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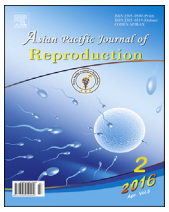

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Reproductive toxicity of aqueous wood-ash extract of *Azadirachta indica* (neem) on male albino mice

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ABSTRACT

Objective: To evaluate the reproductive toxicity of aqueous wood ash extract of *Azadirachta indica (A. indica)* in male albino mice.
Methods: Four different dose levels of 0, 5, 50 and 100 mg/kg body weight were administered to 20 male mice, with five mice per group for seven days that were sacrificed 35 days thereafter. Gonadosomatic index, sperm motility, sperm count, sperm morphology, serum follicle stimulating hormone (FSH), leuitinizing hormone (LH) and testosterone assay, and histopathology of testes were carried out.
Results: Though no toxic effect on testicular weight, FSH, LH and testosterone ($P > 0.05$), significant decrease in sperm motility, live/dead sperm and sperm count, with significant increase of abnormal sperm were recorded ($P < 0.05$). Dose dependent histopathological damage of testes was obtained ($P < 0.05$).
Conclusion: Aqueous wood ash extract of *A. indica* have damaging effects on sperms and testicular tissues, which could impair reproduction.

1. Introduction

Aqueous wood ash extract of *Azadirachta indica* (*A. indica)* and other plants have been used as food additive and for medicinal purposes, such as stomach ache treatment among some ethnic groups in Middle-Belt region of Nigeria [1]. *A. indica,* which belongs to the plants Family Meliaceae has been well known in the traditional system of medicine for more than 2000 years as one of the most versatile medicinal plants having a wide range of biological activity [2]. Various useful products such as antimalarials, spermicidals, antituberculosis agents, antipyrrhetics, antiviral drugs, antiseborrhoeics, antiallergic medicines, antienzymic, and antifungal agents, have been extracted from the *A. indica* [3]. *A. indica* have antiseptic, anti-helminth, antifungal, antibacterial, antipyretic, antimalarial, anti-diabetic and anti-fertility properties [4].

In recent years, there has been growing concern about the deleterious effects of chemicals on developing male reproductive system [5]. In the male reproductive system, weight loss of the gonads as well as reduced sperm count and epididymal sperm motility are considered standard criteria for the characterization of toxic agents that may cause fertility problems in the treated subject [6,7].

Sperms morphology serves as an important and sensitive indicator of chemical toxicity on the reproductive cells. They can be used to evaluate the spermatogenic damage, fertility and heritable genetic changes, which provide a direct measure of the quality of sperm production in chemically treated animals [8–10].

The testis is surrounded by a dense connectival capsule, called the tunica albuginea. From the internal surface of the tunica albuginea, the connective tissue septa depart toward the mediastinum within which the anastomotic network of ducts, the testis, is located. Spermatogenesis occurs within the seminiferous tubules which are located within the network of testicular lobules. Control of the reproductive process is finely regulated by the neuroendocrine system through the hypothalamus and pituitary axis [11]. Though several researches have been reported on *A. indica* products, there is dearth of information on reproductive toxicity of aqueous wood ash of *A. indica*. Hence, this study has been able to report the damaging effects on sperms and testicular tissues of aqueous wood ash extract of *A. indica*, which could impair reproduction.

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2. Materials and methods

2.1. Plants and study design

Fresh *A. indica* (Neem) wood was collected from Katsina State, North-Western Nigeria. Stalk of the plant, carrying leaves and flowers, were collected and taken to the herbarium of Department of Biological Sciences, Ahmadu Bello University, Zaria for authentication and voucher number of 90051 was obtained. The wood processing to ash and sub sequent analysis was carried out as described by [1].

Reproductive toxicity study in male was carried out using methods described by [10,12–14]. A total of 20 apparently healthy adult male mice were divided into 4 groups with 5 mice in each group. Group 1, serving as control was orally administered distilled water while 2, 3 & 4 received 5, 50 and 100 mg/kg bw aqueous wood ash extract of *A. indica*. They were kept in plastic cages with five 5 mice per cage in an environment of approximately 12 h light/dark cycle, a temperature of (24 ± 3) °C. The mice were supplied with a standard diet and water *ad-libitum*.

2.2. Biochemical and morphological analysis

At 5 weeks (35 days) from the first administration, the mice were sacrificed by cervical dislocation and their caudal epididymis surgically removed, sperm smears were prepared from the epididymis. Gonadosomatic index, sperm motility and sperm count [12], Sperm morphology [15] FSH, LH and testosterone hormones were measured using microplate immunoenzymometric assay method (ELISA). Histopathology of testes was also carried out.

