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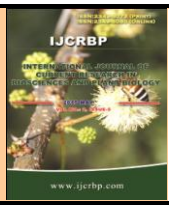

Original Research Article

Effect of *Azadirachta indica* A. Juss. on Some Biochemical and Haematological Parameters of Wistar Rats

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A b s t r a c t

The aim of this study was to evaluate the effects of the extract of the leaf *Azadirachta indica* on biochemical and haematological indices of Wistar rats.16 adult rats (weighing between 195 and 400grams were randomly but equally divided into 4 groups of 4 rats per group. Rats in group I (control) were administered with distilled water and animal feed. While rats in group II to IV were respectively administered with the extract of *Azadirachta indica* orally at the dose levels of 100mg / kg, 200mg / kg and 300 mg / kg once daily for 14days. On the 15th day post administration, rats of all groups were sacrificed and their blood samples were collected through cardiac puncture into EDTA sample bottles for haematological analysis, but collected into a sterile plain container for biochemical analysis. The results showed that AST (11.7±0.3, 15.6±0.3, 19.9±0.5) ALT (14.5±0.4, 19.1+0.3, 24.9±0.5) Urea (32.8±0.6, 45.7±0.6, and 49.6±1.1) Creatinine (1.2±0.1, 2.1±0.1, and 2.5±0.1) were significantly increased in all the experimental groups compared to control (AST 6±0.4, ALT 10.910.9, Urea 20.9±1.6, Creatinine 0.6±0.1). Arbitrarily, there was decrease in body weight of experimental groups compared to the control. While that of Hb (15.7± 0.7, 15.1± 0.6 and 15.0±0.5) and PCV (41.9±0.8, 41.5±0.7, 41.4±0.8) were non-significantly increased in all the experimental groups compared to control (Hb 14.9±1.0, PCV 41.2±1.0) indicating that extract of *Azadirachta indica* causes non-significant increase in Hb and PCV. In conclusion, low dose of aqueous extract of *Azadirachta indica* causes increase in the cellular components of blood but higher doses may result in decrease of some or all of these blood parameters.

K e y w o r d s

Aqueous extract
Azadirachta indica
Haematology
Phytochemicals
Plant extracts

Introduction

Plants shave been man's companion since anyone can remember and has been used as drugs since they are Plants less toxic than those of the synthetic drugs. The use of plants as a medication from the beginning has served as most useful therapeutic weapon in fighting different human and animal diseases (Ogbonna et al., 2008). Many bioactive chemical substances that produce definite physiological and biochemical actions in human body are contained in most plants (Cho et al., 2004). These bioactive constituents are alkaloids, Flavonoid, Phenolic compound Tannin, etc. (Edeoga et al., 2005). Natural product derived from plants has diverse pharmacological properties including antioxidant and anti tumor activity (Karthi et al., 2007). Therefore, plants remain the main source and valuable in the traditional medicine in treating a number of diseases (Ogbonna et al., 2008). These plants can be administered in most diseases condition over a long period of time without proper dosage monitoring and consideration of its toxic effect that may result from prolong use. So practitioners should be informed about reported cases of renal and hepatic toxicity as a result of ingestion of medical herbs. One such plant is *Azadirachta indica* also called Dogonyaro in northern Nigeria. *Azadirachta indica* A Juss. known in vernacular as neem belongs to the family Meliacae. It is widely distributed in Asia, Africa and other tropical parts of the world, almost every part of the plant is used in the indigenous system of medicine for the treatment of various human illness especially against diseases of bacterial and fungal origin (Fujiwara et al., l982).

In fact, in India it is considered to be the village pharmacy and has a key role in Ayuruedic medicine and agriculture since time immemorial. The plant is a large evergreen tree growing up to 10-11m tall. The leaves are divided into numerous leaflets each resembling a full growing leaf (Chatterjee and Pakrashi, 1994). The neem tree flowers from January through April in Northern hemisphere. The flowers are pentamerous, small whitish pink flower buds opens in the afternoon and evening producing a strong scent at night. The flowers are approximately 5mm long and have a sweet Jasmine-like fragrance and produces ample quantity of nectar (Loke et al., 1992). In traditional Ayuruedic medicine, a decoction made from the bark, leaf, root, Fruits and flowers is used in the treatment of blood morbidity, biliary afflictions, itching skin and peptic ulcers (Mitra et al., 1963). The bitter astringent bark is used as decoctum for haemorrhoids. The leaves are steeped for malaria. Neem juice gotten from the leaves, infusion or ointment has been applied externally to wounds and carbineles. The twigs are used to clean teeth, firming gums and preventing gum disease. Neem oil gotten from the seeds is commonly used for hair dressing and is strongly believed to be antifungal and antiviral. Neem oil has been used to treat leprosy and serves as vehicle for other active ingredients (Mitra et al., 1963).

