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Sub-acute toxicity studies of methanol extract of *Hyphane the baica (L) Mart* fruit pulp on normal wister Albino rats

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ABSTRACT.

The effect of methanol extract of Hyphane thebaica (L) mart fruit pulp was investigated. Twenty white albino rats of wister strain were divided into four groups of five rats each. Group one serves as control while groups 2, 3 and 4 were administered daily with 200, 400 and 800mg/kg orally of 70% methanol extract of H. the baica fruit pulp. All the rats were fed with normal diet (ECWA Mash, Jos, Nigeria) and water adlibitum for 28 days. Results revealed significant (p>0.05) body weight increase in all the experimental groups. However, in weeks 3 and 4, there were decrease in feed intake (p<0.05) in the 800mg/kg group when compared with control. In the result of liver function there was no statistical difference (P> 0.05) in the level of total protein, AST, total bilirubin and conjugated bilirubin in treated groups when compared with control group. The group administered 400mg/kg body weight dose has increased (p<0.05) in the level of ALP, while 800mg/kg dose increased significantly (p<0.05) in both ALT and ALP. However, urea, creatinine, sodium and bicarbonate were not affected. It was concluded that the methanolic fruit pulp extract of H. the baica at higher doses; its use should be excised with caution.

Key words

Hyphane thebaica ; sub-acute toxicity studies

Introduction

The history of medicinal uses of plants is intimately connected with the history of mankind. Ancient men lived at the mercy of nature. From the earliest times, tribal priest and medicine men used various plants usually in association with rituals to cure diseases. Today it is believed that over 30% of prescription drugs in developed countries are of plant origin (Iwu *et al*, 1999). In fact, the primary healthcare of 70 to 80% of the world's population is based on the use of traditional system of medicine (LMPTK, 2006). In sub-Saharan Africa, more than 80% of the population relies on medicinal plants and traditional medicine as their primary source of heath care (WHO, 2003).

Hyphaene thebaica (L) Mart is a plant used for its fruits, leaves and roots for medicinal purpose in the North East Arid zone of Nigeria. The plant, *Hyphaene thebaica* is a member of the Palmae (Arecaceae) family. The fruit is being used as condiment to enhance flavor and the fruit pulp extract is also used in the treatment of bilharzias, bleeding especially after child birth and also as haematinic agent (Adaya *et al;* 1977; Von Maydell, 1986). Zannah *et al* (2008) reported that the aoueous suspension of the root of *Hyphane thebaica (L)* Mart was hyponatremic, hypocholesterolemic, hepato and nephro toxic. *In* recent study, using methanol extract of the fruit of *H. the baica*(L)Mart reduced fasting hyperglycaemia(Shehu *et al*,2014)

Ethno medicine is concerned are safety, quality and efficacy. The present study investigated the effect of methanol extract of the plant on some tissues in rats to establish safety or otherwise.

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MATERIALS AND METHODS

Sample Collection and Identification

Fresh fruit of *Hyphaene thebaica,* was collected from Konduga local government area of Borno state, Nigeria. The plant was authenticated by plant taxonomist with Department of Biological Science, University of Maiduguri.The fruit were cleaned, debris removed, shade dried and ground into powder using mortar and pestle.

Extract Preparation

Hyphaene thebaica fruit pulp powder (500g) was macerated with one liter of 70% methanol in a glass jar for 2 days at room temperature. The extract was filtered and concentrated to dryness under reduced temperature and pressure on rotary evaporator**.** The percentage yield was calculated as 31.65%.

Experimental Animals and Treatment

White Wister strain Albino rats weighing between 120 and 200g were used for the study. The rats were obtained from the Animal house of the Veterinary pharmacology department, university of Maiduguri. They were maintained under standard condition of light (12 hour light). The rats were fed with standard rats diet (growers mash, ECWA feed Nigeria Ltd) and water ad libitum. Twenty white Albino rats were divided into 4 groups of 5 rats each. Group 1 serves as control while groups 2, 3 and 4 were administered daily oral doses of 200, 400 and 800 mg/kg of 70% methanolic extract of *H.thebaica* fruit pulp for 28 days.

Biochemical Analysis

Serum Alanine (ALT) and Aspartate (AST) Amino transferases were assayed by the method of Reitman and Frankel (1957). Serum Alkaline phosphatase (ALP) by the method of Mc Comb and Browser (1972). Serum total protein and albumin were assayed by the methods of Henry *et al,* (1974) as reported by Kaplan *et al,* (1988).Total bilirubin and conjugated bilirubin (Koch and Doumas 1982). The diacytylmonoxine and Jaffes reaction as described by Kaplan *et al,* (1988) were used in assaying serum urea and creatinine respectively .Bicarbonate by Van Slyke and Cullen (1977). Serum sodium and potassium levels were estimated by Flame photometric method, (Kolthof, 1976).

