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Hepatoprotective Activity of *Hibiscus cannabinus* (Linn.) Against Carbon Tetrachloride and Paracetamol Induced Liver Damage in Rats

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Abstract: In the present study the hepatoprotective activity of a daily oral dose (1.6 g kg^{-1}) of aqueous leaf extract of *H. cannabinus* was investigated over a two week period in albino rats. Liver damage in rats was induced using Carbon tetrachloride (CCl_4) and Paracetamol (PCM). This was confirmed by increased plasma transaminases activities, total bilirubin concentration and Thiobarbituric Acid Reactive substance (TBRs, a measure of lipid peroxidation). Histopathological examinations substantiated this liver damage with fatty deposits, severe inflammation and severe necrosis. The aqueous leaf extract of *H. cannabinus* showed a significant ($p<0.05$) hepatoprotective activity against this damage in lowering the plasma transaminases and bilirubin concentration significantly ($p<0.05$). Absents of necrosis in liver cells of rats pretreated with extract indicated a protective effect. The extract also inhibited lipid peroxidation, suggesting a possible mechanism of action. The results obtained confirm the hepatoprotective activity of *H. cannabinus*.

Key words: *Hibiscus cannabinus*, hepatoprotective, transaminases, lipid peroxidation

INTRODUCTION

Liver diseases resulting from liver damage is a global problem. A major causative factor is the increasing alcohol consumption in developed countries[1]. Malnutrition, anaemia, infection and availability of over the counter hepatotoxic drugs are the most frequent causes of liver damage in developing countries[2]. It is well recognised that free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorder, arthritis, inflammation and liver diseases[3]. Chemicals and drugs such as CCl_4 and PCM catabolised radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, cause the swelling and necrosis of hepatocytes and result to the release of cytosolic enzymes such as AST, ALT and ALP into the circulating blood[1,4]. Therefore, CCl_4 and PCM induced liver injury has been employed as a convenient model for investigating radical-induced damage and its prevention in animals.

Many plant species are known in folk medicine to be used for the treatment of liver disease because of their hepatoprotective activities. The fruits of *Solanum nigrum* have been shown to possess a hepatoprotective activity on experimental animals[1]. Verbenalin, an extract of *Verba officinalis* has also been reported to exhibit a hepatoptrotective activity on experimental liver damage in rodents[4].

Hibiscus cannabinus is a plant that is widely distributed and commonly known as kenaf. It is used as vegetable, blood tonic and a remedy for liver diseases. An earlier study revealed its antioxidant activity in the protection of the cell membrane integrity from the effect of oxidants[5]. The present study evaluates its hepatoprotective activity using a rat model of CCl_4 and PCM induced hepatotoxicity.

MATERIALS AND METHODS

Animals: Male albino wistar rats (120-150 g) were fed and had access to water *ad libitum*. They were housed in wire-meshed cages on a 12 h light/dark cycle during the entire experimental period.

Plant material: Fresh leaves of *Hibiscus cannabinus* were collected in Obili-Yaounde. Identification of the plant was confirmed in the National Herbarium Yaounde, Cameroon, where voucher specimen (No. 42841/HNC) has been kept.

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Table 1: Effect of *H. cannabinus* extract on plasma transaminases, bilirubin of CCl_4 and PCM intoxicated rats

Treatments	ALT (units mL^{-1})	AST (units mL^{-1})	Bilirubin (mg mL^{-1})
Control	51.30±5.05	56.00±03.94	9.09±1.47
H. cannabinus	53.40±7.70	56.80±10.38	10.70±2.46
H. cannabinus+CCl_4	111.50±1.47^{ce}	98.20±12.39^{ce}	22.71±2.72^{ce}
CCl_4	237.75±10.82^{c}	167.40±08.36^{c}	42.68±7.78^{c}
H. cannabinus+PCM	102.20±10.49^{cb}	93.00±08.24^{cb}	27.58±5.82^{cb}
PCM	178.60±10.64^{c}	168.40±07.13^{c}	38.27±1.29^{c}

Table 2: Effect of *H. cannabinus* extract on plasma lipids and lipid peroxidation of CCl_4 and PCM intoxicated rats

Treatments	Cholesterol (mg dL^{-1})	Triglyceride (mg dL^{-1})	Lipid peroxidation x10^{-6} (units)
Control	67.03±10.36	27.72±3.86	0.26±0.02
H. cannabinus	68.19±04.79	24.17±3.11	0.26±0.03
H. cannabinus+ CCl_4	59.00±03.71^{ce}	27.69±4.95	0.35±0.02^{ce}
CCl_4	64.79±06.73	26.98±5.89	0.44±0.04^{c}
H. cannabinus+PCM	56.25±03.59^{c}	24.65±3.63	0.36±0.02^{cb}
PCM	59.16±05.49	25.07±4.23	0.46±0.02^{c}

Values are mean±SD for 5 rats per group; ^{c} significant VS control; ^{e}significant VS CCl_4, ^{b}significant vs PCM, $p< 0.05$

Preparation of plant material: The plant material was prepared as earlier described[6]. In brief, dried *Hibiscus cannabinus* leaves were ground. The ground material was then extracted in boiling water. This was filtered and the filtrate concentrated using a rotary evaporator with the aid of a vacuum pump. The concentrate was further evaporated to dryness in an oven at 40°C and then ground. The extract was stored in the refrigerator until required.