The specific objectives of the study include:

1. To investigate the effect of *Azadirachta indica* on the following biochemical parameters (urea, creatinine, AST, ALT).
2. To investigate the effect of *Azadirachta indica* on the following haematological parameters (haemoglobi, packed cell volume, PCV).
3. To determine possible toxicity and its relationship with dose.

Materials and methods

Plant materials

Collection of leaves: The leaves of *Azadirachta indica* were collected from Aro Farm at Orji Owerri, North Imo State. The leaves were identified at the Department of plant science and Biotechnology Imo State University, Owerri, Imo State.

Processing of leaves: Fresh green leaves of Azadirachta indica were collected and washed with clean water without squeezing to remove debris and dust particles. The washed green leaves were dried under the sun for several days and then grinded with an electric grinder into powdered form.

Extraction procedure: Powdered leaf (50 g) was weighed and then dissolved in 500ml of distilled water. It was boiled at 100°C for ten min. and allowed to cool, after which it was filtered to get a clear filtrate.

Experimental animals

The rats numbering 16 adult males and females weighed between 195 and 400g. The rats were

purchased from Emii Veterinary services no 120 Royce Road Imo State and transported in a ventilated cage to the animal house of Imo state University Owerri. They were kept for 3 weeks. One week was used for their stabilization.The rats were fed with formulated feed bought at Fidelity Agrollied, Kanu Nwankwo Lane of Egbu, Imo State. The feed was formulated for the rats to be healthy and have enough blood.

Experimental design

The animals (rats) were grouped into four (4) groups. Each group was consisting of 4 animals.

Group I : Animals fed with normal diet and distilled water which serves as control.
Group II : Animals received normal diet with the neem extract (100mg/kg).
Group III : Animals received normal diet with the neem extract (200mg/kg).
Group IV : Animals received normal diet with the neem extract (300mg/kg).

The animals were separated in cages labeled with group 1-4 with the control. The control group continues to receive normal diet food and distilled water without any extract throughout the experimental period. The leaf extract dose toxicity and (100mg/kg, 200mg/kg and 300mg/kg) was given to the rats in group 2-4 for 2 weeks.

Route of administration: The neem extract was fed to the rat using a syringe without needle orally.

Collection of blood samples

After 2 weeks of administration, the animals were sacrificed. 10mls of the blood were collected through cardiac puncture and was immediately transferred to a sterile container and allowed to clot for 30 min. for biochemical analysis, while the rest was transferred to an EDTA bottle for haematological analysis.

Laboratory procedure

The reagents used were commercially purchased and manufacture standard operating procedure (SOP) were strictly followed.

Determination of aspartate amino transferase (AST): Using Randox kit with catalogue number 2533AS by Reitman and Frankel method. Into the test tubes labeled Test (T) reagent blank (B). 0.5ml of AST substrate was added to each tube 0.1ml of distilled water was added to the test tube labeled blank. At timed intervals (10 seconds) 0.1ml of serum was also added to the test tube labeled T and mixed. The two tubes were incubated exactly for 30 min. at 37°C. Then 0.5ml of AST colour reagent was added to the 2 test tubes and mixed properly and allowed to stand at room temperature for 20 min. Then 5ml of 0.4 NaOH was added and mixed properly. The absorbance of the sample was then read against the reagent blank after 5 min. using 546 nm wavelength.

Determination of alanine amino transferase (ALT): Using Randox kit with catalogue number 2305AL by Reitman and Frankel method. Into the test tubes labeled Test (T) reagent blank (B). 0.5ml of ALT substrate was added to each tube. 0.1ml of distilled water was added to the test tube labeled blank and mixed. 0.1ml of serum was also added to test tube labeled Test (T) and mixed. The two test tubes were incubated for 30 min. After exactly 30 min., 0.5ml of ALT colour reagent was added to the 2 tubes and mixed properly and allowed to stand for 20 mins. Then 5ml of 0.4 NaOH was added to the test tubes and mixed properly. After which the absorbance of the sample was then read against the reagent blank after 5 min. using 546 nm wavelength. Result was read using the calibrated chart provided by the manufacturer.