Statistical analysis

The data obtained were presented as Mean and Standard error of mean (Mean ± SEM). Differences among mean were analysed using analysis of variance (ANOVA), by computer statistical software graphpad instat® (2003). Probability value (P Value) ≤ 0.05 was considered significant.

RESULTS

Results of body weight changes following oral administration of different doses of *H. thebaica* fruit methanolic extract for 28 days is presented in Fig 1. The result shows some body weight in all the experimental groups. An increase from 146.00±5.43 to 156.33±2.67 was seen in the group administered 800mg/kg body weight dose. The feed intake pattern also showed (Fig 2) that at 800mg/kg body weight dose group, there was a significant decrease ($P<0.05$) at 3rd and 4th weeks of treatment compared with control group.

The mean water intake showed no statistical difference ($P> 0.05$) from the first to the third week of extract administration, but there was mean reduction in the fourth week ($P<0.05$) of 800mg/Kg body weight group (fig 3)

In the indices of liver function (Table 1), there was no statistical difference ($p>0.05$) in the level of total protein, AST, Total Bilirubin and Conjugated Bilirubin in all the

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treated groups when compared with control group. The group administered 400mg/kg body weight dose has statistically increase ($P<0.05$) in the level of ALP, while the 800mg/kg statically increased at $P<0.05$ both ALT and ALP. The kidney function test (Table 2) showed a statistical decrease ($p<0.05$) in Potassium when compared with the control group. However, urea, creatinine, sodium and bicarbonate were not affected.

DISCUSSION

Subacute toxicity level of methanolic fruit pulp of *H. thebaica* was also investigated. The experimental groups showed body weight increase throughout the experimental period even though is not significant. However, in weeks 3 and 4, there were decrease in feed intake ($p<0.05$) in the highest dose (800mg/kg), when compared with control. This observation may be attributed to the tannin content in the methanolic extract of *H.thebaica* fruit (results not presented), made it less palatable as evident by the reduced feed intake at that week. . When animals lose appetite (anorexia); weight loss is bound to ensure due to disturbances in carbohydrate, protein or fat metabolism (klassen, 2001). Nohra *et al, (*1966) showed that tannic acid reduced feed intake, metabolizable energy and depressed nitrogen retention in chicks, which gradually lead to growth depression. Barnabas *et al, (*1985) also reported that tannin lowers digestibility of proteins and amino acids thus affecting the level of protein in rats. This finding contradicts a similar research using aqueous root suspension where body weight in treated groups showed significant increase compared to control at the doses of 250, 500 and 1000 mg/kg (Zanna *et al,* 2008).

The administration of various doses of methanolic fruit extract of the plant did not produce any significant ($p>0.05$) difference in the levels of AST, total protein, albumin, total bilirubin and conjugated bilirubin. However, the 800 mg/kg body weight doses showed increased in the level of ALT compared with the control ($p<0.05$). Also at 400mg /kg and 800 mg/kg, there was statistical increase in the level of Alkaline Phosphatase (ALP). Elevation of AST levels is seen in patients with acute myocardial infarction , skeletal muscle damage, acute hepatic necrosis, intrahepatic cholestasis, post hepatic jaundice or cirrhosis(Odutola; 1992). Although there was increase in the AST but it was not statistically significant ($p>0.05$). The elevation in the levels of ALT is suggestive of liver damage. However, ALT is widely a serum marker for liver disease, (Kim et al; 2004). Increase in ALP level is usually a characteristic finding in abstructive hepatobiliary disease as found in cholestic liver disease (kaneko. et al; 1997) or may be indication of bone disorder involving osteoblastic activity (schalm *et al,* 1975).

Indices of renal function (urea, creatinine, Na^+ and HCO_3) were not significantly affected by extract treatment. In a similar study using ethanolic fruit pulp extract of the plant, Kamis, *et al,* (2000), reported that at high concentration (1, 2.5 and 5g/kg) the plant extract is hypolipidemic, hepatoxic and nephrotoxic. However, Modu *et al,* (2000) using aqueous pulp extract (1, 2.5 and 5g/kg) of *H.thebaica* found the extract to be hypolipidemic but nontoxic to both liver and kidney.

