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Full Length Research Paper

Safety evaluation of hydroalcoholic extract of *Cochlospermum planchonii* rhizome in rats

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Plant materials have long been known to contain biochemical substances that could have adverse effects on animal tissues. This study was initiated to investigate the effect of hydroalcoholic extract of *Cochlospermum planchonii* rhizome on biochemical and haematological indices of hepatotoxicity in adult albino Wistar rats. Four groups of five rats per group were used. Group A was given only distilled water and served as control, while groups B, C and D were given 125, 250 and 500 mg/kg body weight of the extract, respectively, by oral administration for four weeks. The results revealed that there was a dose-dependent significant increase ($p<0.05$) in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, triglycerides and total bilirubin of all the treatments groups when compared with the control group, while the high density lipoprotein cholesterol of all the treatment groups was significantly ($p<0.05$) reduced. The 500 mg/kg body weight of the extract produced a significant decrease ($p<0.05$) in PCV and haemoglobin concentration. The levels of low density lipoprotein cholesterol, total cholesterol, glucose, albumin, globulin and total protein of all the treatment groups were not significantly ($p>0.05$) altered. These results showed that hydroalcoholic extract of *C. planchonii* rhizome might be injurious to the liver, if administered at a high dose and over a prolonged period of treatment.

Key words: *Cochlospermum planchonii*, hydroalcoholic, haematology, biochemistry, hepatotoxicity.

INTRODUCTION

Throughout the ages and in many cultures, the use of medicinal plants for the treatment of different types of diseases is well known (Adebayo et al., 2006). In recent times, research findings indicate the positive role of traditional medicinal plants in the prevention, treatment and control of some metabolic disorders including diabetes mellitus, cardiovascular diseases and certain types of cancers (Zhang, 1996).

However, care must be taken not to consume plants or plant products that could have harmful effects on the body. Poisonous plants are among the causes of economic loss to livestock farmers, so they are considered when investigating animal diseases and report showed that the use of gum acacia was successful in the treatment of haemorrhage, but the treatment was found to be injurious to the liver (Taylor, 1969). All plant species contain poisonous, medicinal and nutritional compounds (Bakare et al., 2010).

Cochlospermum planchonii (Cochlospermaceae) is a bushy-plant with bright yellow flowers of about 50 cm in height and is widespread in the Savannah and shrubland of West Africa. In Nigeria, it is called *N'Dribala* (Fulani), *Rawaya* or *Kyamba* (Hausa), *Abanzi* (Igbo) and *Sewutu* (Yoruba). The traditional use of this plant as an alternative therapy for the treatment of non-severe malaria (Benoit-Vical et al., 2003; Traore et al., 2008) and diarrhoea (Magaji et al., 2010) have been reported. The decoctions of the whole roots are used as remedy for gonorrhoea, jaundice and gastro-intestinal disease (Mann

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et al., 2003). Experimental studies have confirmed hepatoprotective property of aqueous extract of *C. planchonii* rhizome used in traditional treatment of jaundice (Aliyu et al., 1995) and bactericidal activity of its essential oil (Ouattara et al., 2007). Anaga and Opara (2009) showed that bioactive compounds of *C. planchonii* root extract are potential for the control of pain, inflammation and diabetes mellitus.

Although, numerous therapeutic benefits are ascribed to *C. planchonii* extracts, there seems to be little attention on the effects of the plant extract on vital organs at high dosage and following long treatment regime. Thus, this study was designed to evaluate the effect of hydroethanolic extract of *C. planchonii* rhizome on vital organs using biochemical and haematological indices of toxicity.

MATERIALS AND METHODS

C. planchonii rhizomes were harvested between May and June, 2009 from a farm in the University of Agriculture, Makurdi, Benue state, Nigeria. The area is located on latitude 7° 44' N and longitude 8° 35' E. It was identified and authenticated by Mr. Patrick Ekwuno of College of Forestry, of the same University. Voucher specimen was deposited at the college herbarium.

Preparation of plant extract

The roots of *C. planchonii* were collected and thoroughly washed with clean water and extracted as previously described (Abu and Uchendu, 2010). Briefly, the roots were cut into smaller pieces and allowed to dry at room temperature for one week. The dried roots were then pulverized into coarse powder and stored in a dry container until when required for use. The hydroethanolic solvent was prepared by mixing 800 cm^3 of ethanol with 200 cm^3 of distilled water, to obtain 20% aqueous ethanol.

