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Reproductive Responses to Varying Doses of *Curcuma longa* Extract in Female Wistar Rats

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Abstract— Background: *The popular use of Curcuma longa (turmeric) has been attributed to its several acclaimed health benefits. Thus, the present study aimed toinvestigate the effect of methanolic extract of Curcuma longa on reproductive hormones and fertility in female Wistar rats.* ***Materials and Methods:*** *A total of thirty five (35) Wistar rats weighing between 120-150g were used in the study, 25 female rats were randomly separated into 5 experimental groups of 5 rats each, and ten (10) male rats served the purpose of pairing with the females for the fertility study. Groups one and two received distilled water and Norgestrel + ethinylestradiol 0.21mg/kg respectively. While Groups 3, 4 and 5 were treated with 250mg/kg, 500mg/kg, and 750mg/kg body weight of Curcuma longa rhizome extract respectively. After a 6-week period of experimental study, the animals were sacrificed under standard procedure and blood samples collected and put into properly labeled plain sample bottles which were thereafter centrifuged at 3000 r/min for 10 minutes. The respective sera were then assayed for FSH, LH, oestrogen and progesterone using the Accubind Elisa Microwells (Monobind) method. Quantitative data obtained from the study were subjected to statistical analyses using the statistical package for social sciences version 20.0 tools.* ***Results:*** *The results showed FSH level in group 5(750mg/kg, CL) to be significantly ($p<0.05$) higher when compared to those of groups 1 and 2. LH levels were also significantly ($p<0.05$) higher in groups 4 and 5 compared to groups 1 and 2. Progesterone level was significantly ($p<0.05$) low in groups 3(250mg/kg, CL) and 4(500mg/kg, CL) compared to that of the standard contraceptive treated group (Group 2) but high in group 5 when compared with the control. Oestrogen level was seen to be significantly ($p<0.05$) low in group 3 compared to those of groups 1 and 2. The outcome on mating success indicated 75%, 100%, 50%, 100%, 100% for groups 1, 2, 3, 4 and 5 respectively and fertility successes of 100%, 0%, 100%, 50%, and 75% for groups 1, 2, 3, 4 and 5 respectively.* ***Conclusion:*** *The attributes of the extract is suggestive of dual effects on the female reproductive system of the Wistar rats: fertility boosting effect at low dose and a seeming declining fertility success effect at higher doses.*

Keywords— *Curcuma longa; fertility; reproductive hormones; reproductive response.*

I. INTRODUCTION

The therapeutic properties of plants have been relied on by man since ancient times for the treatment of diseases as well as to regulate or boost fertility in ethnomedicinal practices. (1), (2). Regulation of reproduction is universal as it cuts across cultures, race, religion and gender (3), (4). Nowadays, improved contraceptives with established scientific mechanisms of action are commonly promoted and used (5). However, contraceptive herbs are often ignored and criticized due to the poor knowledge of their modes of actions and safety (6), (7). Conversely, the exploration and use of safe therapeutic plants are encouraged even by the World health Organization (WHO) (8), (9). In fact, due to rising adverse effects from huge and chronic dependence on different synthetic, it is now popularly suggested that safe and effective local plants can be substituted for synthetic drugs in the treatment of various sicknesses and diseases (10). (11), (12).

Today, the rekindled interest in the use and consumption of standardized herbal extracts in many parts of the world including developed nations is mainly due to reduced side effects, availability and affordability of herbs (13). Herbs such as garlic, ginger, ginseng, etc., have become popular for treatment of many health conditions (1), (14). *Curcuma longa* (*C. longa*) is a notable herbaceous plant with increasing popularity due to it many therapeutic benefits (15). It is commonly called turmeric, is a fat-soluble plant with polyphenolic pigmentation known as curcuminoids. This pigmentation is responsible for the plant's intensely yellow colour (16) and it was first isolated in 1842 (17) and it is a perennial plant that belongs to the *Zingiberacea* family. It is mainly cultivated in south and southeast tropical Asia, where it is known to originate 18).

The reproductive system, which is essential for the continuation of life, like other biological systems, can be prone to insults from severe environmental, exogenous and other factors (19). Hence the need to explore beneficial ways or agents that can boost optimal functioning of the reproductive system in especially fertility seeking individuals (20), (21), (22).

C. longa is known to possess constituents or metabolites that are active in relieving conditions such as malaria, diabetes, cancer, diarrhea, hypertension, etc. it is also believed to alter or modulate some functions of the reproductive systems (23), (2), (24), hence the need to scientifically investigate the methanolic

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extract of *Curcuma longa* on some reproductive hormones and fertility in female Wistar rats.

