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The Effect Of Hexane Extract Of *Azadirachta indica* Leaves On testicular Sperm Reserve In Mice

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ABSTRACT

The 50% lethal dose (LD_{50}) of hexane leaf extract of *A.indica* solubilized in 10% tween 20 was determined as 182mg/kg body weight. 25 adult male albino mice (34.1-53) were randomly divided into five groups containing five mice each. The mice in groups I-IV were given graded doses of the extract @ 1,7,3,3,6,7 and 8.33mg/kg body weight respectively by intraperitoneal route. They were treated 3 times a week for 4 consecutive weeks. Group V mice, which served as control received corresponding volumes of 10% tween 20 solutions. The animals were weighed, sacrificed and the testes weighed. The left testis was macerated in 2mls of semen diluting fluid until a homogenous mixture was formed. Improved Neubauer's chamber was used to determine the testicular sperm reserve. The right testis, liver, kidney and spleen were routinely prepared for light microscopy. The control mice had significantly higher ($P<0.05$) testicular sperm reserve than the treatment group mice, which received the highest dose of the extract. Changes in the structure of testes and other viscera were not observed. These results suggest that intraperitoneal injection of the extract reduced testicular sperm reserve in mice in a dose dependent manner.

Key words: Albino mice, Hexane extract, Neem leaves, Sperm reserve, Testes.

The population of developing countries is fast increasing without corresponding increase in food production. This calls for urgent birth control measures to be taken particularly in African countries including Nigeria. The most common birth control measures are in women. They are not yielding the expected results because of poverty, high illiteracy rate and cultural beliefs. There is the need for alternative measures, which will be natural, easily available, cheap and applicable in men.

Neem (*Azadirachata indica*) has very high population density in Nigeria, the resources of which have been exploited beyond being provider of shade, firewoods, wind braker as well as aforestation programmes (Fajinmi et al 1989). The common names of neem tree include "holytree", "magic tree", "wonderfulplant" and the vernacular names are "Dogoyaro" – Hausa, "Ogwoakum" – Ibo and "Afor-Onyigbo" – Yoruba. Several investigations established the occurrence of different flavonoids in various parts of neem tree (Shin-foon 1984 Ukponu 1985). Phytochemical analysis of neem plants revealed that all parts of the plant contain some bitter principles generally known as triterpenes or limonoids (Naganishi 1975, Devakumar and Sukh 1993).

Neem plant has been used in folkloric medicine for many centuries (Pendse et al 1963). The chewing stick from neem stems is effective in preventing periodontal diseases. The aqueous extract of the plant is traditionally used in treatment of malaria and zone

of inflammatory skin diseases (Tella 1976, Okpanyi and Ezeuwku 1981). There are reports that neem seems, leaves and bark contain active agents with demonstrated antifungal (Khan and Wassilew 1987), antiviral (Kaii-a-kamb et al 1992) and antibacterial activities (Sai Ram et al 2000).

Reports on contraceptive activities of neem extract have focused mainly on females (Riar et al 1984, Sinha et al 1984, Sharma et al 1996). There are scanty information on the effect of neem extract on male fertility in vivo. This work was therefore, designed to study the effect of hexane extract of *A. indica* leaves on testicular sperm reserved in mice.

MATERIALS AND METHODS

The Plant and Preparation of Oil Extract:

The leaves of *A. indica* were collected from a farm near the faculty of Veterinary Medicine, University of Nigeria, Nsukka. About 500g of wet leaves were collected from parent plant *A. indica* tree, sun-dried and ground to fine power. 75g of the powder were measured into two separate bottles each containing 300mls of hexane and soaked for 48 hours. The bottles were shaken vigorously at regular intervals.

The solution was filtered and the filtrate evaporated @ 40^0C in a hot air oven overnight. The residue – the oil extract was collected into smaller corked bottles and stored in a refrigerator until use. About 1.0g of the oily paste were solubilized in 2mls of tween 20 and the solution made up to 20mls with distilled water. This gave a stock solution of 50mg/ml concentration.

The Experimental Animals:

Fifty-five adult male albino mice obtained from the laboratory animal unit of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the experiment. The animals were housed in stainless steel cages and fed commercial diet and water *ad libitum* throughout the period of the experiment.

