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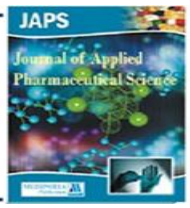

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Effects of Sub-Chronic Administration of *Diospyros Mespiliformis Hochst* (Ebenaceae) Root Extracts on Some Biochemical Parameters in Mice

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ABSTRACT

The effects of medium term administration of crude *Diospyros mespiloformis* root extracts on some biochemical parameters were investigated in mice. Forty mice were divided into two groups of twenty animals each. Animals in group I were gavaged with the root extracts at 400mg/kg/body weight for five weeks. Group II received normal saline (0.09%w/v NaCl) and served as controls. Whole body weights, fresh organ weights, packed cell volume (PCV) and some serum biochemical parameters were analysed using standard methods.
Results showed minimal variation in whole body weights and packed cell volumes of animals given the extracts. Also values for some organ weights, triacyglycerides (148.25± 2.78 mg/dL), and Alkaline Phosphatase (41.50± 1.71 mg/dL) were not significantly ($p > 0.05$) different between test and control animals in the final week. However, heart (0.74%), lungs (4.43%), glucose (113.92 ± 2.43 mg/dL), total proteins (4.75 ± 1.25mg/dL), Aspartate Transaminase (40.50 ± 1.50 µL) and Alanine Transaminase (43.52 ± 4.50µL), were significantly ($p < 0.05$) different between the animals administered *D. mespiliformis* and controls. These results are early indications that long term consumption of *D. mespiliformis* could predispose to adverse tissue effects.

Keywords: *Diospyros mespiliformis, Serum, Triacylglycerides, Aspartate Transaminase, Alanine Transaminase.*

INTRODUCTION

Diospyros mespiliformis Hochst (Ebenacea) also called “African Ebony” or Kanya by the Hausa of Nigeria is a large deciduous tree confined to tropical and subtropical regions including Africa and Asia (Dalziel, 1955; Mohamed *et al*., 2009). Mature trees average 4-6 metres occasionally reaching 5 metres in height. Foliage is dense and dark green with elliptical leaves and fruits often eaten by grazing animals (Belemtougri *et al.*, 2006; Watt and Brandwijk, 1962). *Diospyros* is reportedly one of the most important genera of *Ebenaceae* which species have been used over the millennia in traditional medicinal systems including Ayurveda, Chinese and African folklores (Mallavadhani *et al.*, 1998; Kantamreddi and Wright, 2007).

The ethnopharmacological uses of different organs of *D. mespiliformis* have been reported in the literature e.g leaf decoctions are used against fever, whooping cough and wounds (Adzu *et al* 2002). Barks and roots are used to treat malaria, pneumonia, syphilis, leprosy, dermatomycoses, diarrhea, facilitation of delivery and as psycho-pharmacological drug (Mohamed *et al.,* 2009). Studies have shown that the leaf extracts of *D. mespiliformis* are effective against *Plasmodium falciparum* invitro (Etkin, 1997), *Plasmodium berghei* in vivo (Adzu and Salawu, 2009) and relieved pains and fever in rodent models (Adzu *et al.,* 2002). Tannins, alkaloids, diosquinone and plumbagin were isolated from *D. mespiliformis* and demonstrated activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* thus providing a basis for the plant usage in herbal medicine *(*Adeniyi *et al.,* 2006; Lajubutu *et al.,* 2006). The global acceptability, application and efficacy of herbal medicaments have proportionately exposed millions of people to the underlying but seldom reported problems of medicinal plant toxicity and misadventuring. Most herbal treatments are in the form of crude extracts administered over a few days or weeks with the likelihood of cumulative intoxication of recipients (Jigam *et al.,* 2011[a], Klink, 1997). The therapeutic window of digoxin is so narrow that maintenance doses could be as small as 50ug/day whereas digitalis leaf often preferred to the pure compounds (digoxin and digitoxin) is often given in doses of 1.5g/day (Gamaniel, 2000). The present study was therefore undertaken to evaluate some biochemical indices in mice sub-chronically administered *D. mespiliformis* root extracts in an attempt to further rationalize its use in herbal medicine.