Experimental design: Olive oil was used as a carrier and *H. cannabinus* extract (1.6 g kg^{-1} body weight/day), CCl_4 (1 mL kg^{-1} body weight/3 times a week o.p) and PCM (2.5 g kg^{-1} body weight/3 times a week o.p) were given for 2 weeks (14 days). The dose of 1.6 g kg^{-1} was chosen after a preliminary toxicity study[6].

Group I: Normal control, received olive oil.

Group II: Plant control, received *H. cannabinus* extract (1.6 g kg^{-1}) + olive oil.

Group III: Received plant extract (1.6 g kg^{-1}) + CCl_4 in olive oil

Group IV: Received CCl_4 in olive oil.

Group V: Received plant extract (1.6 g kg^{-1}) + PCM in olive oil.

Group VI: Received PCM in olive oil.

Animals were sacrificed by cervical dislocation on day 15 after an overnight fast. Blood was collected in heparinised tubes after cutting the jugular vein and centrifuged at 3000 rpm for 15 min to obtain plasma. The plasma was used for the determination of alanine Aminotransferase (ALT) and aspartate Aminotransferase (AST)[7], total bilirubin[8], Lipid peroxidation[9], cholesterol and triglycerides (Sigma kits). Liver samples collected in 10% formalin were used for histopathological examination[10]. Paraffin sections were stained with haemotoxylin and eosin (H and E) and examined microscopically (Leitz Wetzla, Germany) at a magnification of x25.

Statistical analysis: Experimental data was analysed using analysis of variance (ANOVA). Duncan's Multiple Range Test was used to determine significant differences between means. The Statistical Analysis Systems (SAS) package was used for this analysis.

RESULTS AND DISCUSSION

Plasma AST, ALT, Bilirubin level (Table 1) and lipid peroxidation (Table 2) increased significantly ($p<0.05$) in rats treated with CCl_4 and PCM when compared to control (vehicle). Pretreatment with *Hibiscus cannabinus* exhibited a significant ($p<0.05$) reduction of these elevated values (Table 1 and 2). Toxins did not have any significant ($p>0.05$) effect on plasma cholesterol and triglyceride concentration which. In the histopathological examination, CCl_4 induced fatty deposits and diffused inflammatory cells (Fig. 1b), PCM induced focal aggregate of severe inflammatory cells and severe necrosis (Fig. 1c) while less inflammation observed in rats pretreated with *H. cannabinus* extract (Fig. 1d).

The liver is an organ with diverse functional activity. The hepatoprotective activity of a drug should therefore be based on its ability to reduce the injurious effect

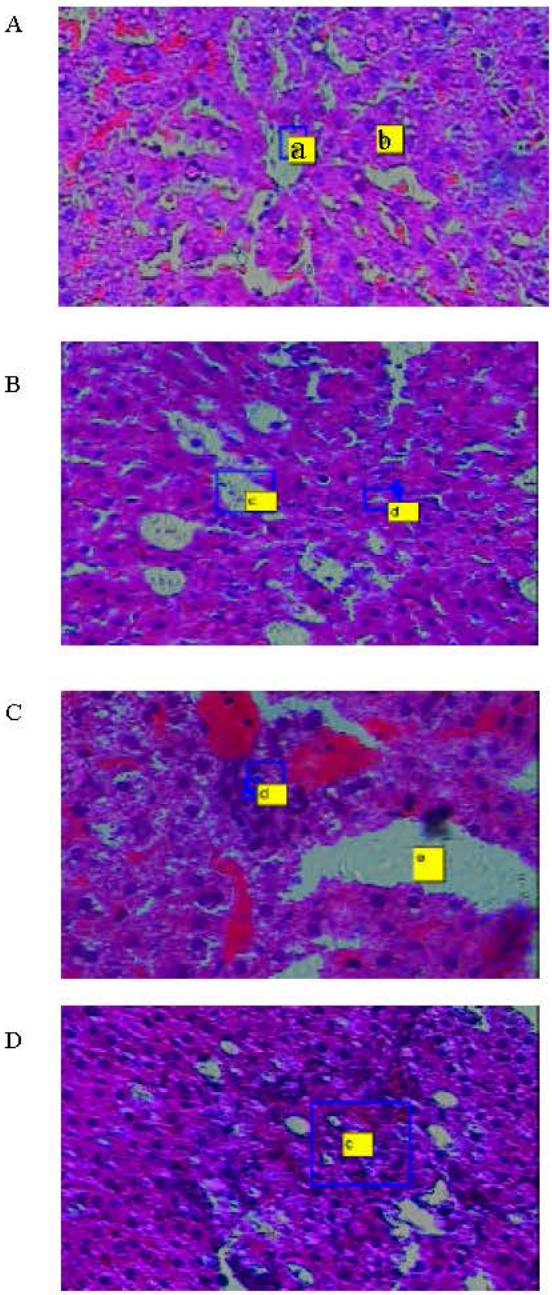

Fig. 1: Hepatotoxins-induced liver damage. A: normal rat liver, a: normal central lobular vein, b: hepatic cells with normal cytoplasm, B: CCl_4 induced c: fatty deposits, d: diffused inflammatory cells. C PCM induced, d: focal aggregate of severe inflammatory cells, e: severe necrosis, D: less severe inflammation and absent of necrosis in liver cells indicate protective effects of *H. cannabinus* leaf extract (1.6 g kg^{-1}) against CCl_4 hepatotoxicity

and/or preserve the architecture and physiological functions of the liver, disturbed by a hepatotoxin[4].

