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RESEARCH ARTICLE

EFFECT OF AQUEOUS LEAF EXTRACT OF NEEM (*AZADIRACHTA INDICA*) ON THE HORMONAL MILIEU OF MALE RATS

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ABSTRACT

The effects of aqueous leaf extract of neem on serum testosterone, follicle stimulating hormone (FSH), luteinizing hormone/interstitial cell stimulating hormone (LH/ICSH), estrogen and prolactin levels in male albino rats were studied. Rats treated with 50, 100 and 150 mg/kg body weight of neem extract intraperitoneally daily for 15 days showed very highly significant ($P< 0.001$) reduction effects on serum concentration of testosterone, FSH, LH/ICSH and prolactin when compared with their control counterparts. On the other hand, the aqueous leaf extract of neem had a very highly significantly ($P<0.001$) increasing effect on serum concentration of estradiol. There were dose-dependent effects of the aqueous leaf extract of neem on the serum concentration of the hormones. These observations suggest that the aqueous leaf extract of neem had strong anti-androgenic property and capability to disrupt hormonal functions. Hence, its indiscriminate use in malaria chemotherapy could increase the in risk of infertility in males.

INTRODUCTION

Neem (*Azadirachta indica* A Juss), locally called "*Dogonyaro*" in Nigeria is used for treatment of malaria (Mackinnon *et al.,* 1997; Okereke, 2003; Obi, 2004), diabetes, digestive disorders and as an herbal pesticide (Okereke, 2003; Obi, 2004). It has also been used locally 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. Some anti-malarial agents have been reported to have anti-fertility actions (Mackinnon *et al.,* 1997; Adeeko and Dada, 1998; Nwanjo *et al.,* 2007; Ekaluo *et al.,* 2008).

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 that serum testosterone concentration in animals treated with higher doses of neem leaves showed significant decreases. 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.

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McGarvey *et al.* (2001), 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. Reductions in levels of testosterone and follicle stimulating hormone (FSH) were responsible for suppressed spermatogenic activities, infertility and reproductive toxicity (Greenspan and Stawler, 1997; Gelain *et al.,* 2005).

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 out to evaluate the effect of aqueous neem leaf extract on the levels of the following reproductive hormones: testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen (estradiol) and prolactin; in male rats as a model.

MATERIALS AND METHODS

Animals

Twenty healthy and sexually mature male albino rats of 12 weeks old were obtained from the Animal House of Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria for this study. The rats were divided into four groups with five rats per group and housed in conventional wire mesh cages under

standard laboratory conditions (temperature 25-30^{0}C, 12hours light and 12hours darkness cycle). They were allowed free access to water and commercial feed throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of animals.

Experimental Design

Four experimental groups of five albino rats each with similar body weights were constituted in a Completely Randomized Design (CRD). Known weights of powdered neem leaves were dissolved in 4.5 ml of physiological saline (0.9% Sodium chloride) and then filtered after six hours of continuous stirring on a magnetic stirrer and then made up to 5 ml with physiological saline to obtain aqueous leaf extract of neem. The rats in group A served as the control and received only 5ml of physiological saline intraperitoneally daily. Rats in group B, C and D rats were administered with 50, 100 and 150 mg/kg body weight of neem extract in 5 ml of physiological saline respectively, daily for 15 days. At the end of the treatment period the animals were sacrificed under chloroform anaesthesia 24 hours after the last treatment. Blood was collected by cardiac puncture for hormonal assay.

Hormonal Assay

The blood samples were spun at 2500 rpm for 10min using Wisperfuge model 1384 centrifuge (Tamson, Holland) at 10-25^{0}C. Serum samples were assayed for levels of testosterone, follicle stimulating hormone (FSH), lutenizing hormone/interstitial cell stimulating hormone (LH/ICSH), estrogen (estradiol) and prolactin using the Microwell enzyme linked immunoassay (ELISA) technique; using analytical grade reagents (Syntron Bioresearch Inc., USA). The differences between the control and treatment groups were subjected to statistical evaluation, for significance using the Analysis of variance (ANOVA) test.

RESULTS

General Observations

General observations showed that all the rats in the study looked healthy and there was a general increase in body weights of all rats in both treatment and control groups during the treatment period. The increases in body weights of the rats indicated that aqueous leaf extract of neem had no adverse effect on growth and body weight of the rats.