100 g of the coarse powdered roots was wrapped with a white piece of cloth, tied and soaked in 500 cm^3 of the 20% aqueous ethanol solvent (ratio 1:5) for 48 h. Then, the extract/solvent mixture was filtered with Whatman No. 1 filter paper. The filtrate was left to dry at room temperature on laboratory bench, to obtain a crude extract, with percentage yield of 17.43% (w/w).

Experimental animals

20 male Wistar strain of albino rats, *Rattus norvegicus albinus,* weighing 153 to 238 g, were purchased from the Animal Breeding Centre, Benue State University, Makurdi, Nigeria. They were kept in healthy environmental conditions; fed with standard feeds purchased from Vital feeds, Jos, Plateau state, Nigeria and clean drinking water *ad libitum*. Five animals were housed in a clean and large cage, making a total of four cages. All of them were handled with care to avoid stress. They were also allowed to acclimatize for two weeks before commencement of the experiment. The body weight of rats was determined with a top-loading weighing balance.

Experimental design and administration of extract

The rats were randomly allocated into four groups of five animals each. Animals in group A (control group) were given equivalent volume of distilled water (per kg body weight), while those in groups B, C and D were respectively administered 125, 250 and 500 mg/kg body weight of the extract re-suspended in distilled water. The oral administration of extract is by daily orogastric intubation for a period of four weeks (Abu and Uchendu, 2010).

Blood collection and analysis

Blood was collected from the rats by cardiac puncture under chloroform anesthesia, on the next day after the last day of administration of the extract. Whole blood was collected with new sterile syringe/needle. 1 cm^3 blood was dispensed into specimen bottles containing the anticoagulant, ethylene diamine tetra-acetic acid (EDTA), while the remaining blood was dispensed into clean Bijou bottles without anticoagulant and left to clot.

Biochemical assay

For biochemical analysis, blood samples in tubes were allowed to stand for 2 h at room temperature and then centrifuged at 3000 rpm for 10 min. The sera was aspirated with clean sterile pipettes and evaluated for biochemical parameters using an automated analyzer (Hitachi 902, Germany). Clinical biochemical parameters determined were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol (TC), HDL-cholesterol, LDL- cholesterol, triglycerides, albumin, total protein, glucose, globulin and total bilirubin using standard assay kits (Roche diagnostics Ltd, United Kingdom).

Haematological assays

The whole blood with EDTA was used to assay for all haematological parameters as described by Schalm et al. (1975). Red blood cells (RBC) and white blood cells (WBC) were counted with improved Neubauer counting chamber (Haemocytometer) using appropriate reagents. Packed cell volume (PCV) was estimated by micro-haematocrit method, then the haemoglobin (Hb) concentration was also determined. The leucocytes differential counts was done with a prepared thin blood film, stained with Giemsa stain, using a light microscope at 100x magnification. The red cell indices such as mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were calculated from the primary values of RBC, PCV and Hb.

Statistical analysis

The results were expressed as mean ±S.E.M (standard error of mean); analyzed by one-way analysis of variance (ANOVA). Means found to be significantly different at $p<0.05$ were separated by Duncan multiple range test. The statistical evaluation was carried out using Graph Pad Prism version 3.0 for Windows (Graph Pad software, San Diego, California, U.S.A).

RESULTS

Biochemical assay

The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglycerides (TG) and total bilirubin of all the

Table 1. Effect of *C. planchonii* extract on some biochemical parameters of albino rats.

Parameter	Group A Control (D/ water)	Group B (125 mg/kg)	Group C (250 mg/kg)	Group D (500 mg/kg)
AST (U/L)	150.70±11.90	311.96±31.07*	296.64±37.3*	289.04±40.1*
ALT (U/L)	67.46±4.20	136.30±14.72*	134.84±16.8*	139.36±11.5*
ALP (U/L)	152.20±8.75	270.60±9.62*	277.80± 6.64*	277.00±8.96*
HDL-C (mg/dl)	62.36±1.50	40.78±3.18*	40.03±2.84*	30.28±4.15*
LDL-C (mg/dl)	10.20±0.56	9.50± 0.74	12.20±1.36	11.60±1.36
T-CHOL (mg/dl)	56.00±3.78	59.80±4.22	55.20± 3.60	56.20± 5.48
TG (mg/dl)	54.00±3.99	164.80±5.94*	167.20±2.30*	166.60±6.65*
Albumin (g/L)	46.32±3.48	43.54±2.64	41.84±2.30	40.74±2.34
Globulin (g/L)	30.67±1.46	43.72±3.19	40.36±3.88	40.63±3.36
T- PROT. (g/L)	76.99±3.33	87.26±4.51	80.55±2.47	79.20±3.39
Glucose (mmol/L)	7.92±0.84	8.24±1.43	7.02±0.92	7.78±0.83
T - BiL (µmol/L)	2.10±0.18	4.11±0.43*	3.90±0.42*	3.92±0.47*

