Toxicity study of *hibiscus cannabinus*

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ABSTRACT

Hibiscus cannabinus is used as a remedy for anaemia and some liver diseases in African folk medicine. The toxicity of dried aqueous extract of *Hibiscus cannabinus* leaves was investigated in mice and Wistar albino rats so as to ascertain its safe use. Mice were used for acute toxicity test while rats were used for both sub-chronic and sub-acute toxicity test. Acute toxicity was determined by LD_{50} while serum biochemical parameters were used as makers of sub-acute and sub-chronic toxicity. The extract given orally was better tolerated than when given intraperitoneal (LD_{50} of 4.47g/kg). In the sub-acute and sub-chronic toxicity investigations, there was no significant ($P>0.05$) difference in some of the serum biochemical parameters between the control and test animals. However, a significant ($P<0.05$) cholesterol, triglyceride and glucose lowering activity were observed in test animals when compared with the control animals. The results suggested that *Hibiscus cannabinus* extract was well tolerated by experimental animals and may also possess hypolipidaemic and hypoglycaemic activities.

Key words: *Hibiscus cannabinus*, toxicity, biochemical parameters.

RESUME

Hibiscus cannabinus est utilisé comme médicament pour traiter les anémies et quelques maladies hépatiques dans le cadre de la médicine traditionnelle africaine. La toxicité des extraits aqueux des feuilles déshydratés de *Hibiscus cannabinus* a été étudiée sur les souris et les rats albinos afin de s'assurer de son innocuité. Les souris ont été utilisées pour l' étude de la toxicité aiguë et les rat pour la toxicité sub-aiguë et sub-chronique. Le paramètre utilisé pour mesurer la toxicité aiguë a été la DL_{50} alors que les paramètres biochimiques du sérum ont été utilisés comme marqueurs de la toxicité sub-aiguë et sub-chronique. L'extrait a été mieux toléré par voie orale que par voie intrapéritonéale pour la quelle une DL_{50} de 4.47g/kg a été obtenue. Pour les études de la toxicité sub-aiguë et sub-chronique aucune différence significative ($P>0.05$) n'a été trouvée dans les paramètres biochimiques du sérum entre les témoins et les essais. Toute fois des baisses significatives ($P<0.05$) en concentrations du cholestérol, des triglycérides et du glucose ont été observées chez les animaux du groupe essai comparées à celles du groupe témoin. Les résultats obtenus ont montré que l'extrait aqueux de *Hibiscus cannabinus* est très bien toléré par les animaux expérimentaux et possède des effets hypolipemiants et hypoglycémiants.

Mots clés: *Hibiscus cannabinus*, toxicité, paramètres biochimiques.

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INTRODUCTION

Hibiscus cannabinus (malvaceae) is a plant common in grassland, secondary regrowth after cultivation and along streams. It occurs naturally in lowland and medium altitude areas up to 2.100m; rainfall 1.000-1.800mm (Katende *et al.*, 1999). *H. cannabinus* tolerates a variety of soils. It is widespread in the tropics. In Africa, it is found in Ethiopia, Zimbabwe, Mozambique, (found in West Nile and Karamoja) and in the northern and eastern Uganda (Katende *et al.*, 1999). *Hibiscus cannabinus* is also widely spread in Cameroon.

The O-hibiscanone and hydroquinone of *H. cannabinus* is produced by the plant in response to infection by the pathogen *Verticillium dahlaie* (Puckhaber *et al.*, 1998). *Hibiscus cannabinus* is highly rich in fibre content such as cellulosic fibres (Eromosele *et al.*, 1999). Its bark fibre serves as a good material in the paper industry rather than the expensive softwood kraft pulps (Mohta *et al.*, 2000). *H. cannabinus* is also rich in nitrogen and phosphorus, thus used in the treatment of polluted water with low nitrogen and phosphorus concentrations (Abe *et al.*, 1999).

H. cannabinus also known as kenaf in Uganda is eaten as vegetable. The seeds are also crushed to a powder to prepare a beverage (Katende *et al.*, 1999). In Northern Cameroon, *H. cannabinus* is also eaten as a vegetable food, and the leaves dried to prepare a beverage, while in the traditional folk medicine of southern Cameroon, the plant is used in the herbal remedy of anaemia and liver disease.

Due to the wide consumption of *H. cannabinus*, in Africa there is the need for a toxicological study so as to guard against food poisoning or drug overdose. The present study report on the acute, sub-acute, and sub-chronic toxicity effects of *H. cannabinus* on experimental animals.

