic death nor abortion in the control and experimental rats suggesting that the garlic extract did not have any abortifacient effect on the pregnancies of dosed rats. Also Ezumi *et al.* could not trace abortion in rats dosed with *Labisia pumila* [22] while Lohiya *et al.*, reported that extracts of *Carica papaya* (pawpaw) caused abortion in langue monkey [23]. There were no significant differences in the litter sizes of the control and experimental rats suggesting that the garlic extracts did not have any effect on the fecundity of the experimental rats. This is similar to the findings by Ezumi *et al.*, [22] that *Labisia pumila* extract did not result in any change in litter size of dosed rats when compared to the control.

There was no change in litter size in rats dosed with aqueous extracts of *Alligator pepper* (Zingiberaceae) and *Aframomum melagnata* [24]. Carina *et al.*, [25] also observed no alterations in litter size in the control and experimental rats dosed with hydro-alcoholic extract of the root of *Petiveria alliacea* L. (Phytolaccaceae). However, there was significant increase in litter size in rats dosed with extracts of *Ficus asperitolia* Pierre *et al.*, [26]. Olayaki *et al.*, [27] also observed increase in litter size in rats dosed with aqueous extract of *Occimum gratissimum* (Efirin). Significant reduction in litter size was observed in Wister rats dosed with extracts of *Dendrophthoe falcate* (L.f) Ettingsh [28]. There were also no significant differences in the live birth weights of the control and rats dosed with aqueous garlic extract. This is similar to the findings by Ezumi *et al.*, [22] that *Labisia pumila* extract did not result in any change in live birth weights of dosed rats when compared to the control. Carina *et al.*, [25] also reported similarly that there were no alterations in the live birth weights of control and rat dosed with extract from the root of *Petiversia alliceae* L. (Phytolaccaceae). However, decreased live birth weight was observed in rats dosed with extracts of *Occimum gratissimum* Olayaki *et al.*, [27] while Pierre *et al.*, [26] reported increase in live birth weights in rats dosed with *Fiscus asperitolia* (Moraceae). Results obtained also revealed that there was no significant difference in neonatal mortality in control and rats dosed with aqueous garlic extract. A similar finding of non-neonatal mortality has been reported in rats that consumed black seed and garlic for two consecutive pregnancies [29]. Histological findings revealed normal volumes of ovaries, secondary and graffian follicles in the control and dosed rats. This is similar to the findings of Monsefi, [15] that extracts of *Anethum graveolens* (Dill) did not result in any change in volumes of ovaries, primary, secondary and graffian follicles. However, decrease in number of developing follicles, graffian follicles, corpus luteum and an increase in number of atretic follicles were reported in the histological findings of rats dosed with seed extracts of *Momordica charantia* Sharanabasappa *et al.*, [16]. Histological findings did not reveal any pathologic lesions in the oviduct, uterus, cervix and the vagina of the control and experimental rats.

The findings of this study did not reveal any toxic or pathologic effect of aqueous garlic extract on the dosed rats. This is similar to the report by Obuchi *et al.*, [30] that garlic does not have any deleterious effect on the female reproduction and could possibly be used in treating diseases associated with pregnancy or during pregnancy.

CONCLUSION

It is concluded that the aqueous extract of *Allium Sativum* does not have any deleterious effect on the reproductive performance of female rat (Wistar strain). However, the significant increase in the lengths of the right uterine horns and decrease in the lengths of the left urine horns ($p < 0.05$) as well as the significant decrease in the weights of the ovaries and the cervixes ($p < 0.05$) of rats dosed with the aqueous extracts of garlic is a relatively new finding and requires further investigation to authenticate the findings.

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