Values were presented as mean ±S.E.M of five replicates. The values with asterisk (*) are significantly different at $p<0.05$, when compared with the control.
TG, Triglycerides; T-PROT, total protein; T-Bil, total bilirubin; T-CHOL, total cholesterol; HDL-C, high density lipoprotein cholesterol; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 2. Effect of *C. planchonii* extract on some haematological parameters of albino rats.

Parameter	Group A Control (D/water)	Group B (125mg/kg)	Group C (250mg/kg)	Group D (500mg/kg)
PCV (%)	43.80±0.58	46.20±0.58	43.80±0.58	36.60±0.93*
Hb concentration (g/dl)	14.14±0.39	15.40±0.19	14.26±0.34	11.76±0.38*
WBC counts ($x10^9$/L)	4.10±0.13	4.23±0.28	4.50±0.14	4.62±0.16
RBC counts ($x10^{12}$/L)	5.43±0.14	4.45±0.48	4.88±0.19	4.86±0.19
Monocytes	3.60±0.40	3.40±0.40	3.60±0.24	3.00±0.44
Lymphocytes	70.40±2.19	70.0±2.45	73.40±3.69	68.60±2.11
Neutrophils	28.60±1.91	28.40±2.32	28.40±1.33	28.80±2.65
Eosinophils	0.60±0.40	0.60±0.40	0.40±0.24	0.60±0.24
Basophils	0.80±0.37	0.40±0.24	0.60±0.24	0.40±0.24
MCV (fL)	79.16±2.71	76.16±1.65	86.40±2.68	74.30±1.87
MCH (pg)	26.12±0.94	25.98±0.70	28.48±0.43	25.96±0.79
MCHC (g/dl)	33.34±0.02	33.32±0.02	33.32±0.04	33.28±0.03

Values were presented as mean ±SEM of five replicates. The values with asterisk (*) are significantly different from the control at $p<0.05$.
MCHC, Mean corpuscular haemoglobin concentration; PCV, packed cell volume; Hb, haemoglobin; RBC, red blood cells; WBC, white blood cells; MCV, mean cell volume; MCH, mean corpuscular haemoglobin; b.wt, body weight ().

treatment groups were significantly higher ($p<0.05$) than the control group. However, the levels of high density lipoprotein cholesterol (HDL-C) of all the treatment groups were significantly lower ($p<0.05$) than the control group. On the other hand, the levels of low density lipoprotein cholesterol, total cholesterol, glucose, albumin, globulin and total protein of all the treated groups were not significantly different ($p>0.05$) from the control group (Table 1).

Haematological assays

The oral dose of 500 mg/kg body weight of *C. planchonii* root extract produced a significant decrease ($p<0.05$) in PCV and haemoglobin concentration. However, there was no significant difference ($p>0.05$) between the treatment and control groups when 125 and 250 mg/kg body weight of the extract were used. All the other haematological parameters were not significantly different ($p>0.05$) from the control group at the various doses used (Table 2).

DISCUSSION

Most reports on toxic effects as a result of the use of herbal medicines and dietary supplements are associated

with hepatotoxicity, although untoward effects on other organs such as kidney, skin, brain and the heart have been published (El Nahhal, 2004; Adebayo et al., 2010). Analysis of blood parameters is relevant in scientific studies for risk evaluation, as changes in haematological and biochemical indices (biomarkers) have high predictive value, because they detect early signs of diseases (Olson et al., 2000).

The higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin and triglycerides as observed in this study might be a sign of hepatocellular damage (Wannang et al., 2007; Hayes et al., 2002; Benjamin, 1978). ALT and AST are located in the cytoplasm and mitochondria of liver cells in high concentrations but low in blood. However, ALT is more liver-specific (Ellis et al., 1978). It is known that increased activities of these enzymes in serum are due to increased membrane permeability and leakage into the blood circulation when there is damage to liver cells (Benjamin, 1978). Thus, rise in ALT and AST levels due to hepatic necrosis may be noticed several days before clinical signs are manifested (Burtis and Ashwood, 2001).

