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Effect of Aqueous Leaf Extract of *Azadirachta Indica* (Neem) on the Reproductive Hormones of Male Albino Wistar Rats

G.N. Azekhumen, *O.E. Osayande and M.O. Jonathan

Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City
*Corresponding author email: omorede@uniben.edu. Tel: +2348034746210

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ABSTRACT: *Azadirachta indica (*AI), is a popular medicinal plant originally grown in India, but is now cultivated in almost every part of the world including Nigeria where it is popularly called "Dogonyaro". The aim of this study was to investigate the effect of aqueous leaf extract of *Azadirachta indica* (Neem) on the reproductive hormones of male albino wistar rats. Twenty (20) male albino wistar rats weighing between (150-200) grams were used. They were randomly allocated to 4 groups with 5 animals in each group. Rats in group A (control) were administered 5ml of normal saline daily. Rats in group B, C and D received 100mg/kg, 200mg/kg and 400mg/kg of aqueous Neem leaf extract in 5ml of normal saline respectively. The duration of the experiment was 15 days. At the end of the experiment, the rats were sacrificed using chloroform anaesthesia. Blood was collected through cardiac puncture, centrifuged and then assayed for serum testosterone, follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH) analysis. There was a significant decrease in FSH in group C and group D respectively compared with the group A (control) ($P<0.05$) but there was no significant difference observed in group B compared with the control ($P<0.05$). Also, ICSH showed a significant decrease in group C and group D respectively compared with the group A ($P<0.05$). There was no significant difference observed in group B compared with the control ($P<0.05$). Serum testosterone showed a significant decrease in group B, group C and group D respectively compared with the group A (control) ($P<0.05$). These observations suggest that aqueous leaf extract of *Azadirachta indica* (Neem) is capable of reducing the serum level of testosterone, FSH and ICSH of male albino wistar rats and may increase the risk of infertility if used indiscriminately for malaria treatment.

Keywords: *Azadirachta indica*, Follicle stimulating hormone, interstitial cell stimulating hormone, Testosterone.

Introduction

Azadirachta indica, A. juss (AI; Family: Meliaceae) is a popular medicinal plant originally grown in India, but is now being cultivated in almost every part of the world including Nigeria (Das *et al*., 2003 ; Sonibare *et al*., 2006) where it is popularly called "Dogonyaro". It is one of the most useful medicinal plants (Kausik *et al*., 2002). Most commonly used for the treatment of Malaria. It is a large evergreen tree growing 10-11 m tall. The leaves are divided into numerous leaflets each resembling a full-grown leaf (Koul *et al*., 1990; Chatterjee *et al.,* 1994). The plant contains different alkaloids which include: nimbitin, azadirachtin and salanin (Koul *et al*., 1990), whose individual effects contribute to the general medicinal properties of the plant.
Mateenuddin *et al.* (1986) have demonstrated the antiesteogenic activity of Neem leaves extract. The ultrastructural changes induced by Neem leaves in the testes of albino rats include vacuolization of sertoli cells, diminished cytoplasmic inclusions in Leydig cells, as well as defects in the mitochondrial sheaths in late spermatids. Thus, Neem leaves may affect spermatogenesis through antispermatogenic and antiandrogenic properties (Kasturi *et al*.,

1988). Aqueous extracts of old and tender Neem leaves were shown to completely immobilize and kill human spermatozoa within 20 seconds (Kasturi *et al*., 1988).
According to Randhawa and Barmar (1996), Neem bark extract and Neem seed oil caused an arrest of spermatogenesis within two months with a decrease in the number of leydig cells which is responsible for the manufacturing of testosterone.
Kasturi *et al*. (1988) observed a reduction effect on serum concentration of testosterone, follicle stimulating hormone (FSH), lutenizing hormone/interstitial cell stimulating hormone (LH/ICSH) and prolactin in animals treated with higher doses of Neem when compared with their control counterparts. This result suggests a possible antiandrogenic property of the Neem leaves. However, Jensen (2002) reported that, studies with different types of mammals treated with Neem did not show changes in libido or hormonal function.
Weber *et al*. (2001) and Pastuszewska *et al*. (2006) reported that plants with high alkaloid content were responsible for increase in serum concentration of estradiol and prolactin that are capable of inhibiting gonadothrophic action of the testes and subsequently the fertility of male animals. The aim of this study therefore was to investigate the effect of aqueous leaf extract of *Azadirachta indica* (Neem) on the reproductive hormones of male albino wistar rats.