Determination of urea: Using Diacetyl monoxime method (Acid method), the reagents and samples were mixed thoroughly and incubated at 100^{o}C exactly for 15min. It was then cooled immediately and after 5 min. the absorbance was read at 560 nm wave length.

Determination of creatinine: Using Jaffe slot Reaction method, 1ml of serum 1% sodium tungstate and 2/3N H_2SO_4 each was pipetted out in the tubes labeled test tubes, mixed thoroughly and allowed to stand for 10 min. It was then centrifuged at 3000 rpm for 5 min. After a clear filtrate was gotten, picric acid and 2.3 N NaOH of 0.5 ml each were added to the clear supernatant , the contents was mixed well and allowed to stand for 15 min, read using blue fitter at 560nm against blank.

Determination of haemoglobin: Cynmethaemoglobin method using Drabkin solution. Four ml of Drabkin solution with 0.02ml of blood sample and with

appropriate blank (without sample) was mixed thoroughly and allowed to stay for 10 min. Finally it was read at 540 nm wave length.

Results

Table 1 shows the liver and kidney parameters before and after administration of *Azadirachta indica*. From the result gotten, it shows that there was a statistically significant increase in the levels of AST and ALT (p<0.05). Moreover urea and creatinine levels were also increased. Table 2 shows the haematological parameter before and after administration of A*zadirachta indica* extract. From the result it could be noted that the level of Hb and PCV was statistically not significantly increase (p>0.05) in the experimental group as compared to the control.

Table 1. Liver and kidney parameters (mean ± SD) before and after administration of the leaf extract of *Azadirachta indica* for the period of 21 days.

Parameters	Control	100mg/kg body wt.	200mg/kg body wt.	300mg/kg body wt.
	Group I	Group II	Group III	Group IV
AST	7.6 ± 0.4	11.7 ± 0.3	15.6 ± 0.3	19.9 ± 0.5
ALT	10.9 ± 0.9	14.5 ± 0.4	19.1 ± 0.3	24.9 ± 0.5
Urea	20.9 ± 1.6	32.8 ± 0.6	45.7 ± 0.6	49.6 ± 1.1
Creatinine	6 ± 0.1	1.2 ±0.1	2.1 ± 0.1	2.5 ± 0.1

Table 2. Haematological parameters before and after administration of the leaf extract of *Azadirachta indica* on wistar rats for the period of 21 days.

Parameters	Control	100mg/kg body wt.	200mg/kg body wt.	300mg/kg body wt.
	Group I	Group II	Group III	Group IV
HB	14.9 ± 1.0	15.7 ± 0.7	15.1 ± 0.6	15.0 ± 0.5
PCV	41.2 ± 1.0	41.9 ± 0.8	41.5 ± 0.7	41.4 ± 0.8

Table 3 shows the body weight of the Wistar rat before and after administration of the *Azadirachta indica* leaf extract and the change in body weight after the experiment. From the result, it shows that there was a statistically significant decrease (p<0.05) in the body weight of experimental group III and IV as compared to control and group II which showed a statistically significant increase (p<0.05) in body weight, indicating that the decrease in body weight is dose dependent.

Table 3. Body weight of the Wistar rats before and after administration of *Azadirachta indica* leaf extract for a period of 21days and the change in body weight.

Weight (g)	Control	100mg/kg body wt.	200mg/kg body wt.	300mg/kg body wt.
	Group I	Group II	Group III	Group IV
Initial weight	198.8 ± 8.5	236.3 ± 12.5	252.5 ± 21.0	330.0 ± 60.2
Final weight	211.3 ± 12.5	243.8 ± 6.3	198.8 ± 18.9	292.5 ± 56.9
Change in body weight	12.5 ± 4.0	7.5 ± 6.2	53.7 ± 2.1	37.5 ± 3.3

Discussion

The biochemical markers such as AST, ALT are used to assess the extent of liver damage and are elevated in hepatic damage. Urea and creatinine are used to assess the renal damage and are elevated if there is any renal damage. In this study, *Azadirachta indica* causes a relative increase in the biochemical markers in rats in Group II given lower doses of *Azadirachta indica* but in the subsequent groups, it causes a significant increase in the levels of serum markers enzyme of hepatic damage AST, ALT indicating that there was hepatic damage. Also there was a significant increase in the levels of urea and creatinine above the normal values which indicate renal damage. Therefore, toxicity of *Azadirachta indica* is dose and time dependent.