MATERIALS AND METHODS

Plant Materials

The root of *Diospyros mespiliformis* was collected in July in Bosso area Minna, Northern Nigeria after due identification by a herbal practitioner and authentication at the Department of Biological Sciences, Federal University of Technology, Minna.

Preparation of Crude Extracts

50g of air dried plant materials were micronized and extracted exhaustively (48 h) in the cold with 2L of methanol, (Sigma-Aldrich Europe). The marc was filtered with muslin cloth and solvent removed under reduced pressure in a rotary evaporator. Brown coloured paste was obtained, freeze dried and weighed prior to analysis.

Animals

Healthy Swiss albino mice of either sex of about 6 weeks old weighing between 20 – 30 g each obtained from National Institute of Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria were used for the experiments. The rodents were conveniently housed under standard environmental conditions (Temperature 27 ± 2°C; 70% relative humidity; 12hrs daylight/night cycle) and had free access to commercial feed pellets and water. Experiments were conducted in strict compliance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (CCAC, 1997).

Safe dose and acute toxicity (LD_{50})

Five groups (A, B, C, D and E) of four mice each were used. The animals were given extracts intraperitoneally (i.p) at doses of 100, 200, 400, 600 and 1500mg/kg body weight (bw) in groups A, B, C, D and E respectively. Extracts were dissolved in dimethylsulphoxide (DMSO) (Sigma chemicals; St. Louis, M. O. USA). A control group was given normal saline (0.9% w/v NaCl) at 20 ml/kg bw. Mice were observed over 72h. Clinical signs and mortality were recorded. LD_{50} was obtained graphically as the intercept of % mortality (y-axis) and dosages (x-axis).

EVALUATION OF THE EFFECTS OF SUB CHRONIC ADMINISTRATION OF CRUDE EXTRACT IN MICE

Forty mice were kept in two groups (A and B) of twenty each. Group A was used as test and gavaged with 400mg/kg bw extract daily while B was control and given 20ml/kg bw normal saline daily. All animals were monitored for different biochemical parameters at weekly intervals for five weeks. Weights of mice were taken with an Avery Balance (W and T) Avery Ltd, Birmingham, UK. Packed Cell Volume (PCV) was determined using the microhaematocrit method (Green, 1976). Serum glucose was assayed with Randox glucose diagnostic kit (Cat/Kat NR GL 2623) based on the glucose oxidase reaction. Total proteins were evaluated with the Randox Protein Diagnostic Kit (TP 245) based on the interaction of cupric ions in alkaline media with protein peptide bonds. The AGAPPE, Triglyceride Kit (Cat. 1121500, Kerala, India) was employed for serum triglycerides. This involve the reactions by lipases, glycerol kinase, glycerol – phosphate oxidase and peroxidase which produced a red quinone dye read at 630nm (Annoni, *et al.;* 1982). Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were analyzed using the DIALAB IVB standard diagnostic kits based on the method of Wolf, (1980). Alkaline phosphatase (ALP) was however determined on the basis of the conversion of P – nitrophenol to its intensely yellow coloured derivative, 4 – nitrophenoxide (Tietz, 1983). The diagnostic reagent (DIALAB Cat D95560) kit was used.

STATISTICAL ANALYSIS

Results are expressed as mean ± standard error of the mean. While student's t-test was used to test for differences between groups using Statistical Package for Social Sciences (SPSS) version 16. A value of $P<0.05$ was accepted as significant and the data compared using Analysis of variance (ANOVA)

RESULTS

The extract yield of *Diospyros mespiliformis* roots in methanol was 1.83g (3.66%), with safe dose of 400mg/Kg bw and LD_{50} of 620mg/kg bw of mice.

Weight variations

Whole body weights of mice (Fig. 1) administered *D. mespiliformis* extract exhibited minor depreciation in weeks one (1) and two (2). It subsequently varied only minimally. However weights of control mice increased steadily with a peak in week three (3) and subsequent drop by week four (4). Fresh organ weights of mice expressed as percentages of whole body weights are in Table 1. Only the heart and lungs of test animals exhibited some variations. The other organs of the test mice were comparable in weight with those of the controls.

