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 (CCL₄) and Paracetamol (PCM). This was confirmed by increased plasma transaminases activities, total bilirubin concentration and Thiobarbituric Acid Reactive substance (TBARS, 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 CCL₄ and PCM catalysed 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 cotylosic enzymes such as AST, ALT and ALP into the circulating blood[4]. Therefore, CCL₄ 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[5]. Verbenalin, an extract of *Verba officinalis* has also been reported to exhibit a hepatoprotective activity on experimental liver damage in rodents[6].

*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[7]. The present study evaluates its hepatoprotective activity using a rat model of CCL₄ 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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Preparation of plant material: The plant material was prepared as earlier described. 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 Hibiscus 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.

Group I: Normal control, received olive oil.

Group II: Plant control, received *H. cannabina* 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)\(^3\), total bilirubin\(^6\), Lipid peroxidation\(^8\), cholesterol and triglycerides (Sigma kits). Liver samples collected in 10% formalin were used for histopathological examination\(^10\). Paraffin sections were stained with haematoxylin 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. cannabina* 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.
and/or preserve the architecture and physiological functions of the liver, disturbed by a hepatotoxin.

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. Research on the mechanism of liver toxicity of the drug has provided a theoretical basis of therapy. The toxic effect of PCM is mediated by its reactive electrophilic metabolite N-acetyl-p-benzoquinone-imine which exerts oxidative stress on cellular lipids and proteins resulting to cellular damage. Accumulation of CCl₄ in the hepatic parenchyma cells results into homolytic cleavage producing trichloromethyl (CCl₃⁺) and the chloride (Cl⁻) free radicals due to the activation effect of cytochrome P₄₅₀ enzymes. The CCl₃⁺ 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. Thus administration of CCl₄ 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 hyperbilirubinemia.

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 CCl₄ and paracetamol on experimental rats. The crude powdered extract of H. cannabinus exhibited a good hepatoprotective activity. It significantly (p<0.05) reduced CCl₄ 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. Bilirubin also measures the binding, conjugating and excretory capacity of hepatocytes and is proportional to the erythrocyte degradation rate. 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 CCl₄. 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\(^{[4]}\). Increased level of lipid peroxidation products in the plasma of CCI\(_4\)- 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 \(H.\) \textit{cannabis}us extract indicates anti-lipid peroxidation effect of \(H.\) \textit{cannabis}us.

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\(^{[10]}\) showed that a block of the secretion of hepatic triglyceride into plasma is the major mechanism underlying the fatty liver induced in rats by CCI\(_4\). In the present study, we observed fatty deposits and diffused inflammation (Fig 1b) in liver tissues of rats intoxicated with CCI\(_4\). 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\(^{[4,22,23]}\). Histopathology examination (Fig. 1d) confirmed the hepatoprotective action of \(H.\) \textit{cannabis}us against all two toxins as less inflammatory cells were observed in rats pre-treated with \(H.\) \textit{cannabis}us 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 CCI\(_4\). We did not obtain any significant alteration in triglyceride concentration between groups of experimental rats.

The results of our investigation demonstrated that \(H.\) \textit{cannabis}us extract was effective in protecting against sub-acute CCI\(_4\) and PCM induced hepatic injury. This process may be due to an inhibitory action against cytochrome P\(_{450}\) enzymes and/or promotion of its glucuronidation activity\(^{[21,22]}\). It could also be that \(H.\) \textit{cannabis}us extract contained some natural antioxidants that scavenge the CCI\(_4\),* and the N-acetyl-p-benzoquinone-imine electrophilic metabolite as soon as they are produced. Further studies are needed to elucidate the mechanisms for the hepatoprotective action of this plant.

REFERENCES


