

Modulations of Neem Leaf Extract on Reproductive Hormones of Male Wistar Rats

Aigbiremolen AA¹ and Odigie MO^{2*}¹Department of Pharmacology and Therapeutics, Faculty of Clinical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria²Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria

*Corresponding author: Odigie OM, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria, E-mail: osgiedeprof@yahoo.com

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Abstract

Reputable for treatment of Malaria, Diabetes, Digestive disorders, Fever, Wounds, Jaundice, and painful menstruation, *Azadirachta indica* (Neem) is locally called “Dogonyaro” in Nigeria and has been reported to be an orally active, safe, and effective plant preparation in fertility regulation. This study investigated the effect of neem leaf extract on male reproductive hormones in wistar rats. Eight wistar rats were divided into two groups of four rats each (n=4). While group 1 rats were fed normal mesh and water (Control), group 2 received 200 mg/kg body weight of neem extract and mesh feed (Extract). Following period of administration, rats were euthanized, and blood samples obtained for analysis of reproductive hormones [Testosterone, Follicle Stimulating Hormone (FSH), and Leutenizing Hormone (LH)]. Student's t-test revealed a decrease in testosterone, FSH, and LH levels of experimental groups as compared with control. Study therefore has shown that neem leaf extract can negatively affect male reproductive hormones.

Keywords: *Azadirachta indica*; Reproductive hormones

Introduction

In the last few decades, global status of herbal medicine has grown as fast as necessitating the World Health Organization (WHO) to set up a Task Force on Plant Research for fertility regulation, with the view to finding new orally active non-steroidal contraceptive compounds 1 and 2. To this point, though very few contraceptives have been developed from plant extracts; several medicinal plants have however been associated with antifertility properties [1]. In China and India, though a large number of plant species with anti-fertility effects have been screened; however, due to incomplete inhibition of fertility or side effects, the search for an orally active, safe and effective plant preparation or its compound is yet to be fully explored for fertility regulation [2,3].

Locally called “Dogonyaro” in Nigeria, *Azadirachta indica* (Neem) belongs to the Maliaceae family of plants, and is useful for treatment of malaria [3,4], diabetes, digestive disorders, and as an herbal pesticide [3,4]. It has also been helpful for the treatment of body heat, fever, wounds, painful periods and jaundice. Neem leaves and bark have been the primary ingredients in ancient medicinal preparations for malaria because of their availability throughout the year [5]. In West Africa and India, aqueous and alcohol extracts of bark and leaves of neem are effective anti-malaria agents, particularly on chloroquine resistant strains [6,7].

In the last two decades research on neem has been intensified and many of its agricultural and medical properties were discovered. Today, what seems a major breakthrough is that; projects for the commercial use of Neem have been successfully introduced in other countries [6]. In the world of microbes for instance, neem has been shown to have high efficacy against most pathogens. As fungicide, over 14 common

fungi species are sensitive to neem preparations [8,9]. On reproductive indices, neem seed oil and leaf extracts have been shown to act as a powerful spermicide with significant inhibition of spermatogenesis, decreased sperm motility, count and cessation of fertility [9]. There is also reported significant reduction in semen volume, sperm count, and higher incidences of morphological abnormalities of spermatozoa, fertilizing ability of rabbit bucks fed neem leaf meal based diets and hatchability of eggs on birds fed neem kernel cakes [10-12].

To this point, Due to the insufficient information on the reproductive toxicity of neem and the need to avoid the risk of infertility resulting from malaria chemotherapy, this study was set up to evaluate the effect of aqueous neem leaf extract on the levels reproductive hormones in male wistar rats.

Aim of the Study

This study aimed at evaluating the risk of infertility resulting from malaria chemotherapy activity of aqueous leaf extract of *Azadirachta indica* (Neem) on male Wistar rats. Specifically, study investigated:

- i. The effect of neem leaves extract on body weight of wistar rats.
- ii. The effect of neem leaves extract on testosterone levels.
- iii. The effect of neem leaves extract on the level of luteinizing hormone (LH).
- iv. The effect of neem leaves extract on the level of follicle stimulating hormone (FSH).

Materials and Methods

Scope of study

Study was limited to rats as its invasive nature would be inappropriate for humans. It was limited to investigating the effects of neem leaves extract on male reproductive hormones.

Study design

Eight male Wistar rats (weighing between 105-250 g) were divided into two groups of 4 rats each (control and extract). The extract was administered orally by the use of oral-gastric cannula;

Control group: Received (feed and water) for 4 weeks (28 days).

Extract Group: Orally received 200 mg/kg body weight of *Azadirachta indica* (Neem) extract for 4 weeks (28 days).

Procedure

Ethical clearance: With rules for handle of laboratory animals strictly adhered to, Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State.

Identification of neem: With assisted efforts from experienced taxonomist from the Department of Botany, Faculty of Sciences, Delta State University, Abraka, Delta State, Fresh healthy leaves of *Azadirachta indica* (neem) were obtained and thereafter, leaves were sun-dried and blended to powder for the extraction.

Preparation of neem extract: Following purchase and identification of *Azadirachta indica* (Neem), leaves were removed from the stem of the tree and then dried for several days. It was then grounded and 200 g of powder was extracted with distil water (1200 ml) with constant

stirring for 4 hours and left overnight before filtering. Extraction of the neem leaves was carried out using 20 g of ground leave sample in soxhlet extractor with distilled water. Recycling of the solvent was allowed to be repeated for complete extraction. The slurry extract were then poured into evaporating dish to evaporate the solvent in the extract over the water bath at temperature of 70°C-75°C. This yielded a 22.5 g of crude extract, which was collected, stored, and subsequently fed to rats.

