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BIOCHEMICAL INDICATORS OF SUBCHRONIC TOXICITY OF AQUEOUS CRUDE EXTRACT OF *Azadirachta indica* LEAF IN MICE

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Abstract
Azadirichta indica (neem) reportedly has vast therapeutic potentials. The extract was found to be composed of tannin, saponin, glycoside, alkaloid, flavonoids and volatile oil. The safe dose of the extract was 150mg/kg body weight (BW) of mice. The sub-chronic toxicological evaluation showed a higher body weight gain in the test animals but a decline in the body weight in the control animals. The packed cell volume (PCV) was higher in test animals than in the control. There was also a significant ($P<0.05$) increase in the serum glucose, triglyceride and enzymes (glutamate oxaloacetate and serum glutamate pyruvate transaminase). There was however a decrease in total proteins as compared with the control. We conclude these findings are indicative of likely toxicological and pathological consequences on long term consumption of crude leaf extracts despite its medicinal properties.

Keywords: *Neem, phytochemical, weight gain, sub-chronic, toxicity*

INTRODUCTION

Medicinal Plants are believed to be relatively safer than the synthetic drugs. Plant constituents closely mimic the natural constituents of the human somatic system (Gamaniel, 2000). However, the toxicity profile of most medicinal plants has not been thoroughly evaluated. *Azadrichta indica* (neem) is a member of the Meliaceae family and is native to India and tropical South East Asia (Lawal *et al.*, 2005). It is a fast growing plant that can survive drought and poor soil, keeping its leaves all year round (Rosnerr *et al.*, 2005). In Nigeria the tree is commonly called 'Dogon yaro' and mostly found in the Northern part of the country. It is extensively used in traditional medicinal practice. Neem is regarded as the 'wonder tree' and nature's drug store because its extracts have vast pharmacological activities (Lawal *et al.*, 2005). Neem has been reported to have antibacterial, antifungal, antimalaria, anti inflammatory, hypolipidemic properties, immune potentials (Raka *et al.*, 2007), anti tumor and anti cancer. It's also used as raw material for several products including pesticides, mosquito repellents and skin disease treatment (Sandre *et al.*, 2000), soap making and tooth paste (Chattapadhyay, 2005). Neem and other plant species are commonly used as a cocktail in the treatment of malaria (Jigam *et al.*, 2010). Aqueous extracts have been reported to be effective for the treatment of heat rashes and boils. Neem is used to make soap and tooth paste (Chattapadhyay, 2005). Important chemicals in neem that contribute to these effects include nimbidin, limonoid, catechin, azadirachtin, nimbin, deacetyl azadiractinol, nimbinin, nimbesterol, salamin, liminoids, quercetin, nimocinol and meldenin (Raveendra *et al.*, 2004). Due to the problem of drug resistance in many diseases like malaria, fungi and bacteria infections to conventional therapeutic drugs, many have been left with no other choice but to use such alternatives as neem. However taking of this phytomedicines without recourse to purification and safe therapeutic dose is a major concern. This study is to ascertain the possible toxicity effect of neem on long term consumption.

MATERIALS AND METHODS

Plant Materials

Neem leaves were collected for screening between June and July from Minna and its environs. The leaves were cleaned and dried at ambient temperature. The dried leaves were crushed and ground with pestle and mortar into powdery form and stored in air tight sample holders until needed.

Plant Extraction

50g of the powdered plant sample was extracted by reflux using 200ml of distilled water for 2 hours. The marc was filtered with muslin cloth. The extract was concentrated by evaporating the solvent over a steam bath and subsequently stored in tightly stoppered sample bottles before use.

Animals

Healthy Swiss albino mice of both sex with average weight ranging from 20-30g were obtained from the National Vetinary Research Institute (NVRI) Vom, Plateau state. The animals were housed in laboratory animal cages under standard environmental conditions of temperature, humidity and allowed free access to commercial livestock pellets and water ad-libitum.