Paracetamol (PCM) and carbon tetrachloride mediated hepatotoxicity were used as the experimental model for liver injury in this study. Paracetamol also known as acetaminophen has been utilized as an analgesic and antipyretic since the mid-1950s and has become more prominently recognized as a potential hepatotoxin in the overdose situation since the original British reports in the late 1960s[11]. Research on the mechanism of liver toxicity of the drug has provided a theoretical basis of therapy[12,13]. The toxic effect of PCM is mediated by its reactive electrophylic metabolite N-acetyl-p-benzoquinone-imine which exerts oxidative stress on cellular lipids and proteins resulting to cellular damage. Accumulation of CCl_4 in the hepatic parenchyma cells results into homolytic cleavage producing trichloromethyl (CCl_3*) and the chloride (Cl*) free radicals due to the activation effect of cytochrome P_{450} enzymes. The CCl_3* alkylates cellular proteins and other molecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen forming lipid peroxides leading to liver damage[14,15]. Thus administration of CCl_4 and PCM at specific doses will produce marked liver damage. This was obtained in our study as evidenced by significant ($p<0.05$) increases in enzyme (ALT, AST) activities, lipid peroxidation and hyperbilirubinaemia.

Studying the hepatoprotective effect of aqueous extract of *H. cannabinus* revealed that 1.6 g extract/kg body weight effectively reduced the effects of both CCl_4 and paracetamol on experimental rats. The crude powdered extract of *H. cannabinus* exhibited a good hepatoprotective activity. It significantly ($p<0.05$) reduced CCl_4 and PCM elevated plasma activities of ALT, AST enzymes.

Bilirubin assay is a sensitive test to substantiate the functional integrity of the liver and severity of necrosis[16]. Bilirubin also measures the binding, conjugating and excretory capacity of hepatocytes and is proportional to the erythrocyte degradation rate[16,17]. An increase in plasma concentration of bilirubin is an indication of liver cell damage. In the present study, the concentration of bilirubin increased significantly with the administration of paracetamol and CCl_4. This increase was significantly reduced by pre-administration of *H. cannabinus* extract, thus preventing the functional integrity of the liver tissue.

Peroxidation of lipids is a complex process mediated via a free radical mechanism and is implicated in innumerable pathological conditions. Under normal physiological conditions, low concentrations of lipid peroxidation products are always seen in tissues and

cells. In pathological conditions, more peroxidation products are formed[18]. Increased level of lipid peroxidation product in the plasma of CCl_4 and paracetamol intoxicated rats is an indication of excessive free radicals formation. The significant decrease in the concentration of lipid peroxidation products in the plasma of rats' pre-administered *H. cannabinus* extract indicates anti-lipid peroxidation effect of *H. cannabinus*.

A number of hepatotoxic agents also cause accumulation of fatty deposits predominantly triglycerides in the parenchyma cells in the liver. This accumulation of triglycerides may be as a result of an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchyma cells into the systemic circulation. Dianzani[19] showed that a block of the secretion of hepatic triglyceride into plasma is the major mechanism underlying the fatty liver induced in rats by CCl_4. In the present study, we observed fatty deposits and diffused inflammation (Fig 1b) in liver tissues of rats intoxicated with CCl_4. Liver from PCM intoxicated rats showed severe inflammation with focal aggregate and severe necrosis (Fig. 1c). Similar results on enzymatic and histopathological changes in hepatic injury have been reported by earlier researchers[1,4,20,21]. Histopathology examination (Fig. 1d) confirmed the hepatoprotective action of *H. cannabinus* against all two toxins as less inflammatory cells were observed in rats pre-treated with *H. cannabinus* extract.

The accumulation of triglyceride in the hepatic cells is paralleled by a decrease in the concentration of triglyceride in fasted rats' lipoproteins. The concentration of triglyceride in fasted rats can be reduced to almost half its normal value within 30 min of exposure to CCl_4. We did not obtain any significant alteration in triglyceride concentration between groups of experimental rats.

The results of our investigation demonstrated that *H. cannabinus* extract was effective in protecting against sub-acute CCl_4 and PCM induced hepatic injury. This process may be due to an inhibitory action against cytochrome P_{450} enzymes and/or promotion of its glucoronidation activity[21-23]. It could also be that *H. cannabinus* extract contained some natural antioxidants that scavenge the CCl_3* and the N-acetyl-p-benzoquinone-imine electrophylic metabolite as soon as they are produced. Further studies are needed to elucidate the mechanisms for the hepatoprotective action of this plant.

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