Although the methanolic extract of fruit pulp is safe in rats treated with 200 and 400mg/kg body weight nonetheless is appears toxic at higher dose. It is recommended that other parts of the plant and different medium of extraction be tried to ascertain safely

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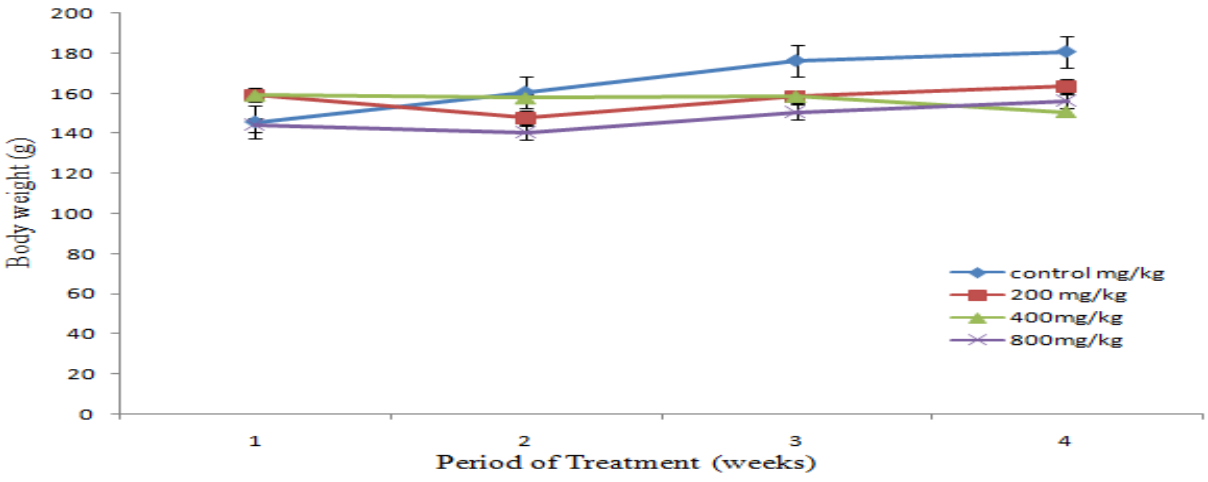

Figure 1: Average Body Weight of Normal Rats (n=5) Orally Administered Different Doses of Methanolic Extract of *H. thebaica* Fruit Extract for 28 Days

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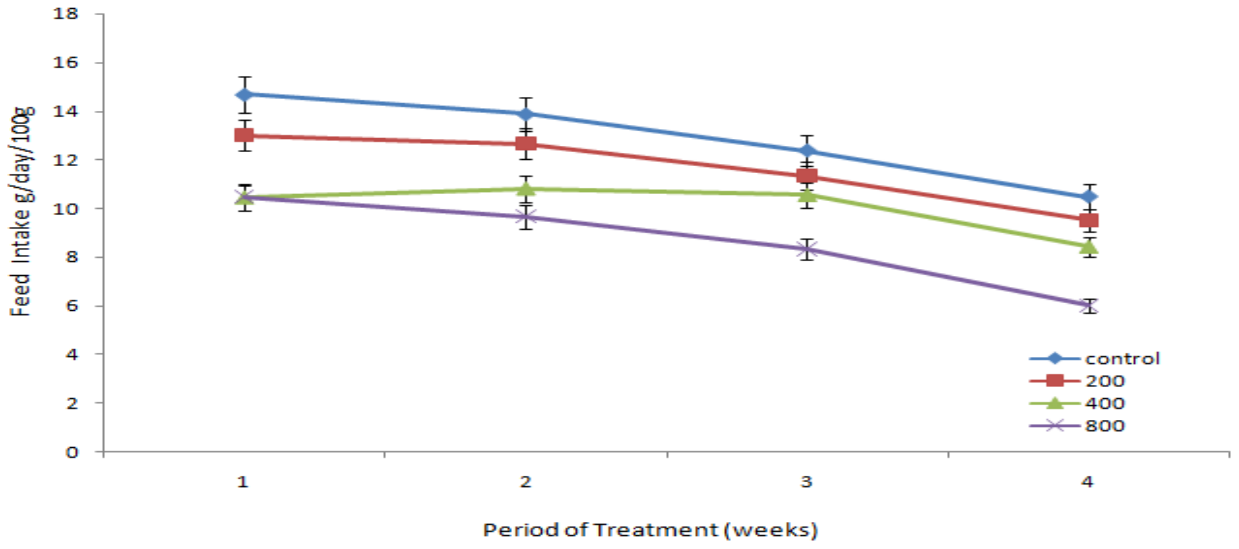