Immediately after sacrificing, the bloods were collected into plain 1.5 mL eppendough tubes. The blood were left to coagulate and then centrifuged at 3 000 rpm for 30 min to separate the serum. The separated serums were stored at −20 °C for subsequent hormonal analyses. The circulating levels of testosterone, FSH and LH were determined using radioimmunoassay kits. TAC was also determined.

The body weight of each mouse was determined immediately before sacrificing. After sacrifice and dissection, the testes were removed and weighed to determine the gonadosomatic index.

Immediately after sacrificing, the caudal epididymis was collected from each mouse to assess sperm motility, count and viability (morphology). The assessment of sperm was carried out according to [12] protocol and data were expressed as the number of sperm per mL. Samples from the testes of the four groups were processed histologically for paraffin sections. 5–7 μm sections was prepared and stained by haematoxylin and eosin stain.

Ethical approval was obtained from the University of Ibadan Animal Care and Use for Research Ethical Committee (ACUREC).

2.3. Statistical analysis

The values are expressed as mean ± Standard error (SE). An analysis of variance (one way ANOVA) was used to determine the significance between different doses of exposure and was followed by Duncan Multiple Range (DMR) test. The significant difference between the groups will be considered significant at $P < 0.05$ level.

3. Results

The results obtained showed significant difference in the serum concentration of leautinizing (LH) and follicle stimulating hormones (FSH), while there was no significant difference in the gonadosomatic index and testosterone concentration among male mice exposed to different doses of aqueous wood ash extract of *A. indica* (Table 1).

Table 1
Gonadosomatic index and hormonal assay of mice exposed to aqueous wood ash extract of *A. indica*.

Groups	Testicular weight % (g/g BW × 100)	LH (IU/L)	FSH (IU/L)	Testosterone (IU/L)
Control	0.64 ± 0.02	8.75 ± 0.48^a	6.75 ± 0.48^a	0.7 ± 0.07
5 AI	0.65 ± 0.04	8.50 ± 0.65^a	6.75 ± 0.63^a	0.68 ± 0.09
50 AI	0.62 ± 0.05	16.50 ± 3.18^b	13.25 ± 1.60^b	0.75 ± 0.23
100 AI	0.72 ± 0.01	10.5 ± 1.19^a	7.75 ± 0.95^a	0.75 ± 0.12

AI: *A. indica* extract (mg/kg); Values are expressed as Means ± SEM (n = 5 per group). Means in same column with different superscript letters are significantly different; $P < 0.05$.
AI: *A. indica* extract (mg/kg).

Table 2
Result of sperm count and motility assay of mice exposed to aqueous wood ash extract of *A. indica*.

Groups	Motility (%)	Live/Dead (%)	Volume(μL)	Count (×10^6/mL)
Control	91.5 ± 1.19^a	98.00 ± 0.00^a	5.10 ± 0.00	137.75 ± 1.84^a
5AI	73.25 ± 2.36^b	94.25 ± 1.65^{ab}	5.13 ± 0.03	123.75 ± 1.55^b
50AI	75.00 ± 2.04^b	96.25 ± 0.63^{ab}	5.10 ± 0.00	122.00 ± 1.63^b
100AI	60.00 ± 0.00^c	91.75 ± 2.36^b	5.13 ± 0.03	95.00 ± 2.45^c

AI: *A. indica* extract (mg/kg); Values are expressed as Means ± SEM (n = 5 per group). Means in same columns with different superscript letters are significantly different; $P < 0.05$.

Table 3
Showing sperm morphology parameters of mice exposed to aqueous wood ash extract of *A. indica*.

Groups	Tailless head	Headless tail	Rudimentary tail	Curved tail	Curved midpiece
0 (DW)	4.25 ± 0.63	4.25 ± 0.25	1.75 ± 0.48	7.00 ± 0.41^a	8.00 ± 0.41
5 AI	4.25 ± 0.48	4.00 ± 0.41	1.75 ± 0.48	8.00 ± 0.41^a	7.00 ± 0.41
50 AI	4.25 ± 0.48	4.00 ± 0.41	1.75 ± 0.41	8.00 ± 0.41^a	7.00 ± 0.41
100 AI	3.75 ± 0.48	5.00 ± 0.41	2.00 ± 0.41	10.0 ± 0.41^b	8.25 ± 0.63

AI: *A. indica* extract (mg/kg); Values are expressed as Means ± SEM (n = 5 per group). Means in same column with different superscript letters are significantly different, $P < 0.05$.