EXPERIMENTAL

Plant material: The whole plant of *H. cannabinus* (malvaceae) was collected in Obili-Yaounde in August 2001. The plant was authenticated at the National Herbarium Yaounde (voucher specimen No.42841/HNC).

Extraction: Shade-dried powdered plant (360g) was soaked in 3 litres of boiling water for 15 minutes. The mixture was allowed to cool and filtered using a glass funnel plugged with cheese cloth. The residue obtained was further extracted twice as before. The resulting filtrate was then concentrated using a rotary evaporator. The concentrated filtrate was then dried in an oven at 40°C to obtain a dry material (96g) giving a percentage yield of 27%

Phytochemical Screening: The extract was then tested for the presence of pharmacologically active compounds applying the method described by Trease and Evans (1978). This methods are based on colour changes which are indications of positive test, thus a qualitative test.

Animals: Male Wistar albino rats (130-150g) and mice raised in the animal house of the Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Cameroon were used. They were kept in wire-meshed cages in a room with a 12 hour light / dark cycle throughout the maintenance and experimental period and given food and water *ad libitum*.

The mice were used for acute (intraperitoneal (i.p) and oral (o.p)) toxicity testing, while the albino rats were used for sub-acute and sub-chronic toxicity studies

Reagents: All reagents were of analytic grade. Kits for urea nitrogen, total cholesterol and triglycerides were purchased from Sigma chemical Co. (St Louis, MO. USA).

Acute toxicity Studies: Mice were divided into 6 groups of 6 mice each. Group one was given 'vehicle' (distilled water) alone and served as the control group. The other groups were given single oral doses of *H. cannabinus* (0.5, 1.0, 2.0, 4.0, and 8.0g/kg) dissolved in 0.5ml of distilled water.

For the intraperitoneal acute toxicity study, mice in the different experimental groups were injected a single intraperitoneal dose of *H. cannabinus* (0.4, 0.8, 1.6, 3.2, and 6.4g/kg) dissolved in 0.5ml distilled water. The animals were observed on a continuous basis for 1 hour and at half hourly intervals thereafter for the next 7 hours, for any change in behavioural activity and then 12 hourly for the next 72 hours for any mortality. The method described by Miller and Tainter (1944) was used to determine the LD_{50} .

Sub-acute and sub-chronic toxicity studies: Experimental rats were divided into 5 groups of 12 rats each and labelled A, B, C, D, E. Group A served as the normal control and was given distilled water throughout the experimental period. Group B, C, D,

E served as the test groups and were given daily oral doses of *H. cannabinus* extract (0.4g/kg, 0.8g/kg, 1.6g/kg and 3.2g/kg respectively) throughout the experimental period.

Six rats per group were sacrificed by cervical dislocation on day 14 (sub-acute toxicity) and day 90 (sub-chronic toxicity) and blood collected by decapitation for biochemical assays. Aspartate aminotransferase (ASAT) and alanine amino transferase (ALAT) activities were measured using the methods of Reitman and Frankel (1957). Creatinine levels were measured using the method of Hare, (1950), total proteins by the method of Gornall *et al* (1949), total bilirubin by the method of Nosslin, (1960) and glucose by the method of Michod and Frei, (1963). Different analytical kits obtained from Sigma Chemical Company were used for the determination of the concentrations of urea (Sigma N0 535-150), cholesterol (Sigma, N0 352) and triglycerides (Sigma, N0 343).

Liver samples were collected in 10% formalin for histopathology studies (Gabe, 1968). Paraffin sections were stained with haematoxylin and eosin (H&E) and microscopically (Leitz Wetzlar Germany) examined at a magnification of x 25 microscope.

Statistical analysis of experimental data were analysed using employing analysis of variance (ANOVA). Duncan's multiple test was used to determine significant differences between means. The statistical analysis system package was used for this purpose.

RESULTS

Table 1: The effect of sub-acute (2 weeks) administration of *Hibiscus cannabinus* extract on serum biochemical parameters of albino rats.