Materials and methods

Preparation and extraction of plant material: The Neem plant was purchased from New Benin market, Benin City (6°20'5.95"N, 5°36'13.49"E). It was then taken to the department of pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria for identification and authentication. The leaves were then washed with distilled water and air dried for 3weeks at room temp (25^0C) before pulverization and maceration using normal saline. In the process, specific mass (301.7 g) of the pulverized (powdered plant) was weighed and poured into the extraction tank. Approximately 2500ml of normal saline was added to the content of the extraction tank. The tank and its content were, periodically agitated for a period of 24 h after which the suspension was filtered using Whatman-No 1 filter paper. The filtrate or extract was then concentrated (evaporated to dryness) at 65 ºC using thermostatically regulated water bath. The yield was weighed, percentage yield calculated and the extract was kept in refrigerator at 4 ºC for subsequent investigation.
Sterilization of materials: The oven and autoclave were used to sterilize various materials. Glassware such as sample bottles, Petri dishes, conical flask used for the research was soaked and washed using detergent and rinsed several times with distilled water. They were then wrapped in the oven in an inverted position at 160 ºC – 170 ºC for 45-60 min.
Experimental animals: A sample size of 20 healthy Wistar rats (150-200 g) was used for this study. The rats were purchased from the Department of Anatomy, University of Benin, Benin City, Nigeria. The experiment commenced after acclimatizing for 2 weeks in the Anatomy animal house and laboratory in the School of Basic Medical Sciences, University of Benin. They were allowed free access to water and commercial feed throughout the period of the experiment. They were cared for in compliance with the international guidelines for animal research study.
Experimental Design: Four experimental groups, each containing five (5) Wistar rats weighting between 150-200 g were constituted in a Completely Randomized Design (CRD). Rats in group A served as the control and received only 5 ml of normal saline orally daily, while rats in groups B, C and D were the experimental groups, and were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of Neem leaf extract in 5 ml of normal saline respectively, daily for 15 days.
Collection and preparation of blood aamples for Analysis: This experiment lasted for 15days. Twenty four (24) hours after the last administration was carried out, a total of twenty (20) rats were weighed and sacrificed under chloroform anaesthesia. Blood was collected through cardiac puncture, centrifuged at 5000 rpm for 15 min and plasma was separated and stored at − 20 °C until ELISA assay for the hormones testosterone, follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH) analysis using appropriate ELISA kits for each hormone.
Statistical Analysis: All results are presented as means ± SEM. The data from the groups were analyzed using ANOVA with the aid of Graph pad prism version 5.0 statistical software. Values of P less than 0.05 ($p<0.05$) were considered to be statistically significant.

Results

General observations showed that all the rats in the study looked healthy and there was a general increase in body weight of all rats in the treatment group but this was not statistically significant ($p<0.05$). There was an increase in the body weight in control group (Group A) during the treatment period as shown in Fig 4, which implies that aqueous leaf extract of Neem had no adverse effect on growth and body weight of the rats. However, there was a general reduction in the levels of male reproductive hormones.

Serum Hormone Level

Follicle stimulating hormone (FSH)

(a) At 100 mg/kg BW (Group B)

The mean serum FSH concentration in treated males in Group B was 0.200±0.027 mIU/L while FSH concentration in control rats was 0.277±0.020 (mIU/L). When the mean serum FSH concentration in group B was compared with control (group A) the decrease was statistically insignificant ($p<0.05$) as shown in Fig 1.

(b) At 200 mg/kg BW (Group C) and 400mg/kg BW (Group D).

The mean serum FSH concentration in treated males at in Group C was 0.153±0.003 mIU/L while mean serum concentration in treated males in Group D was 0.107±0.013 mIU/L. When the mean FSH concentration in test group C and group D were compared with FSH concentration in control (Group A) rats there was a significant decrease ($p<0.05$) as shown in Fig 1.