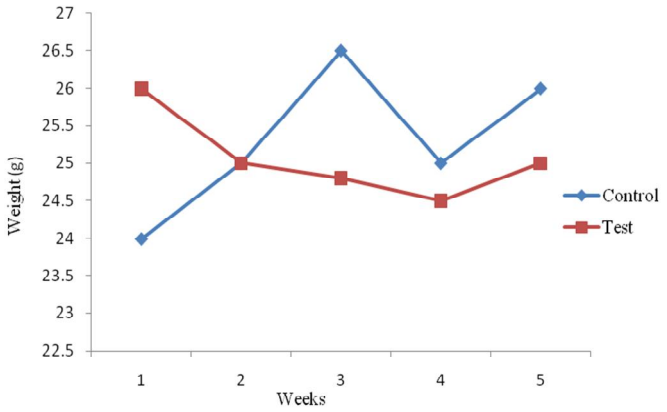

Fig. 1: Weight variation in mice administered *D. mespiliformis* extracts.

Table. 1: Fresh organ weights of mice administered *D. mespiliformis* extracts.

Organ	Weight (%)	
	Control	Test
Heart	0.59	0.74*
lungs	2.12	4.43*
Kidneys	1.98	2.16
Liver	6.43	6.40
Stomach	1.92	2.17
Intestine	14.80	15.20
Spleen	0.71	0.69

* Significant

Packed Cell Volume

The Packed Cell Volume PCV (Fig.2) of test animals exhibited only minor variations in the course of study. These changes were comparable to those for controls.

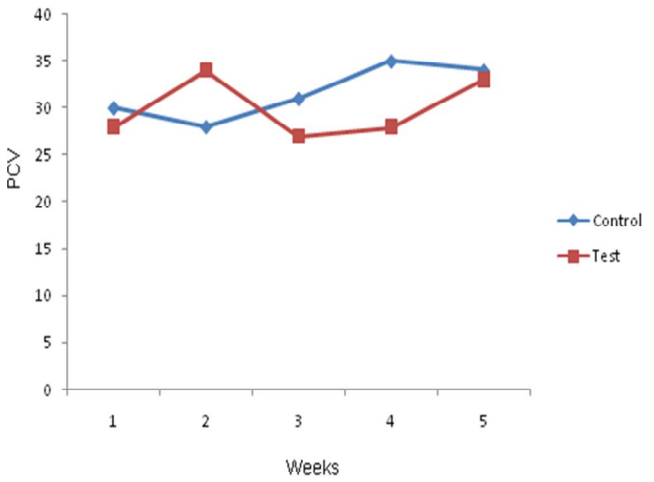

Fig. 2: Variations in the Packed Cell Volume of mice administered *D. mespiliformis*

Serum Glucose, Total Proteins and Triglycerides

Table 2 contains values for glucose, total proteins and triacylglycerides. Glucose levels in the test mice were significantly ($p<0.05$) elevated in week 3 (139.00$\pm$ 4.43mg/dl) and week 5 (113.92 $\pm$ 2.43mg/dl) respectively. However total Protein levels declined in the third week (4.75$\pm$1.25mg/dl). No significant ($p>0.05$) changes were observed in triacylglyceride levels between test and control mice over the five-week duration of the study.

Serum Transaminases (AST and ALT) and Alkalline Phosphatase (ALP)

The values for Transaminases (AST and ALT) and ALP are in table 3. AST was significantly ($P<0.05$) higher in the test mice (40.50 $\pm$ 1.50 µ/L) compared with levels in controls (32.75 $\pm$ 1.11 µ/L) in week 5. Similarly, serum ALT levels were significantly ($P<0.05$) elevated in week 3 (42.25 $\pm$ 1.93 µ/L) and week 5 (43.52 $\pm$ 4.50 µ/L) in the test animals compared to controls. There were no significant ($P>0.05$) differences in ALP levels between test and control mice over the 5 week study period.