Parameters	Group 1 (control, H2O)	Group 2 (0.4g/kg)	Group 3 (0.8g/kg)	Group 4 (1.6g/kg)	Group 5 (3.2g/kg)
ALAT (units/ml)	54.00 ± 3.6	54.25 ± 3.73	52.00 ± 3.48	54.20 ± 2.46	53.70 ± 2.85
ASAT (units/ml)	81.80 ± 3.42	80.50 ± 3.32	82.50 ± 2.07	80.80 ± 3.39	81.00 ± 2.69
Creatinine (mg/l)	13.42 ± 0.79	13.26 ± 0.66	14.03 ± 0.34	13.58 ± 0.66	13.57 ± 0.66
Urea (mg/dl)	27.10 ± 1.63	26.71 ± 1.51	28.10 ± 2.28	26.60 ± [illegible]	26.83 ± 1.8
Total protein (mg/ml)	6.19 ± 0.75	5.56 ± 0.40	6.30 ± 0.49	5.89 ± [illegible]	6.39 ± 0.6
Total Bilirubin (mg/l)	8.82 ± 0.93	9.50 ± 0.823	8.33 ± 2.11	11.76 ± [illegible]	8.68 ± 1.47
Glucose (g/l)	1.08 ± 0.06	1.06 ± 0.07	0.92 ± 0.05	0.97 ± [illegible]	1.02 ± 0.07
Cholesterol (mg/dl)	76.34 ± 2.10	75.58 ± 3.15	69.85 ± 2.58 [illegible]	6.36 ± 2.08	2.34 ± 2.4
Triglyceride (mg/dl)	69.49 ± 1.33	66.97 ± 3.91	66.06 ± 2.91	64.13 ± 1.24	61.16 [illegible]

Values are mean ± Standard Deviation for six rats per group., [s] Significantly lower than control (group 1). $P < 0.0$[illegible]

Table 2: The effect of sub-chronic administration (12 weeks) of *Hibiscus cannabinus* extract on serum biochemical parameters of albino rats.

Parameters	Group 1 (control,H2O)	Group 2 (0.4g/kg)	Group 3 (0.8g/kg)	Group 4 (1.6g/kg)	Group 5 (3.2g/kg)
ALAT (units/ml)	24.30 ± 2.99	25.50 ±0.58	25.7 ± 2.27	24.70 ± 1.86	24.63 ± 2.28
ASAT (units/ml)	72.00 ± 2.97	73.00 ±1.24	72.80 ± 1.70	74.50 ± 3.23	73.00 ± 1.64
Creat (mg/l)	18.06 ± 1.19	18.79 ±1.03	18.63 ± 1.19	18.95 ± 1.48	16.89 ± 1.19
Urea (mg/dl)	19.94 ± 2.09	18.31 ±2.36	18.09 ± 2.15	20.11 ± 1.54	19.51 ± 1.46
Total protein (mg/ml)	6.52 ± 0.71	6.072 ±0.12	7.34 ± 0.23	6.82 ± 0.75	6.29 ± 0.35
Total Bilirubin (mg/l)	11.50 ± 1.33	12.47 ±0.90	12.67 ± 2.11	12.47 ± 1.82	14.03 ± 3.19
Glucose (g/l)	0.99 ± 0.08	0.64 ± 0.14[s]	0.69 ± 0.10 [s]	0.45 ± 0.03 [s]	0.67 ± 0.1 [s]
Cholesterol (mg/dl)	64.66 ± 2.93	56.69 ±0.79 [s]	61.98 ± 2.24	60.91 ± 1.73	58.62 ±1.47 [s]
Triglyceride (mg/dl)	63.84 ±7.86	46.61 ±2.38 [s]	36.12 ±1.41[s]	60.4 ± 1.55	51.36 ±2.14 [s]

Values are mean ± Standard Deviation for six rats per group., [s] Significantly lower than control (group 1). $P < 0.05$

The sub-acute (2weeks) administration of *H. cannabinus* extract brought about changes in serum biochemical parameters of albino rats (Table 1). A significant ($P<0.05$) decrease of serum total cholesterol was observed in rats that were administered 0.8g/kg, 1.6g/kg and 3.2g/kg of the extract. A similar decrease was observed in serum triglyceride levels . The other parameters were not altered significantly ($P>0.05$).

The effect of sub-chronic administration (12 weeks) of *H. cannabinus* extract on serum biochemical parameters of albino rats are presented in Table 2. Sub-chronic administration of *H. cannabinus* had no significant ($P>0.05$) effect on ALAT and ASAT. Similar results were obtained for creatinine, urea, total protein and bilirubin. Serum glucose, total cholesterol and triglyceride were however significantly ($P<0.05$) reduced by the extract (Table 2).