Interstitial cell stimulating hormone (ICSH)

(a) 100 mg/kg BW (Group B)

The mean serum LH concentration in treated male Wistar rats in Group B was 0.1433±0.012 mIU/L when compared with control (Group A) which was 0.203±0.026 mIU/L shows no significant difference ($p<0.05$) as shown in Fig 2.

(b) 200 mg/kg BW (Group C) and 400 mg/kg BW (Group D)

The mean serum ICSH concentration in treated male Wistar rats in Group C was 0.093±0.007 mIU/L and Group D was 0.060±0.023 mIU/L. When mean serum LH/ICSH level in test group C and D were compared with ICSH concentration of control (Group A) which was 0.203±0.026 mIU/L a significant decrease was observed ($p<0.05$) as shown in Fig 2.

Testosterone

(a) 100 mg/kg BW (Group B), 200 mg/kg BW (Group C) and 400 mg/kg BW (Group D).

The mean serum testosterone concentration in treated male rats in Group B, C and D was 1.270±0.134 ng/ml, 0.497±0.012 ng/ml and 0.363±0.073 ng/ml respectively, while testosterone level in control was 3.770±0.654 ng/ml. When testosterone level in treated males in Group B, C and D was compared with Group A (control), there was a significant decrease observed ($p<0.05$). Also, there was a significant decrease observed in Group C and D when compared with Group B ($p<0.05$). However, there was no significant change when Group C was compared with Group D ($p<0.05$) as shown in Fig 3.

Hormonal Assay

Table 1: Comparism of the mean values of the serum level of FSH, ICSH and testosterone following the administration of aqueous Neem leaf extract in male Wistar rats for 15 days

Parameter	Group A	Group B	Group C	Group D
FSH (mui/L)	0.277 ± 0.020	0.200 ± 0.027	0.153 ± 0.003*	0.107 ± 0.013$^{*\#a}$
LH/ ICSH (mui/L)	0.203 ± 0.026	0.143 ± 0.012	0.093 ± 0.007$^{*\#}$	0.060 ± 0.023$^{*\#}$
Testosterone (ng/ml)	3.770 ± 0.654	1.270 ± 0.134*	0.497 ± 0.012$^{*\#}$	0.363 ± 0.072$^{*\#}$

The results are expressed as Means ± SEM.

*$P<0.05$ indicates significant difference when group B, C and D were compared with group A (control).

$^{\#}P<0.05$ indicates significant difference when group C or group D were compared with group B.

$^{a}P<0.05$ indicates significant difference when group D is compared with group C.

Serum FSH Concentration

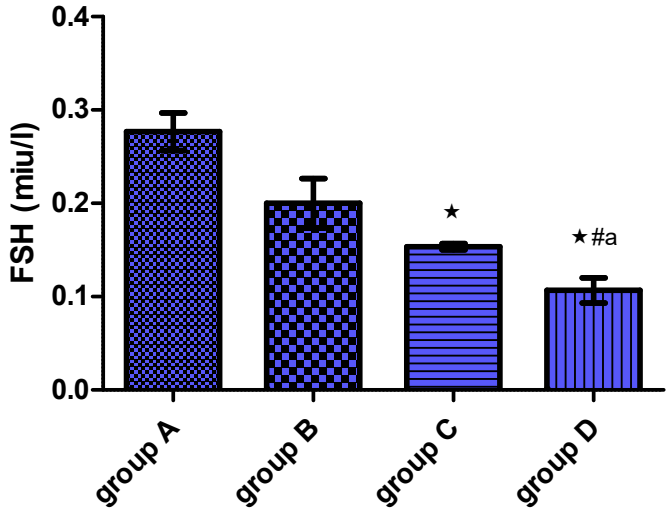

Fig 1: Bar charts showing the follicle stimulating hormone (FSH) level of male Wistar rats following the administration of Neem leaf extract at different doses after 15 days. There was a significant decrease in group C and group D respectively compared with the group A (control) ($P<0.05$) but there was no significant difference observed in group B compared with the control (P<0.05).