Sample collection : At the end of the 4 weeks period administering test substance, blood samples were collected from the orbital sinus of all animals through ocular puncture. Serum was separated through centrifugation at 6000 rpm for 15 mm, and biochemical tests were conducted on blood samples to obtain hormonal levels.

Statistical analysis

With data represented as mean and standard mean error, Statistical analysis was done using the student t-test. Statistics was carried out with SPSS 22 software. A p-level<0.05 was considered as statistically significant.

Results and Discussion

Medicinal plants are commonly used in developing countries as an alternative to orthodox therapy. In Africa alone, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. Regrettably, only a few of such African medicinal plants have received scientific examination [13].

The process involved in the fertility of mammalian species is very subtle and delicately balanced by changing levels of hormonal factors. Basically, this research was directed to investigating the role that neem leave extract can play in alteration of the male primary hormones which aid their reproductive function.

Reproductive hormones							
Hormones	Control	Experimental	t Stat	P (T<=t)	t-crit	P value	Remark
LH MIU/ML	4.68 ± 0.75	2.73 ± 0.49	2.498	0.044	2.353	0.05	Sign
FSH (MIU/ML)	2.80 ± 0.34	1.79 ± 0.21	5.035	0.008	2.353	0.05	Sign
Testosterone (ng/ml)	3.79 ± 0.50	3.05 ± 0.30	2.851	0.033	2.353	0.05	Sign

Note: Values are expressed as mean ± Standard error of mean (S.E.M), n=4. P<0.05: Significant as determined by student t-Test: Paired Two Sample for Means. Significant difference (**P<0.05): (*) increase when compared control group to *Azadirachta indica* groups, (^) decrease when compared control group to *Azadirachta indica* groups

Table 1 : Showing the effects of *Azadirachta indica* leaves extract on the serum hormone levels in Wistar rats.

From the study Table 1, the levels of FSH were slightly lower as compared to the untreated (control rats). While the levels of LH and testosterone was higher in treated and untreated animals. These hormonal levels reflect the neem leave extract treatment. Male animals treated at higher dose have shown the lower levels of all male reproductive hormones including FSH, LH and testosterone as compared to untreated/control male rats. This finding is in agreement with the findings of Parshad, et al. [14] who did report a significant decrease in serum testosterone level after oral administration of neem extract in male wistar rats for 4 weeks. The antifertility effect at high dose in male rats may be because of various possibilities. Lower testosterone levels may result in delayed maturation of spermatozoa, or

lower FSH levels, though not significant statistically. This may have affected the sertoli cell function present in the seminiferous tubules resulting in disturbed facilitatory function of these cells which is essential for maturation and release of spermatozoa in the tubular lumen.

Again from Table 2, Serum luteinizing hormone (LH) levels of control group (4.68 ± 0.75) show moderate significant increase (p<0.05) when compared to *Azadirachta indica* treated rats group (2.73 ± 0.49). Meanwhile, follicle stimulating hormone (FSH) level of control groups (2.80 ± 0.34) shows significant increase (P<0.05) when compared to *Azadirachta indica* treated rats group (1.79 ± 0.21), and

Testosterone level of control groups (3.79 ± 0.50) shows significant increase ($P < 0.05$) when compared to *Azadirachta indica* treated rats group (3.05 ± 0.30). This result shows that *Azadirachta indica* moderately affect serum hormones in Wistar rats.

Tables 3 and 4 show the effects of *Azadirachta indica* (neem) leaves extract on body weight of wistar rats. From the table, a mild elevation

was observed with experimental group (23.60 ± 5.11) as compared with control group (23.23 ± 2.65) body weight change, but this did not attain significance at ($p < 0.05$).

Body weight (g)								
	Control	Experiment	Df	t-cal	t-tab	t-crit	P-value	Remark
Weight change	23.23 ± 2.65	23.60 ± 5.11	3	-0.05	0.482	2.353	0.05	Not sign

Note: Values are expressed as mean ± Standard error of mean (S.E.M), n=4. *P<0.05: Percentage change between initial and final weight determine

Table 2: Effect of *Azadirachta indica* leave extract on body weight of wistar rats.

Control group body weight (g)			
	Initial	Final	Weight change
1	184	203	19
2	137.1	157.3	20.2
3	129.3	160.1	30.8
4	123.9	146.8	22.9
N=4	143.58 ± 13.75	166.80 ± 12.40	23.23 ± 2.65

Note: Values are expressed as mean ± Standard error of mean (SEM), n=4

Table 3: Change in body weight of control Wistar rats.

Experimental group body weight (g)			
	Initial	Final	Weight change
5	154.6	184.5	29.9
6	175.9	210	34.1
7	130	141.9	11.9
8	149.8	131.3	18.5
N=4	152.58 ± 9.42	166.93 ± 18.39	23.6 ± 5.11

Note: Values are expressed as mean ± Standard error of mean (SEM), n=4

Table 4: Change in body weight of Experimental Wistar rats.

Significance of the Study

In view of the adverse effect of neem and insufficient information on the anti-fertility and reproductive toxicity actions of neem, present investigation was therefore undertaken to determine the effects of aqueous leaf extract of neem on antifertility parameters specifically; serum testosterone levels, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in male Wistar rats as a model to reduce the risk of infertility resulting from malaria chemotherapy.

Conclusion

Neem leave extract has an antifertility potential in males, the significant effect has only been shown that high dose of neem leaves extract and levels of FSH are slightly lower as compared to the untreated (control) rats. How be it, the levels of LH and testosterone are higher in treated and untreated animals. Thus, high dose used in present study may be considered as sufficient to produce antifertility effect in male wistar rats.

Recommendations

Due to the antifertility effect of *Azadirachta indica* (Neem) leaves extract on wistar rat, its use is not recommended for human and most especially on daily basis. However, further study is recommended to determine its safety margin.

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