DISCUSSION

Total body weight was unaffected in the animals thus signifying normal feed intake and bioavailability of nutrients. A weekly body weight measurement has been recommended for mice under chronic toxicological assessment. Body weight and feed utilization are often sensitive to xenobiotics and occasionally are the only significant toxicological findings with materials of low toxicity. Stress, diarrhea and dehydration are other factors that influence weight change (Jigam *et al*., 2011[b]; EPA\OPPTS, 1988). Some variations were obtained in the weights of heart and lungs of animals dosed with *D. mespiliformis* but not kidneys, liver and spleen, organs critically involved with detoxification and hematopoiesis (Abdelgadir *et al*., 2010). The observed minimal changes in the packed cell volume of treated animals is indicative of the insignificant levels of iron chelating principles in extracts of *D. mespiliformis*. Spleen which is a hematopoietic organ, was also unaffected (Mitruka and Rawnsley, 1977; Jigam *et al*.,2011[b]). Significant ($p<0.05$) elevations in serum glucose levels could be attributed to effects of *Diospyros* on the pancreas, insulin production and glycogenesis (Gad, 2001). These findings should however be necessarily interpreted in conjunction with histopathological examinations (Pearse, 1985; Abdelgadir *et al*., 2010). Total protein levels depreciated significantly ($p<0.05$) in the test animals in the third week, an effect that was reversed in the final week. Decrease in serum proteins could generally be early indications of liver damage renal failure or nutritional deficiency (Sood 2006). Reports exist of similar effects of some plant extracts in experimental animals (Sodipo *et al*., 2011). The significant elevation ($p<0.05$) of serum transaminases (AST and ALT) obtained in mice treated with the extracts is noteworthy as these enzymes are indices of hepatic injury although ALT is more liver specific. A variety of plants have been reported with this effect. ALP levels were not however increased despite the fact that the enzyme is usually encountered in obstructive jaundice and cirrhosis

of the liver (Sodipo *et al.*, 2011; Jigam *et al.*, 2011[b]). Enzymes are sensitive indices of cellular injury and are elevated above normal from tissue leakage before changes are noted with clinical and histological tests (Obatomi *et al*., 1995).

CONCLUSION

The medicinal properties of *Diospyros mespiliformis* have been widely documented. However, the potential toxicity of crude extracts especially with chronic intake cannot be ignored. Histopathological studies are hence warranted to validate such findings. Bioactive principles(s) could be further isolated to serve as lead compounds or templates for the synthesis of more potent drugs with reduced toxicity.

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Table. 2: Serum glucose, total proteins and Triacylglycerides in mice administered *D. mespiliformis* extracts.

Parameters	Week				
	1	2	3	4	5
Control Glucose (mg/dl)	103.06±2.45	95.45±3.94	95.55±2.44	94.00±2.48	94.00±2.16
Test	105.80±3.56	102.50±2.18	139.00±4.43*	103.25±4.03	113.92±2.43*
Control Total proteins (mg/dl)	7.94±0.34	7.61±0.33	9.50±2.22	7.53±0.29	7.83±0.42
Test	8.01±0.08	7.84±0.42	4.75±1.25*	7.92±0.42	8.20±0.42
Control TAGs (mg/dl)	147.25±5.17	145.50±4.25	137.52±0.42	142.50±2.06	142.52±3.87
Test	151.50±3.10	144.00±3.16	137.90±0.52	143.50±2.50	148.25±2.78

n= 20 *p<0.05

Table. 3: Serum AST, ALT and ALP in mice administered *D. mespiliformis* extract.

Serum Enzymes (u/L)	Week				
	1	2	3	4	5
Control AST	29.50±1.71	29.00±0.87	29.50±2.22	29.50±1.55	32.75±1.11
Test	26.75±0.75	29.00±1.08	34.75±1.25	31.00±1.29	40.50±1.50*
Control ALT	33.75±1.60	33.00±1.47	33.50±1.47	32.50±1.50	30.60±2.43
Test	32.75±1.11	36.75±0.75	42.25±1.93*	36.50±2.06	43.52±4.50*
Control ALP	36.00±1.41	36.50±0.96	34.00±1.41	34.00±1.47	38.16±1.14
Test	34.25±0.85	32.50±1.89	38.00±0.82	36.75±1.89	41.50±1.71

n=20 *p<0.05.

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