II. Materials and Methods

Research Design—The current study was an experimental and laboratory based study that used male and female Wistar rat models. The study was conducted in the Animal House of the Faculty of Basic Medical Sciences (FBMS), University of Port Harcourt, Nigeria. Prior to the commencement of the study, ethical approval was sought and obtained from the University of Port Harcourt Central Ethic Unit. The study lasted for a period of 56 days (14 days of acclimatization and 42 days of experimentations).

Collection, Identification and Preparation of Plant Extract

Rhizomes of *Curcuma Longa* were bought from Fruit Garden Market, Port Harcourt and voucher sample was deposited at the University of Port Harcourt Herbarium housed in the Department of Plant Science and Biotechnology for identification and authentication. Consequently voucher number—UPH/P/167 was issued. Thereafter, the rhizomes of *C. longa* were washed, air-dried and pulverized into powder form. The powdered form of the *C. longa* rhizome was then soaked in methanol for 72hours with periodic macerations. The extract obtained was filtered through Whatman paper to isolate the filtrate from the residue. The filtrate was concentrated in a rotatory evaporator to a minutest volume. A semi-solid form of the extract was recovered after the sample was put in a water bath for final evaporation of the methanol solvent. This method of extraction was adopted from an earlier study by Bekinbo et al., (24). The extract was then collected in clean and well labeled sample bottles and refrigerated at 4^{o}C prior to the commencement of experimentations.

Handling of Study Animals

A total of thirty five (35) Wistar rats weighing between 120-150g were used in the study, (25 of these rats were female and were randomly separated into 5 experimental groups of 5 rats each; the remaining 10 rats were males and only served the purpose of mating at the fertility experimental phase of the study). The animals were obtained from and housed in the FBMS Animal House under the 12 hour light/dark lighting condition and a humidity of about 25^{o}C. The animals were allowed access to normal pellet diet and tap water *ad libitum* throughout the period of the study.

Experimental Protocol

Prior to the fertility test phase of the study, all 25 female Wistar rats were randomly separated into 5 groups of 5 rats each. The remaining 10 male Wistar rats were separated from the female rats until the time of their mating. The doses of the extract were determined based on an earlier established LD_{50} of above 1000mg/kg by Govind, (25). The route of administration of the extract and the standard drug was per oral using oral gavage and the vehicle for the extract was dimethyl sulphur-oxide (DMSO). The treatment of the test groups of animal with the standard and different doses of the extract of *C. longa* was daily for 42 days.

Fertility Test Study

Pre-oestrus female rat were paired with male rats for 10days in a 2:1 ratio. Positive mating was confirmed by the existence of spermatozoa in vaginal smear or copulatory plug in vagina of female rats. Semen positive female rats were observed and the resultant pregnancies noted.

Reproductive parameters were computed using the following functions below (26), 27), (28):

Fertility success =Number pregnant /Number mated x 100

Mating success = Number mated/Number paired x 100

Animal Grouping

Group 1: served as Control and received 1ml Distilled water.
Group 2: served as Standard contraceptive treated group and received 1ml of 0.21mg/kg Norgestrel + ethinyestradiol
Group 3: served as treatment and received with 250mg/kg *C. longa* extract
Group 4: served as treatment group and received 500mg/kg *C. longa* extract
Group 5: served as treatment group and received 750mg/kg *C. longa* extract

Harvesting of Samples from the study animals

At the end of treatments, blood sample was collected via cardiac puncture and put into properly labeled plain bottle and thereafter centrifuged at 3000 r/min for 10minutes to separate serum from cells. The serum was then tipped into a separate vial and later subjected by Monobond Inc. ELISA method for assessment of LH, FSH, Progesterone and Oestrogen, following the kit manufacturer's guide.

Statistical Analysis

Quantitative data from the laboratory analysis of samples were statistically analyzed with statistical package for social science (SPSS) version 20.0. The ANOVA and Post Hoc LSD multiple comparison analyses were done and $p<0.05$ was considered to be statistically significant.

III. Results

As shown in Table 1, the levels of FSH and LH indicated dose-dependent increases across the different doses of the extract treated animals when compared to both the control and standard drug groups. On the other hand, the oestrogen levels of groups 3 and 4 (i.e. 250 and 500mg/kg treated groups) had significantly ($p<0.05$) decreased levels when compared to that of control. Only group 3 had significant decrease ($p<0.05$) in oestrogen level when compared to group 2 (the standard control). More so, the level of progesterone in group 3 was found to be significantly lower ($p<0.05$) when compared to that of group 1 rats (control). However, the progesterone levels in groups 4 and 5 (i.e. 500 and 750mg/kg Methanolic extract of Curcuma longa respectively treated rats) were remarkably raised ($p<0.05$) when compared to that of group 1. Both the 750mg/kg Methanolic extract of Curcuma longa (group 5) and the synthetic drug (group 2), had similar levels of increases in their progesterone concentrations when compared to that of group 1.