Acute Toxicity Test (Determination of LD_{50})

Thirty adult male albino mice (27-50.5g) were used for the test. The mice were randomly divided into six groups containing 5 mice each. The mice were graded doses of the extract as follows: Group (1-IV) mice received 50,75,125,200,250 and 300mg/kg body weight respectively by intraperitoneal route. The animals were observed for clinical signs of toxicity such as depression, stretching, dizziness, weakness, sunken gait, nervous signs and death within 24hrs. The LD_{50} was determined by plotting the graph of percentage mortality against log dose and the antilog dose, which caused 50% mortality, determined (Fig.1).

Effects Of A. Indica Extract On Testicular Sperm Reserve:

Treatment of Mice:

Twenty-five adult male albino mice (34-54.3g) were randomly divided into five groups of five mice each. The mice were treated as follows:

Groups I-IV mice were injected i/p with graded doses of the extract. They received doses of 1.7,3.3,6.7 and 8.33mg/kg body weight respectively. The extract was given three times a week for four consecutive weeks. These amounted to cumulative doses of 20,40,80 and 100mg/kg body weight respectively for the treatment period. The animals in group V which served as control received corresponding volumes of 10% tween 20 solution.

Processing of Testicular Material:

The mice were weighed, sacrificed and the testes dissected out and weighed. The left testis was macerated in 2mls of semen diluting fluid until a homogenous mixture was formed. The mixture was mounted in improved Neubauer's Chamber and the spermatozoa counted under light microscope at x 400 magnification. (Obidike et al 2001).

***Quantitative Measurement*:**

The testicular sperm reserve was determined by multiplying the number of counted spermatozoa by weight of testis, volume of semen diluting fluid and a diluting factor of 50,000 (Obidike et al 2001).

The testicular body mass indices in percentage were calculated by dividing the weight of both testes with the body weight and multiplying by 100. The data obtained were analyzed using one-way ANOVA and Duncan's Now Multiple Range Test to test the level of significance.

Processing of tissues for light microscopy:

The right testis, liver, kidney and spleen were fixed in Bouin"s fluid and processed routinely for light microscopy. They were stained with Haematoxylin and Eosin. Photomicrographs were prepared.

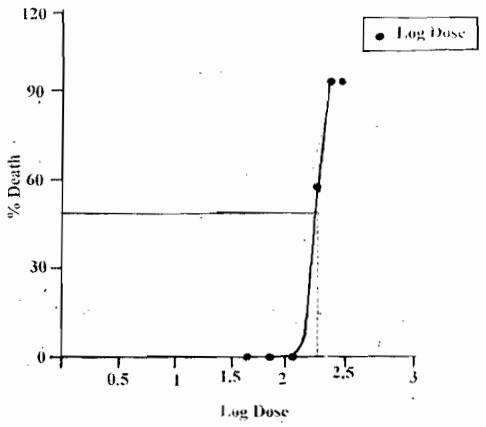
RESULTS

Plant extraction:

The hexane extract of *A. indica* leaves was black in colour, pasty and oily to touch with a characteristic unpleasant smell. The yield was 3g i.e 0.6% w/w.

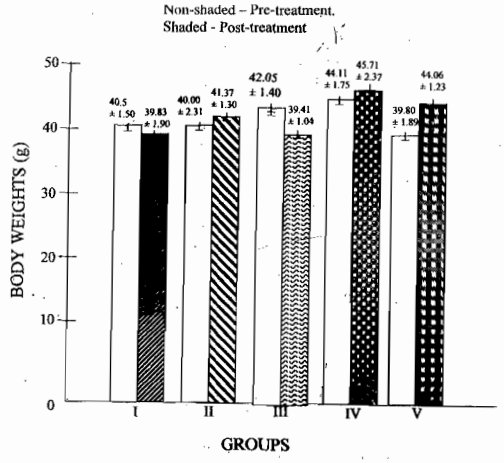
Table 1: Toxicity Effect of Hexane Extract of A. Indica Leaves in Mice.

Dose	Group	No of Animals	No of Deaths	%Death	Log Dose
50	I	5	0	0	1.7
75	II	5	0	0	1.9
125	III	5	0	0	2.1
200	IV	5	3	60	2.3
250	V	5	5	100	2.4
300	VI	5	5	100	



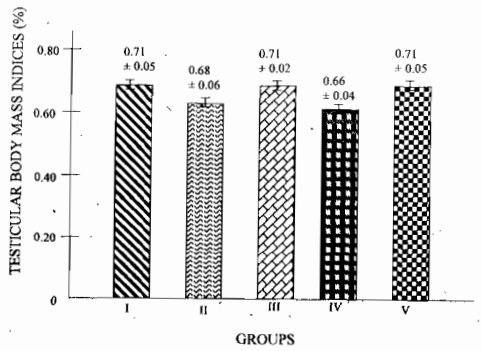
Effective dose ED_{50} = 2.26;
Anti-log = 182mg/kg.