Phytochemical Analysis

Standard screening test were used to detect secondary metabolites such as alkaloids, tannins, saponins, glycosides, volatile oil, anthraquinones etc in the crude extracts (Trease and Evans, 1989).

Safe Dose and acute toxicity (LD_{50}) of Neem crude extract

Five groups of four mice each were used. The extracts were suspended in water at concentrations of 200, 400, 600, 800 and 1000 mg/kg body weight (bw) and administered to the animals intraperitoneally. The control group was given normal saline (0.9% NaCl) at 20ml/kg bw. The mice were observed for 24-72 hours with clinical signs and mortality recorded. LD_{50} was obtained as the intercept of % mortality and dosage.

Sub-chronic Toxicity assay of *Azadirachta* (Neem) crude extract

150mg/kg bw of *Azadirachta indica* leaf extract was administered to five groups of five mice for six weeks. The weights of the mice were recorded weekly using an Avery balance (W and T Avery Ltd Birmingham UK.). Dead animals were autopsied, surviving animals were sacrificed and histopathological studies were carried out. The Serum was obtained as described by Monica (1999) and Lawal *et al.* (2005) and used for the determination of serum glucose (Aliyu *et al.*, 2005; Trinder, 1974), total protein using Doumas method (Cheeseebrough, 1991), triglycerides and serum enzymes were analysed by standard biochemical methods of Reitman and Frankel (1957).

Statistical Analysis

Results are expressed as mean ±SEM between control and experimental animals in the groups. Statistical significance was analysed by one way ANOVA and Duncans post Hoc Test between homogenous subsets. Values of $P<0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Table 1: Phytochemicals of aqueous leaf extracts of *Azadirachta indica*.

Phytochemicals	Inference
Alkaloids	
Tannins	+
Glycosides	+
Saponins	–
Volatile Oils	+
Flavonoids	+

(+) Present (-) Not detected

Table 2: Average Organ Weight of mice treated with aqueous leaf extracts of *Azadirachta indica*

Organ	Control (g)	Test (g)
Heart	0.20±0.01	0.15± 0.02
Liver	1.07±0.01	1.21±0.05
Lungs	0.15±0.01	0.18±0.02
Kidney	0.32±0.02	0.35±0.05
Spleen	0.08±0.03	0.13±0.04
Stomach	0.47±0.01	0.51±0.27
Intestine	2.11±0.01	3.14±0.54

Table 2: Safe Dose of aqueous leaf extract of *A. indica* in mice

Dose (mg/kg bw)ip	Clinical observation	Mortality	% Mortality
200	Normal	0/4	0
400	Drowsy	0/4	0
600	Somnolence	0/4	0
800	Laboured breathing	2/4	50
1000	Tarchycardia and mortality	2/4	50

Table 3: Biochemical Parameters of Sub-chronic Assay of aqueous leaf extract of *A. indica* in mice.

Parameter	Control	Test
Glucose (g/dl)	81.09±2.32	95.16±6.7
Total Protein (mg/dl)	6.36±2.06	6.00±0.99
Triglycerides (mg/dl)	130.29±10.77	153.55±10.99
Glutamate oxaloaceate transaminase (U/L)	33.26±0.015	45.50±0.099
Glutamate pyruvate Transaminase (U/L)	28.92±0.076	36.40±0.004

The results of the assays are presented in Tables 1, 2, 3, and Figures 1 and 2. Phytochemicals detected in the extracts of *Azadirachta indica* include tannins, saponins, glycosides, alkaloids, flavonoids and volatile oils (Table 1). This is supported by earlier reports of (Hassan *et al.*, 2006; Wasagu *et al.*, 2005) that these secondary metabolites are found in *Azadirachta indica*. These compounds form the basis of the pharmacologic effects of such plants and lend credence to the medicinal uses of the plant. Alkaloids rank topmost among efficient and therapeutic plant components. Pure alkaloids and their derivatives are used as basic medicinal agents (Jigam *et al.*, 2010). Alkaloids and saponins have been reported to have antibacterial activity (Hassan *et al.*, 2006). Quinine is antimplasmodial, colchicine is used for gout, Morphine is analgesic, reserpine is a tranquilizer (Haidet, 2004; Jigam *et al.*, 2009).