This finding is in line with Akah et al. (1992) who studied the hepatotoxic effect of *Azadirachta indica* leaf extract on rats and suggested that in high doses the aqueous extract may have some hepatobiliary toxic effect. The study shows that the toxicity caused by the leaf extract was dose dependent.

Studies have it that at specific doses of the leaf extract of *Azadirachta indica*, ALT, AST, Urea and creatinine were altered. The alterations in the biochemical parameters have consequential effects in the normal functioning of the organs of animals and therefore the ethanolic extract of *Azadirachta indica* stem bark may not be completely safe as an oral remedy. Nonetheless, a gradual recovery has been reported in the biochemical parameters after cessation of the treatment suggesting that the effects are transient and reversible. In its haematological effect, this study showed that Azadirachta Indica causes non-significant increase in the concentration of blood parameters such as Hb and PCV. This finding is supported by Ekaidem et al. (2010) who showed that PCV, Hb values were not significantly different among the test and control.

It is also in line with Koofreh et al. (2010) who reported that Hb and PCV were non-significantly increased in all the experimental groups compared to the control. This study equally observed a loss of body weight in some of the experimental groups (III and IV) indicating that the loss of body weight is as a result of high dose of the leaf extract.

Conclusion

Although fresh leaf extract of Azadirachta indica exhibits strong medicinal properties and many individuals use it in high doses to fight against diseases. It is concluded that the present results have it that Azadirachta indica extracts have a strong biochemical effect on the liver, kidney, of these lower mammals even when use in small dose and so should be taken with caution if absolutely necessary.

References

Akah, P.A., Offiah, V.V., Onugu, E., 1992. Hepatotoxic effect of *Azadirachta indica* leaf extract in rabbits. Fitoter. 63(4), 311-319.

Chatterjee, A., Pakrashi, S., 1994. The Treatise on Indian Medicinal Plants.: Publications and Information Directorate, New Delhi. 76p.

Cho, E., Seddon, J., Ronser, B., Willet, W., Hankinsons, S., 2004. Prospective study of intake of fruits, vegetables vitamin and caroterudes and related muscucopathy. Archoptha 122, 853-892.

Edeoga, H.O., Okwu, D. E., Mbaoble, B. O., 2005. Phytochemical constituents of some Nigeria medicinal plants. Afr. J. Biotechnol. 4(7), 685-688.

Ekaidem, I.S., Alangwho, H.D., Akpan, U.F., Usoh, O.E., Etim, P. E., 2010. Effects of ethanol extract of *Azadirachta indica* leaves on some immunological and haematological parameters of diabetic Wistar rats. Afr. J. Pharm. Pharmacol. 4(3), 104-108.

Fujiwara, T., Takeda, T., Ogihara, Y., Shimizu, M., Nonura, T., Tomita, Y., 1982. Studies on the structure of polysaccharides from the bark of *Melia azadirachta*. Chem. Pharm. Bull. 30, 4025-4030.

Karthi, K.S.,Vigneswari, K., Jegatheesari, K., 2007. Screening of antibacterial and antioxidant activities of leaves of *Eclipta prostata*. Sci. Res. Essay 2(4), 101-104.

Koofreh, D., Uduak, O., Ekpenyong, C., Akpan, U., Amadi, D., 2010. Effect of ethanolic extract of leaf of *Azadirachta indica* on some hematological parameters in albino Wister rats. Global J. Med. Sci. 9(1 & 2), 1-4.

Loke, J. H., Heng, C. K., Rejab, A., Basinin, N., Mardi H. C. A., Ooi, P.A.C., Lim G.S., Teng P.S., 1992 : Studies on neem (*Azadirachta indica* A. Juss) in Malaysia: In: Proceedings of the 3rd International Conferences on Plant Protection Society. pp.103-107.

Mitra, C. K., Patel, M. S., (1963) Neem. Indian Central Oil Seeds Committee, Hyderabad. pp.69-74.

Ogbonna, S., Adekunle, A. A., Bosa M. K., Enweru, V. N., 2008. Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. Afr. J. Biotechnol. 7(6), 701-705.