Serum ICSH Concentration

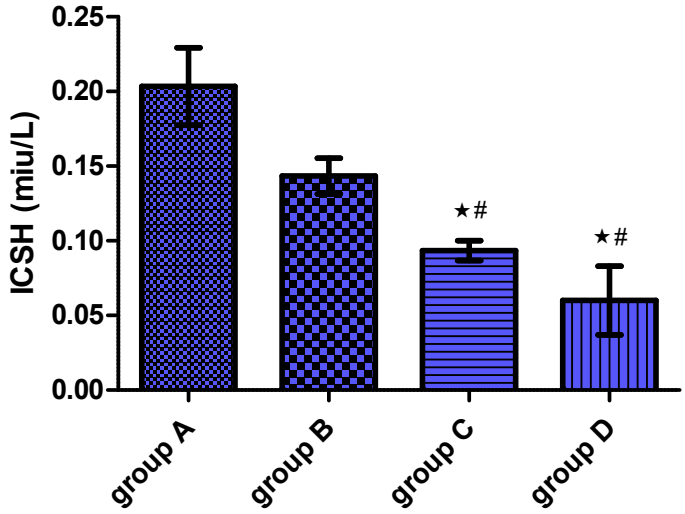

Fig 2: Bar charts showing the interstitial cell stimulating hormone (ICSH) level of male Wistar rats following the administration of Neem leaf extract at different doses after 15 days. There was a significant decrease in group C and group D respectively compared with the control. ($p<0.05$) but there was no significant difference observed in group B compared with the control ($p<0.05$).

Serum Testosterone Concentration

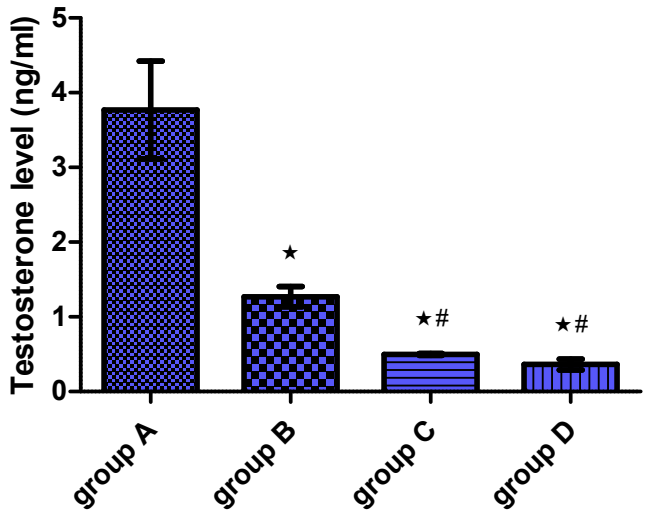

Fig 3: Bar charts showing the testosterone level of male Wistar rats following the administration of Neem leaf extract at different doses after 15 days. There was a significant decrease in group B, group C and group D when compared with the group A (control) ($p<0.05$). Also, there was a significant decrease observed in group C and group D compared with group B ($p<0.05$), however, there was no significant change between group C and group D ($p<0.05$).