DISCUSSION

The results of the phytochemical screening of *Hibiscus cannabinus* aqueous extract indicated the presence of some pharmacologically active principles such as phenolic compounds, tannins, saponins, alkaloids and steroids which may confer medicinal properties on this plant.

Acute oral administration of aqueous extract of *Hibiscus cannabinus* produced no visible signs of toxicity in experimental mice except for an initial prostration and huddling observed at the higher doses of 4g/kg and 8g/kg body weight. No mortality was recorded even at a dose of 8g/kg body weight. Doses higher than 5g/kg body weight are generally not considered as dose related toxicity (Hayes, 1987), because it is difficult for an individual to take 5g/kg body weight dose of a plant extract. This allows us to infer that the *H. cannabinus* extract is not toxic via the oral route. However, when the *H. cannabinus* extract was administered intraperitoneally, writhing and abnormal gait were observed even at a low dose level of 1.6g/kg. Other toxic manifestations such as grooning and sniffing and an increased reactivity to the environment were observed at an administered dose of 3.2g/kg. This was followed by convulsion and death. The LD_{50} of *H. cannabinus* extract administered via the intraperitoneal route was established to be 4.47g/kg body weight. This shows that the *H. cannabinus* extract is better tolerated when administered via the oral route, this route being the normal route of administration in situations of daily use.

The liver is an organ with diverse functional activity. Its hepatic cells participate in a variety of metabolic activities, thus the presence of a host of enzymes. The activity of two of these enzymes ALAT and ASAT serve as the standard tests for hepatocellular damage with increases in the activity of these enzymes in the serum serving as an indication of toxicity. This is the case in the administration of toxins to experimental animals where there is a significant increase in the serum level of these enzymes (Nadeen et al., 1997, Singh et al., 1998). Hyperbilirubinaemia is also a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis (Zimmerman, 1973). Bilirubin also measures the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degradation rate (Edmondson and Peters, 1985). Sub-acute and sub-chronic administration of aqueous leaf extracts of *H. cannabinus* to experimental rats had no significant effect on the ALAT, ASAT activity as well as the bilirubin concentration. The histological examination of the liver tissue supported this finding, with no alteration observed when samples of the test groups were compared to those of the control group. Thus treatment of experimental animals with *H. cannabinus* did not have any hepatotoxic effect since the activity of serum aminotransferases, bilirubin concentration as well as the histology of the liver remained unchange throughout the study period.

Creatinine and urea are two serum metabolites that are indicative of renal function. Though these metabolites are end products of protein metabolism, their concentrations remain fairly constant under normal conditions unless renal function changes (Whitby *et al.*, 1988). Serum Urea concentration may also increase in congestive cardiac failure and gastrointestinal haemorrage (Whitby *et al.*, 1988). Treatment with *H. cannabinus* did not alter any of these parameters significantly. This indicates that the extract did not alter renal function which subsequently may not be apparent from histological sections.

A number of factors have been identified as contributing to the formation of artherosclerotic plaques and the resulting impairment of coronary arterial blood flow. Among these, blood levels of lipids and lipoproteins play an important role (Shaila *et al.*, 1997). Sub-acute administration of *H. cannabinus* significantly reduced the serum level of cholesterol and triglyceride in a dose dependent manner in experimental groups compared to the control group. Lowering the serum

cholesterol level is an approach to the prevention of artherosclerosis. The leaf extract of *H. cannabinus* may therefore have implications in the management of artherosclerosis.

Hyperglycaemia is indicated in impaired insulin action and/ or inadequate insulin secretion (Zimmerman *et al.*, 1996, Bailey, 2000). The management of hyperglycaemia as is observed in cases of diabetes is by the oral administration of hypoglycaemic agents. Many hypoglycaemic agents of plant origin are being used in traditional medicine and some have been investigated (Saravana *et al.*, 2002, Olajide *et al.*, 1999). *H. cannabinus* administration at sub-chronic levels significantly reduced the concentration of serum glucose and could thus find applications in the management of hyperglyceamia.

From the results obtained, it can be concluded that the oral administration of the leaf extract of *H. cannabinus* may not be toxic at acute, sub-acute and sub-chronic levels. However, further toxicity studies on other organs are needed so as to ensure the safe use of this plant. Further research is also needed to identify other potential uses of *H. cannabinus* like in the treatment of atherosclerosis and diabetes melitus.

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