Fig. 1: Graph of % Death against Log Dose



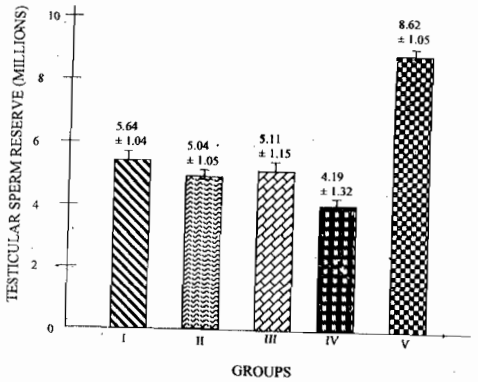
Values represent mean ± S.E for each measurement.
Degrees of freedom = 20. P > 0.05

Fig. 2 Comprison of Body Weight (g) of control and Treated



Values represent mean ± S.E. for each measurement.
Degrees of freedom = 20 P > 0.05

Fig. 3 Comparison of Testicular Body Mass indices(%) Using Anova



Values represent mean ± S.E. for each measurement.
Degrees of freedom = 20 P < 0.05

Fig. 4 – Comparison of Testicular Sperm reserve (10^6)Using Anova

Fig. 5- Control group mice Sections of Testes Showing intestitial cells (1) and seminiferous Tubules (s) x 100

Fig. 6 Treated group mice. Sections of Testes. Showing intestitial cells (1) and seminiferrous tubules(s) x 100

Fig. 7 – Control mice. Sections of the liver showing Veins(v), plates of hepatocyles (h), sinusoids (Arrows) x 100

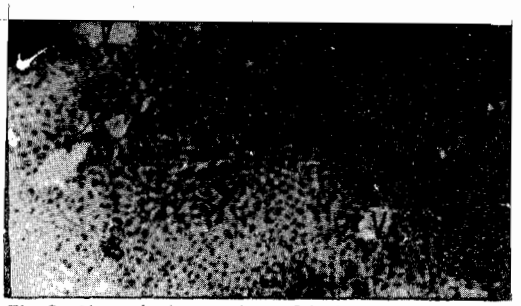


Fig. 8 – Treated mice sections of the live showing veins (v), hepatocytes (H) and sinusoids (Arrows) x 100

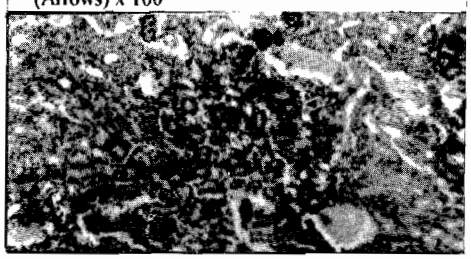


Fig. 9 – Control mice- Sections of the renal cortex Showing glomeruli(G) and renal tubules (T) x 100

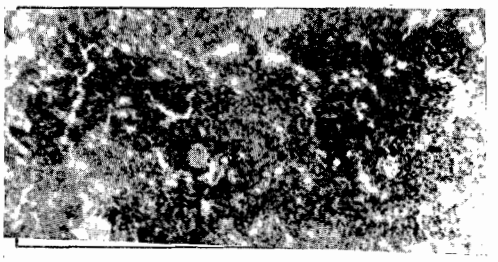


Fig. 10 – Treated mice. Sections of the renal cortex showing glomeruli (G) and renal tubules ® x 100

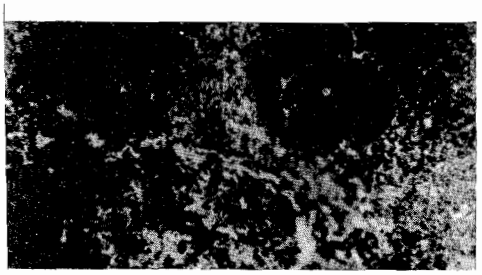


Fig. 11 – Control mice. Sections of the spleen showing Renal corpuscles © and red pulp ® x 100