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The data in Figure 1 indicates the effects of methanolic extract of *Curcuma longa* on fertility. The fertility test results revealed the effects of the

Curcuma longa extract treatments on mating success, and fertility success in female Wistar rats. The study animals were grouped as follows:

TABLE 1. Mean values of female sex hormones in study animals treated with the extract

S/N	Groups	FSH (UL/L)	LH (UL/ L)	Oestrogen (pg/mL)	Progesterone (ng/mL)
1	**Control**	0.136 + 0.012	0.228 + 0.009	97.400 + 0.245	1.950 + 0.005
2	**Norgestrel+ethinylestradiol 0.21mg/kg**	0.176 + 0.033	0.210 +0.018	97.200 + 0.374	5.916 + 0.187*
3	**250mg/kg *C. longa* extract**	0.232 + 0.048	0.242 + 0.026	94.000 + 1.183*[a]	1.930 + 0.007[a]
4	**500mg/kg *C. longa* extract**	0.242 + 0.045	0.392 + 0.035[a*]	95.400 +0.510*	2.844 +0.628[a]
5	**750mg/kg *C. longa* extract**	0.460 + 0.060*[a]	0.612 + 0.038[a*]	98.000 + 0.632	5.4260 + 0.314*

*Mean values are significant at $p < 0.05$ when compared to control.
[a]Mean values are significant at $p < 0.05$ when compared to Norgestrel+ethinylestradiol (0.21mg/kg) group.

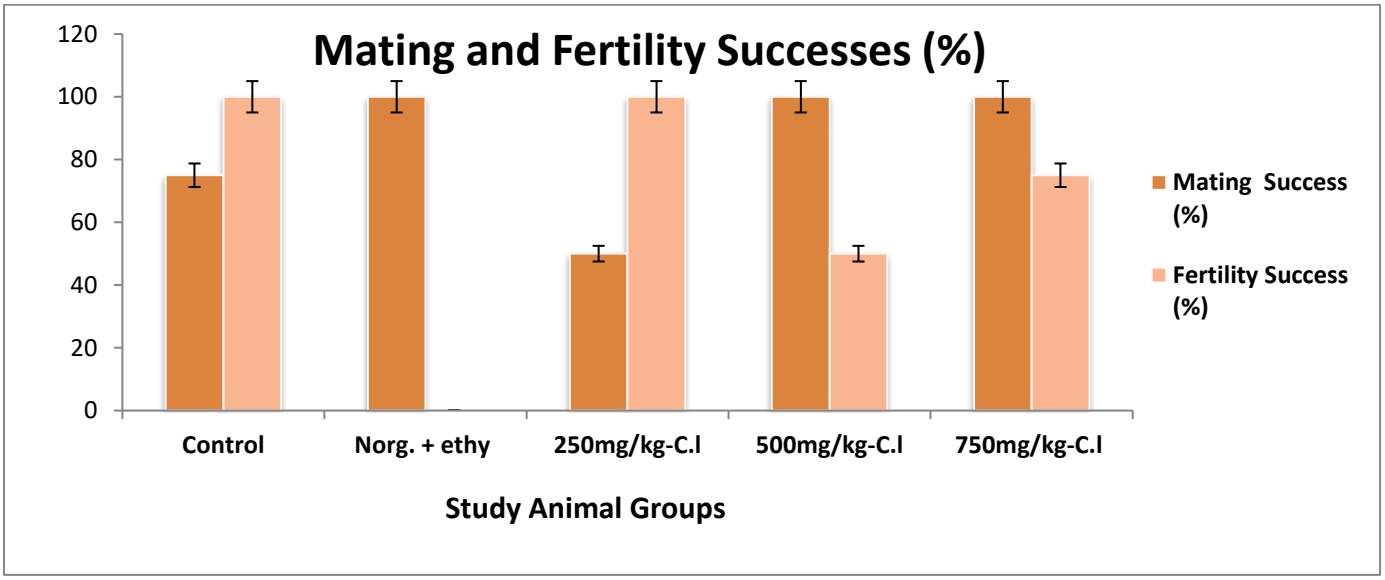

Fig. 1. Mating and Fertility Successes (%) in Female Wistar rats Treated with methanolic extract of *Curcuma longa.*
Note: Norg. + ethy = Norgestrel+ ethinylestradiol (0.21mg/kg) treated group; C.I = *Curcuma longa* methanolic extract.

Group 1: Control- untreated female rats
Group 2: standard synthetic contraceptive treated group
Group 3: 250mg/kg body weight *Curcuma longa* extract treated female rats
Group 4: 500mg/kg body weight *Curcuma longa* extract treated female rats
Group 5: 750mg/kg body weight *Curcuma longa* extract treated female rats
The outcome on mating success indicated 75%, 100%, 50%, 100%, 100% for groups 1, 2, 3, 4 and 5 respectively. On the other hand, the result on fertility success revealed 100%, 0%, 100%, 50%, and 75% for groups 1, 2, 3, 4 and 5 respectively.