Body Weight

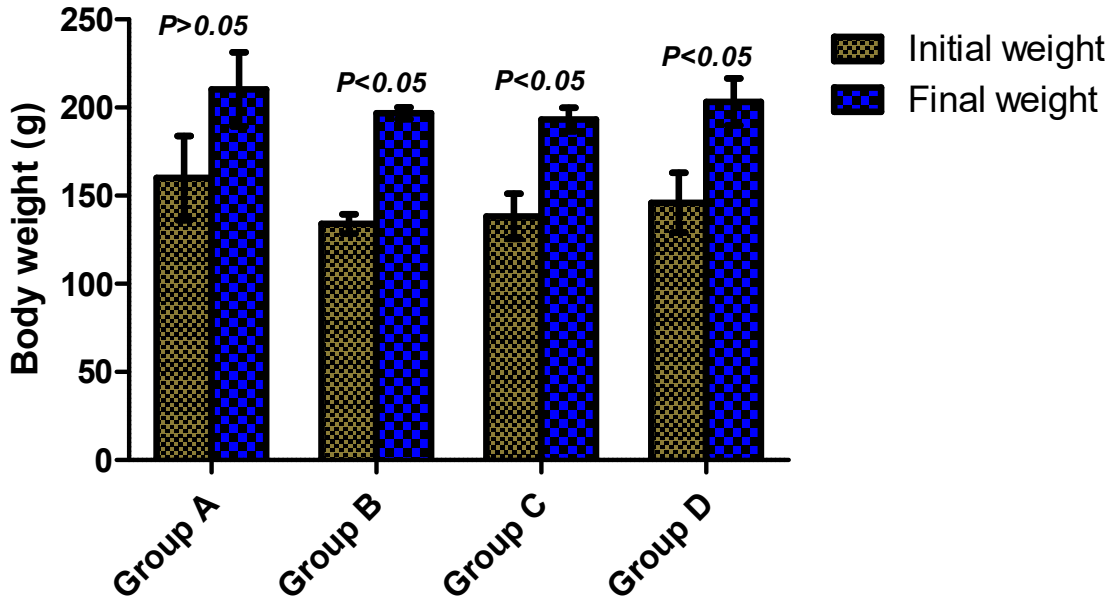

Fig 4: the body weight of male Wistar rats before and after the administration of different doses of Neem leaf extract. A significant increase ($p<0.05$) in the final weights compared with the initial weights in group B, group C and group D was observed, but there was no significant change between the initial and the final weight of rats in the group A.

Discussion

The neurons of the hypothalamus synthesize and secrete gonadotropin-releasing hormone, which induces the production and release of ICSH and FSH from pituitary gland.

A significant decrease in serum FSH level in group C and group D was observed when compared with group A (control) ($p<0.05$). There was no significant difference in serum FSH level in group B when compared with group A (*control*) ($p<0.05$). This insignificant difference in serum FSH concentration in group B implies that at 100 mg/kg BW the *Azadirachta indica* leaf extract has little effect on FSH. Whereas the significant decrease in serum FSH concentration when administered at 200 mg/kg BW and 400 mg/kg BW implies that the decrease in FSH was dose dependent.

This study shows no significant difference in serum LH/ICSH in group B when compared with group A (control) ($p<0.05$). However, there was a significant decrease in group C and group D when compared with group A (control) ($p<0.05$). This result shows the dose dependent effect of *Azadirachta indica* leaf extract at medium and high dose of 200 mg/kg BW and 400 mg/kg BW respectively on serum LH/ICSH level. This significant decrease in serum ICSH level as seen in group C and D is in line with Raji *et al*. (2003) who reported a dose dependent decrease in serum ICSH level after administering Neem stem bark ethanol extract intraperitoneally.

This dose dependent decrease of FSH and ICSH is in line with the work of Akpantah *et al*. (2010) after administering 200 mg/kg BW and 400 mg/kg BW of *A. indica* methanol leaf extract. This reduction may be as a result of inhibitory effect of Neem leaf extract on the hypophysis, which in turn results in reduced gonadotrophs which secretes FSH and ICSH. The reduction of gonadotrophs may be because of reduced basophil population (Akpantah *et al*., 2010).

In this study, we observed a significant decrease of serum testosterone level in groups B, C and D respectively when compared with group A (control) ($p<0.05$). Also, a significant decrease in serum testosterone level was observed in group C and D compared with group B ($p<0.05$), however, there was no significant changes between group C and group D ($p<0.05$).

This decrease in serum testosterone as observed in this study agrees with the report of Kasturi *et al*. (1988). It is also in line with the work of Parshad *et al*. (1994) who reported a significant decrease in serum testosterone level in test group when compared with the control after the oral administration of crude Neem extract in male wistar rats for 10 weeks.

This decrease may be due to the possible antiandrogenic property of Neem leaf and decrease in the number of Leydig cells which is responsible for the manufacturing of testosterone. The decrease in serum testosterone level may also be as a result of reduced follicle stimulating hormone secretion. This assumption is based on the fact that testosterone activity is largely influenced by androgen- binding protein whose formation from Sertoli cells is stimulated by FSH.

Conclusion

This study shows that aqueous leaf extract of *Azadirachta indica* (Neem) is capable of reducing the serum level of testosterone, follicle stimulating hormone and interstitial cell stimulating hormone of male albino Wistar rats and may increase the risk of infertility if used indiscriminately for malaria treatment. However, more study needs to be done to ascertain the mechanism of action of aqueous leaf extract of *Azadirachta indica* on male reproductive hormones.

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