Serum Hormone Levels

Table 1 shows that aqueous leaf extract of neem had very highly significantly (P<0.001) reduction effects on serum concentration of testosterone, follicle stimulating hormone (FSH), lutenizing hormone/interstitial cell stimulating hormone (LH/ICSH) and prolactin in treated male albino rats when compared with their control counterparts. While on the other hand the aqueous leaf extract of neem also had a very highly significantly (P<0.001) increasing effect on serum concentration of estradiol. There was also a very highly significantly (P<0.001) effect between different concentration groups with exception of control and 50 mg/kg BW for Prolactin.

There was a dose-dependent effect of the aqueous leaf extract of neem on the serum concentration of the hormones as shown on Fig. 1. Increase in dose had positive effect only on the serum concentration of estradiol, while it had negative effects on serum concentration of testosterone, FSH, LH/ICSH and prolactin.

Table 1. Effect of aqueous neem leaf extract on the serum level of hormones in male rats

Hormone	Concentration of aqueous neem leaf extract (mg/kg BW)				LSD
	0(Control)	50	100	150	Value
Testosterone (ng/ml)	$2.70^{d} \pm 0.007$	$2.40^{c} \pm 0.011$	$2.04^{b} \pm 0.008$	$1.80^{a} \pm 0.016$	0.0143
Estradiol (pg/ml)	$0.68^{a} \pm 0.014$	$0.86^{b} \pm 0.013$	$1.03^{c} \pm 0.008$	$1.21^{d} \pm 0.019$	0.0202
Prolactin (ng/ml)	$0.85^{c} \pm 0.009$	$0.84^{c} \pm 0.010$	$0.82^{b} \pm 0.007$	$0.53^{a} \pm 0.015$	0.0143
FSH (Miu/ml)	$1.80^{d} \pm 0.015$	$1.67^{c} \pm 0.005$	$1.26^{b} \pm 0.017$	$0.82^{a} \pm 0.015$	0.0202
LH/ICSH (mIU/ml)	$1.21^{d} \pm 0.008$	$1.18^{c} \pm 0.018$	$1.07^{b} \pm 0.008$	$1.00^{a} \pm 0.040$	0.0202

[abcd] [Values across the table with dissimilar superscript are significantly different at 0.1% based on ANOVA]

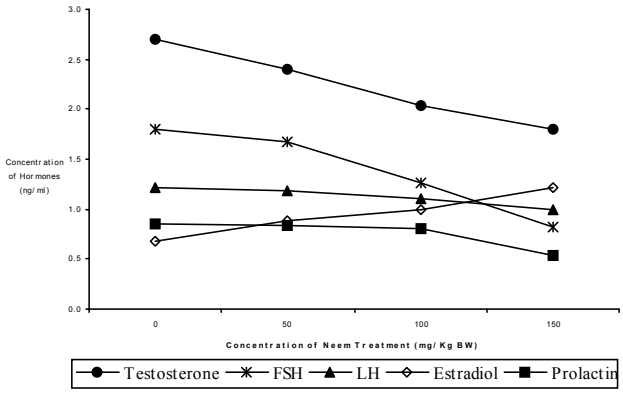

Figure 1: Effect of Neem Treatments on Hormonal Profile of Male rats

DISCUSSION

The male rats treated with aqueous leaf extract of neem showed a dose-dependent effect on the serum concentration of the hormones which was contrary to the report of Jensen (2002). The decrease in serum

testosterone concentration agrees the report of Kasturi *et al.* (1988), which suggests a possible antiandrogenic property of the neem leaves and attributed the decrease to the number of leydig cells which is responsible for the manufacturing of testosterone (Randhawa and Barmar, 1996). The significant increase in serum concentration of estradiol can be ascribed to the high alkaloid content of the extract (McGarvey *et al.,* 2001; Weber *et al.,* 2001; Pastuszewska *et al.,* 2006) and this is capable of inhibiting gonadothrophic action of the testes and subsequently the fertility of the male animals. This study shows that aqueous leaf extract of neem is capable of disrupting the normal hormonal milieu of male rats and may increase the risk of infertility as a result of malaria chemotherapy.

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