IV. Discussion

The outcome of the present study revealed markedly raised levels of FSH, LH and Progesterone but selectively appreciably decreased levels of oestrogen (i.e. in 250 and 500mg/kg Methanolic extract of Curcuma longa treated rats). The significant reduction of oestrogen levels may be linked to the activity of phytoestrogen properties of flavonoids reported to be present in the *Curcuma longa* extract (29), (30). This may be so, as phytoestrogens with their structural similarity to oestrogen and possibly acting as an exogenous oestrogen, thus, the concomitant reduction of oestrogen secretion (29), (31).

These phytoestrogens are noble estrogens found in variety of plants, are known to have weak affinity compared to natural or endogenous oestrogen, it however have a significant effect in the regulation of reproduction (31), (32). This property of phytoestrogen may be likened to a shielding effect on the natural oestrogen levels in the rats, thus, the significant reduction in the endogenous level of oestrogen. It is thus suggestive to state that low to moderate doses (i.e. 250 and 500mg/kg) doses of Methanolic extract of Curcuma longa may possess a shielding effect on natural oestrogen level, thus, the simultaneous increases in FSH and LH. The above findings validates earlier claims by research scholars over the years, stated that alkaloids and tannins, saponins and flavonoids are active constituents in *Curcuma Longa* and have antifertility effects (16), (23).

The upsurge in FSH, LH and progesterone and decreased oestrogen levels as observed in this study, suggest the bivalent properties of *Curcuma Longa* in comparison to the standard contraceptive treated group, this position is consistent with the submissions of Reed and Carr, (33). Therefore, the reduced oestrogen levels at low dose may have stimulated negative

feedback such that decreased oestrogen stimulated increased FSH, LH and progesterone levels, which may have in turn led to the maximal fertility success in the 250mg/kg *C. longa* treated rats.

The findings of the present study is consistent with the position of Al-Taee *et al.,* (34), which stated that aqueous extract of *Curcuma longa* increased FSH and LH in mice; but contrasts with Priya *et al.,* (35) who said that, oestrogen in ethanolic extract of *Curcuma long* and *Carum Carvi* increased significantly. The finding of the present study is also in agreement with Sitrotkin *et al.,* (32), who observed that the ovaries of turmeric-treated rabbits after 294 days released more progesterone. It however contrasted with the position of Tharkur *et al.,* (29) which explained that, ethanolic and aqueous extract of *Curcuma longa* increased serum oestrogen levels in rats, but reduced FSH, LH and Progesterone.

On the outcome of the present study on mating success, it was found to be decreased in rats that received higher doses of the Methanolic extract of Curcuma longa and this could be due to inhibited sexual behaviour evident in the reduced oestrogen levels.

Fertility success was peak (100%) at the lowest dose of the extract (250mg/kg bw) treated rats. This could be perhaps due to low oestrogen that possibly stimulated increased FSH, LH and progesterone thereby boosting fertility (36). At higher doses, oestrogen levels were seen to be raised and may thus be responsible for the anti-fertility effect of the extract as fertility success was lower in the 500 and 750mg/kg *C. longa treated* rats. From the foregoing result, the low dose of the *C. longa* extract could have boosted fertility, and the anti-fertility effect possibly due to raised oestrogen level. This finding is in line with the earlier report of Amah-Tariah et al. (22), which also found that, higher doses of another fertility-boosting herb, *Fleurya aestuans,* can result in anti-fertility effect. Again, the finding corroborates with another study (37), which explained that the extract of *Curcuma longa* in male rats showed anti-fertility effect. It however contrasts with Kumar and Sakhya, (38)'s submission which showed that aqueous and Petroleum ether extracts of *Curcuma longa* rhizomes showed 100% anti-fertility effect when administered orally in rats. It validatess the fact that alcoholic extracts of *Curcuma longa* maintain gestation in rats. Therefore as indicated by the result of the present study, mild to moderate doses of the present extract may be potent enhancer of fertility success in female Wistar rats.

V. CONCLUSION

This study has shown that the methanolic extract of *Curcuma longa* may be effective in regulating female reproductive hormonal levels in a dose related manner in Wistar rats. Following its effects on the different female reproductive hormones and the fertility success of the study rats, the methanolic extract of *Curcuma longa* can be said to enhance fertility at moderate or low dose in female Wistar rats. However, higher doses showed decreased fertility success in the study animals. In conclusion, the attributes of the extract is suggestive of dual effects on the female reproductive system of the Wistar rats: fertility boosting effect at low dose and a seeming declining fertility success effect at higher dose.

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