The elevated level of alkaline phosphatase observed may be indicative of intra-hepatic cholestasis and pathological condition. Alkaline phosphatase is a marker of obstructive jaundice or intra-hepatic cholestasis (Davern and Scharschmidt, 2002). The bile duct obstruction induces synthesis of this enzyme by biliary tract epithelial cells, leading to very high level of the enzyme in blood circulation. The response of the liver to any form of biliary tract obstruction is to induce synthesis of ALP, and drugs have been known to cause intra-hepatic obstruction of bile flow (Burtis and Ashwood, 2001; Wannang et al., 2007). Also, mild elevation of ALP is seen in parenchymal diseases of the liver caused by infectious or toxic hepatitis, due to the effect of drugs or xenobiotics (Vasudevan and Sreekumari, 2007).

The decreased level of HDL-cholesterol and elevation of triglycerides are predictive of the risk of developing impaired cardiovascular system in the animals treated with plant extract. It has been reported that low HDL-cholesterol and high triglycerides levels could increase the risk of atherosclerosis, a major cause of cardiovascular diseases (Cecil et al., 1995). Since AST is one of the markers of cardiac diseases, the elevated level observed is also a sign of risk, as elevated level of the enzyme is seen in myocardiac infarction (Vasudevan and Sreekumari, 2007). Thus, it would appear that the harmful effect of the plant extract is not only on hepatocytes, but on the heart and cardiovascular system; since AST and triglycerides were elevated, while HDL which is synthesized in the liver was reduced. HDL normally removes cholesterol from the walls of the arteries and transports it to the liver, thus reducing the risk of atherosclerosis (Haskell et al., 1984). However, deceased level may indicate risk. The insignificant effect on the levels of LDL-cholesterol, total cholesterol, albumin, globulin, total protein and glucose suggests that the deleterious effect on the animals' livers has not yet affected the metabolism of these biomolecules, within the period of our investigation.

The deceased level of haemoglobin (Hb) concentration and packed cell volume (PCV) after treatment with 500 mg/kg body weight of *C. planchonii* root extract may be an indication of rapid haemolysis leading to haemolytic anaemia. Haemoglobin estimation measures the amount of Hb in grams per 1 dl of whole blood and provides an estimate of oxygen-carrying capacity of the RBCs, while the PCV, also known as haematocrit, measures the total number of erythrocytes in 100 ml of whole blood. It is well known that certain chemical substances could cause increased haemolysis of red cells, releasing haemoglobin into the plasma, which is quickly degraded to form bilirubin by the reticulo-endothelial system (Murray et al., 2006; Vasudevan and Sreekumari, 2007).Therefore, it appears that the plant extract has harmful effect on red blood cells and Hb metabolism and might reduce the oxygen-carrying capacity of the rats' erythrocytes, which is an indication of anaemia (Breazile, 1971).

The elevation of serum bilirubin in the treated animals suggests that the plant extract could increase haemolytic breakdown of erythrocytes. The haemoglobin released from RBC is degraded to form bilirubin. It was reported that approximately 85% of the total bilirubin produced was derived from the heme moiety of haemoglobin, released from aging erythrocytes which are destroyed by the reticulo-endothelial system, while the remaining 15% was derived from break down of red cells precursors and heme-containing proteins (Murray et al., 2006; Burtis and Ashwood, 2001). These metabolic processes occur normally, but elevated level of serum bilirubin may occur when there is rapid haemolysis, such that the liver which is involved in bilirubin metabolism cannot adequately control it (Murray et al., 2006; Ogbe et al., 2010).

Experimental studies have confirmed hepatoprotective property of aqueous extract of *C. planchonii* rhizome, which is used in traditional treatment of jaundice (Aliyu et al., 1995), contrary to this investigation, suggesting that high dose of hydro-ethanolic extract of *C. planchonii* rhizome over a prolonged period of treatment could be injurious to the liver. Although, method of extraction could elicit varied physiological activities, histopathological studies could further confirm the hepatotoxicity of hydro-ethanolic extract of *C. planchonii* rhizome in rats.

Therefore, this study revealed that the hydro-alcoholic extract of *C. planchonii* rhizome may be injurious to the liver. So, those who use the crude plant extract for treatment of diseases, should exercise caution to avoid overdose.

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