The safe dose determinations presented in Table 2 shows the Safe dose of the extract was 600mg/kg bw with LD_{50} of 900mg/kg bw. Doses below 800mg/kg bw were safe and free of any adverse clinical symptoms. Not all medicinal plants are safe for consumption in the crude form, some level of toxicity may arise as a result of potential toxic compounds they contain (Amdur *et al.*, 1991). According to Clack and Myra (1975), any substance with LD_{50} above 1000mg/kg bw is regarded as safe, thus neem extract with LD_{50} of 900mg/kg (from this study) is potentially toxic. Various toxic effects of neem including liver and kidney damage have been reported (Ibrahim *et al.*, 2006).

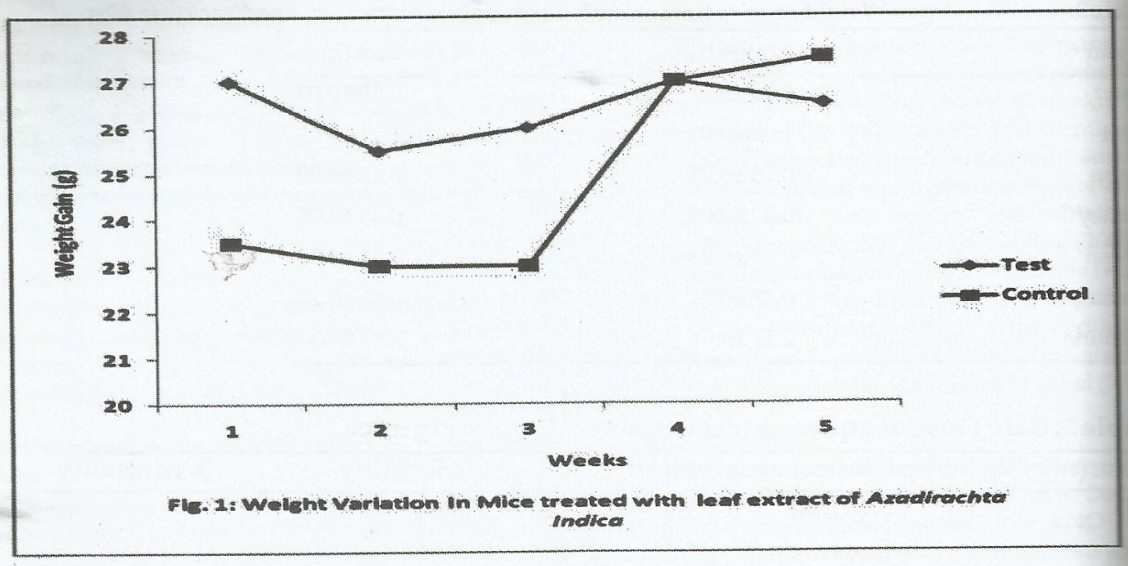

Fig. 1: Weight Variation in Mice treated with leaf extract of *Azadirachta Indica*

A slight increase in weight of test animal compared to the control was observed, (Figure 1). The decrease in weight within the test group during the 40 days of extract administration compared with control could be due to reduced feed and water intake. The secondary metabolites may have caused loss of appetite resulting in decreased weight gain in the test animal. Tannins inhibit growth by decreasing the digestion coefficient of most nutrients and the coagulation of proteins Sotohy (1997) as sited by Jig[illegible] *et al.* (2011). Also an appreciable difference in aver[illegible] organ weight of the test animals to the control [illegible] obtained. The alteration in these organs may be due[illegible] their exposure to the toxic principles in the extract[illegible] they are sites of biotransformation and excretion. Some alkaloids and saponins have cytotoxic effect[illegible] organs; they damage the cells of the liver, kidney, lungs, and heart (Harborne, 1972).