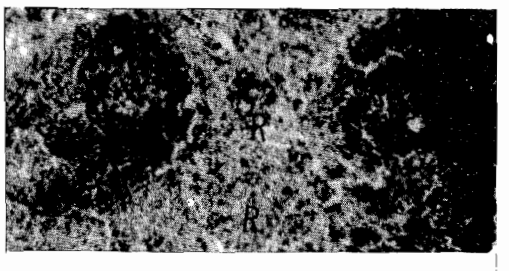


Fig. 12 – Treated mice. Section of spleen showing renal corpuscles © and red pulp ® x 100

Acute Toxicity Test (LD_{50}):

The result of the acute toxicity test is presented in Table 1. The doses of 300 and 250mg caused 100% mortality and at 200mg/kg 3 out of 5 experimental animals died (60% mortality) within 24 hrs post injection. The lethal Dose (LD_{50}) was determined to be 182mg/kg body weight (Fig.1).

Effect of Hexane Extract of *A. indica* on Weights and Testicular Sperm Reserves:

Comparison using one-way ANOVA showed that there was no significant difference ($P>0.05$) between the body weights of control and treated group mice before and after treatment (Fig.2).

There was also no significant difference ($P>0.05$) between the testicular body mass indices of the groups (Fig.3). Comparison of testicular sperm reserves showed that the groups differed significantly ($P<0.05$). Further comparison using Duncan's New Multiple Range Test showed that the control group had significantly higher ($P<0.05$) testicular sperm reserve than group IV which received the highest dose of the extract. However, there was no significant difference ($P>0.05$) between control and other treatment groups (Fig. 4).

Effect of hexane extract of *A. indica* on histology of testes and other viscera:

The histology of the testes in both control and treated mice (Figs. 5, 6) showed normal interstitial tissues (I) and seminiferous tubules (S) with spermatocyttes and spermatids at different stages of development similar to the description of Banks (1993).

The liver of control and treated mice showed lobules containing central veins (v), plates of hepatocytes (H) radiating peripherally and sinusoids between them (Figs. 7, 8). A section of the kidney of

control and treated mice (Figs. 9, 10) showed renal cortex with many glomeruli (G) and some tubules (T). Their spleen (Figs. 11, 12) contained splenic corpuscles (C) and red pulp (R).

DISCUSSION

The study showed that intraperitoneal injection of hexane leaf extract of *A. indica* significantly reduced testicular sperm reserve in mice in a dose dependent change in the sperm count of rats (Sadre et al 1983). This difference might have resulted from extraction method, dosage and route of administration.

Sadre et al gave the rats 2ml/kg of the water extract of neem for six weeks. In the present study, however, the animals were given neem oil intraperitoneally. It is thought, therefore, that a higher dose of neem oil administered intraperitoneally can be readily absorbed into the blood and transported to the testes where mature spermatozoa may be affected. This present observation agrees with the findings of Riar et al 1991 and Sharma et al 1996 that neem oil when applied intravaginally possessed spermicidal activity by damaging the plasma membrane of spermatozoa.

During the 4 weeks of study there was no significant difference between testicular body-mass indices of the control and treated groups. There was also no significant difference between their pretreatment and post-treatment body weights. There was no manifestation of toxicity as shown by the normal histology of the visceral organs. These suggest that the extract at the given doses was not toxic in mice.

The histology of the testes of both control and treated mice showed normal interstitial tissues, seminiferous tubules with spermatocytes and spermatids at different stages of development as earlier observed by Sadre et al 1983. The decrease in testicular sperm reserves in mice due to i/p injection of neem oil may be due to inhibition of the process of spermatogenesis or due to spermicidal effect. The later may be more probable hence the histology of testes was not altered. Male contraceptives will be generally accepted only when they can produce the desired effect, preserve virility and do not produce their toxic effect. Unfortunately most male contraceptive methods have serious side effects.

manner. This was due to the fact that the sperm reserves in control mice were significantly higher than those of treatment group IV (100mg.kg). However there was no significant difference between the control group and other treated groups (20, 40 and 80mg/kg). This observation was at variance with the report that oral feeding of cold-water extract of neem leaves produced no comparable

These side effects are not exhibited by the neem extract.

In conclusion, this study has demonstrated that intraperitoneal injection of hexane leaf extract of *A. indica* reduced significantly testicular sperm reserves in mice in a dose dependent manner.

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