Figure 2: Feed Intake (g/day/100g) of Normal Rats (n=5) Orally Administered Different Doses of Methanolic Extract of *H. thebaica* Fruit Pulp for 28 Days

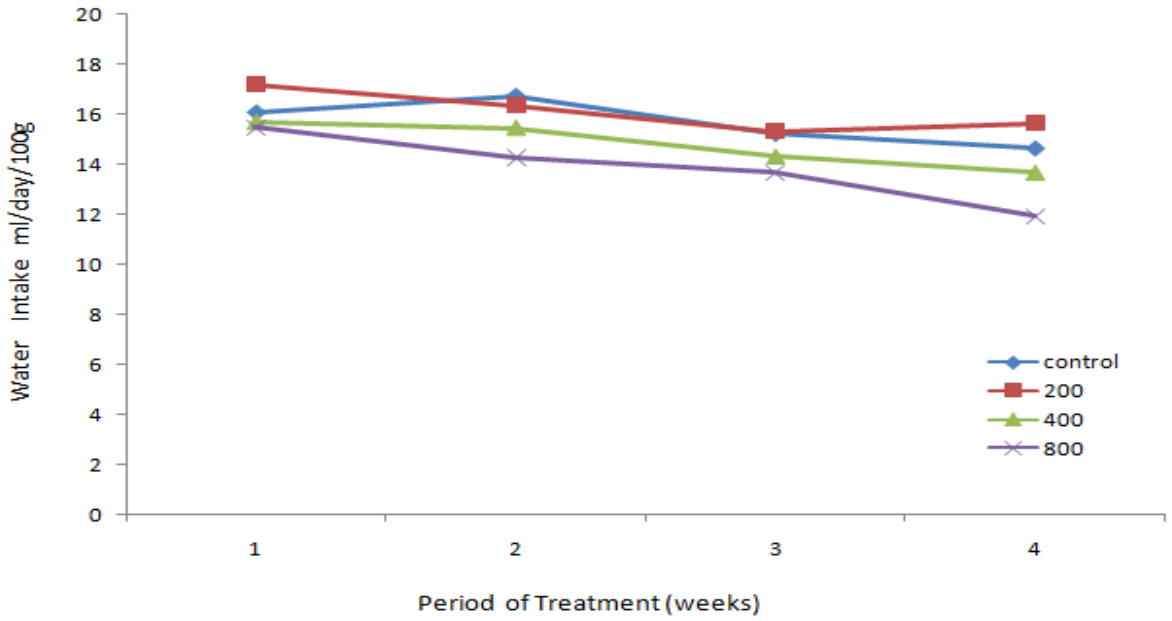

Figure 3: Water Intake (ml/day/100g) of Normal Rats Orally Administered Different Doses of *H. thebaica* Fruit Pulp Methanolic Extract for 28 Days

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Table 1. Effect of oral administration of different doses of *H. thebaica* fruit pulp methanolic extract on some indices (mean ± SEM) of liver function in normal rats (n=5) for 28 days

Doses (mg/kg)	AST (iu/L)	ALT (iu/L)	ALP (iu/L)	T. Protein (g/L)	Albumin (g/L)	T.Bil. (µmol/L)	Conj. Bil (µmol/L)
Control	161.62±3.38	37.23±2.21	332.37±15.4	69.40±1.69	31.80±0.97	17.00±0.05	8.60±1.20
200	172.80±8.66	36.80±4.47	380.32±14.59	68.40±1.57	31.20±0.73	16.00±1.80	9.60±1.81
400	188.34±15.45	50.28±4.87	461.00±20.44*	66.00±1.34	30.80±0.49	20.25±1.31	8.56±0.08
800	195.89±16.81	60.61±8.23*	475.51±32.48*	63.40±2.11	28.80±0.97	18.03±2.08	9.00±0.00

* $p<0.05$ significantly different from control

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Table 2. Effect of oral administration of different doses of *H. thebaica* fruit pulp methanolic extract on some kidney function indices (mean ± SEM) in normal rats for 28 days

Doses (mg/kg)	Urea mmol/L)	Creatinine (µm/L)	Na^+ (mmol/L)	K^+ (mmol/L)	HCO_3 (mmol/L)
Control	5.88±0.34	80.60±5.11	136.80±0.49	6.52±0.07	22.40±0.24
200	6.00±0.37	80.60±2.84	136.40±0.68	5.86±0.06*	22.00±0.04
400	6.42±0.15	90.80±7.17	137.00±0.80	5.92±0.12*	22.20±0.49
800	6.54±0.28	93.80±8.54	138.40±0.75	5.72±0.10*	22.60±0.51

* $p<0.05$ significantly different from control