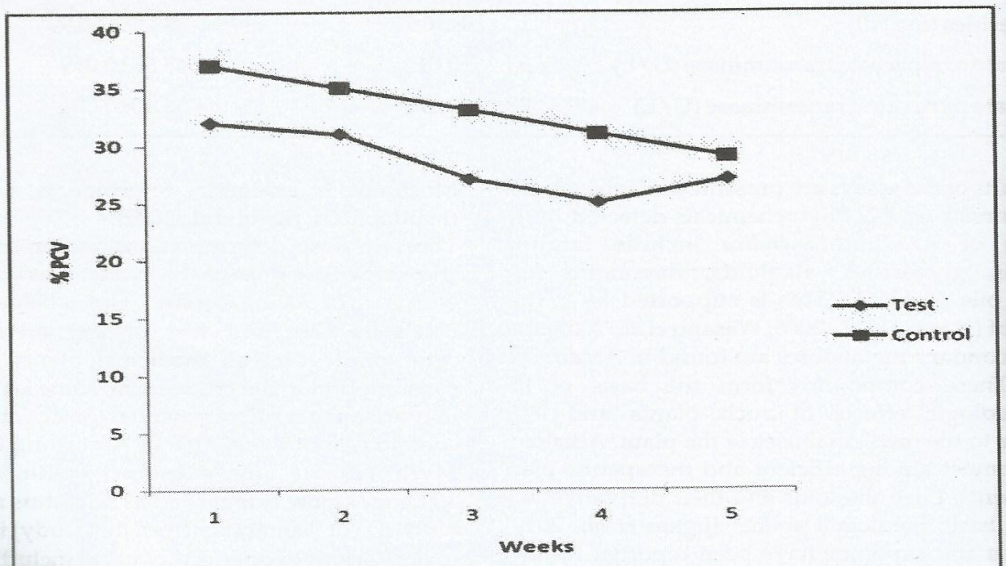

Fig 2: Percentage packed cell Volume (PVC) of Mice treated with leaf extract of *Azadirachta Indica*

The packed cell volume shows a marked decrease in the test animal as compared to the control, (Figure 2). Antinutritive compounds chelates mineral such as iron and adversely affect the bioavailability of vitamins required for hemopoiesis (Jigam *et al.*, 2011b). Saponins cause haemolysis of the red blood cells (Clark and Myra, 1975).

A lower total protein in test animals compared to control was obtained (Table 3). Decreased serum protein is indicative of kidney and liver excretory and synthetic dysfunctions respectively (Cheesbrough, 1991; Gad, 2001). The finding in this study is in agreement with the observations of Lawal *et al.* (2005).

The biochemical parameters in Table 3 show a significant ($P<0.05$) difference in the serum enzymes and a non significant ($P<0.05$) difference in the serum glucose, protein and triglycerides in the test animals compared to controls. Alteration of serum glucose other than those associated with stress are uncommon and reflect an effect on pancreatic islets, this implying the test plant extract appears not to have a toxic effect on the pancrease. Alterations in total protein are usually associated with decreased production by the liver or increased loss from kidney (Gad, 2001).

Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) are maker enzymes of liver and kidney functions (Lawal *et al.*, 2005). The predominant source of SGPT is the liver followed by the kidney. The significant elevation of the SGOT and SGPT are as a result of possible necrotic injury and cholestasis (Bhanwra *et al.*, 2000; Hassan 2006). Liver toxicity is ascertained if an increase in SGPT is three times its level at the onset (Hosein, 2001).

These findings did not provide evidence of clinical safety of *Azadirachta indica* plant extract at higher concentrations on long term consumption. It is hence necessary to be cautious when using Azadirachta *indica* in herbal medicine.

CONCLUSION

From this study, it is concluded that crude extracts of *Azadirachta indica* need to be purified and standardized before it is used as phytomedicine, to prevent possible organ damage.

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