Research Article

Morinda lucida Stem Bark Protects Paracetamol Induced Liver Damage

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ABSTRACT

The present study evaluated the protective effect of *Morinda lucida* (*M. lucida*) stem bark extract on the liver against paracetamol induced toxicity. The experimental design constituted 4 groups of 5 animals each serving as normal control (received distilled water), pathology control (received paracetamol, 2.5 g/kg p.o) and the remaining two groups received simultaneously paracetamol (2.5 g/kg, p.o.) and *M. lucida* extract (100 and 500 mg/kg p.o.). Each treatment was given once daily for 15 days. Animals were sacrificed and blood collected for measurement of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, cholesterol, triglycerides and glucose concentration. Malondiadéhyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) were estimated in rat liver homogenate and histopathology of liver was studied. It was observed that paracetamol induced a significant ($P<0.05$) increase in ALT and AST activities, cholesterol, triglyceride and MDA concentrations. On the contrary significant ($P<0.05$) decrease in CAT, SOD and GSH were obtained in liver hemogenate. Histological evaluation supported this change with evidence of inflammation of the liver tissue of the intoxicated animals. However, pre-administration of *M. lucida* stem bark extract inhibited the deleterious effect of paracetamol considerably by preventing the increase in AST and ALT activities, cholesterol, triglyceride and MDA induced by paracetamol. And also prevented the collapse of the antioxidant defense by significantly ($P<0.05$) maintaining the CAT, SOD and GSH activity towards normal. The effect of *M. lucida* was dose dependent with the dose of 500 mg/kg body weight showing the best protective effect. It is suggestive that the aqueous extract of *M. lucida* Benth. (Rubiaceae) stem bark could protect the liver cells from paracetamol-induced liver damages.

Keywords: *Morinda lucida*, Hepatoprotective properties, Antioxidants enzymes, Paracetamol

INTRODUCTION

The key organ regulating homeostasis within the body is the liver. Hence, liver diseases are detrimental to the body functioning and may result into serious impediment ranging from severe metabolic disorders to even death.[1] Consumption of alcohol, toxic chemicals and drugs such as high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, and oxidized oil can lead to liver injury and hence, their management is a critical concern in medical science.[2]

Paracetamol (acetaminophen) is an over the counter antipyretic and analgesic which may produce acute liver damage if abused. Chemicals such as CCl4 and PCM intoxication are known to catalize radical induced lipid peroxidation, liver damage causing the swelling and necrosis of hepatocytes releasing cytosolic enzymes such as AST and ALT into the circulating blood.[3,4] Hence, CCl4 and PCM induced liver injury have been employed as a convenient model for the investigation of radical-induced damage and its prevention.[5] The mode of paracetamol induced hepatotoxicity is base on the release of a toxic metabolite N-acetyl-P-benzoquinone imine (NAPQI) when metabolized by hepatic cytochrome P-450.[6]

Considerable attention in recent years have been turned to plant derived natural products such as flavonoids, terpenoids and steroids due to their diverse pharmacological properties including antioxidant and hepatoprotective activity.[7] Such plants include *M. lucida* Benth (Rubiaceae) commonly known as Brimstone tree. *M. lucida* is a medium-sized tropical tree measuring approximately 15 m in height and widely used in West and Central Africa traditional medicine. *M. lucida* is implicated in the treatment of different types of fevers, jaundice, hypertension, cerebral congestion, dysentery, diabetes and gastric ulcer.[8] Earlier research has shown that the leaves and roots of *M. lucida* posses hypoglycemic/anti-diabetic and anti Salmonella typhi activities.[9-12] Isolated uterine smooth muscle contractility,[13] toxicity and mutagenic studies,[14-17] reducing and antioxidant properties have all been reported.[18] Anthraquinones, anthraquinols, alkaloids, tannins, flavonoids and saponins have earlier been reported to be responsible for the biological properties of *M.* lucida.[8]

The present study was therefore undertaken to investigate the hepatoprotective potential of aqueous extract of *M. lucida* stem bark against paracetamol-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Reagents

Paracetamol (acetaminophen) was purchased from a regular pharmacy in Yaoundé. Standard assay kits of aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, triglyceride and glucose were purchased from Dialab Laboratories, Austria.

Animals

The male Wistar Albino rats (150-200 g) used in this study were raised in the animal house of the Institute of Medical Research and Medicinal Plants Studies. The animals were housed in wire meshed cages and maintained at 24 ± 2°C under 12h light dark cycle. The animals were fed *ad libitum* with standard rat diet and were allowed free access to tap water. They were allowed to acclimatize for one week before the experiment. The practice and principles of the 1996 Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act that pertains to research of this nature were applied in this study. The Ethical Review Board of the Institute of Medical Research and Medicinal Plants Studies, Yaoundé, Cameroon approved this study.

Preparation of Plant Extract

Fresh stem bark of *M. lucida* was harvested from an uncultivated farmland on the outskirt of Yaoundé, Cameroon in the month of September. Plant identification and voucher specimen (specimen no: 2528 SRFK) referencing was done at the national herbarium of Cameroon. The *M. lucida* stem bark was chopped into tiny bits of about 2 cm. They were subsequently dried in a hot air oven and ground to powder using a grinding machine. 210 g of the ground sample was immersed in distilled water for 48 hr. The extract was filtered with a sieve of 80 µm pore size and the filtrate was concentrated with the aid of a rotary evaporator and freeze dried to obtain 13.20 g of the crude aqueous extract.

Experimental Protocol

Animals were divided into four groups of five rats each and fed orally as below for 15 days.

Group-I

Served as normal control received distilled water.

Group-II

As pathology control (PCM 2.5g/kg body weight).

Group-III

PCM (2.5g/kg body weight) as in group-II + *M. lucida* extract 100 mg/kg weight.

Group-IV

PCM (2.5g/kg body weight) as in group-II + *M. lucida* extract 500 mg/kg weight.

At the end of the treatments animals were sacrificed by cervical dislocation under light ether anesthesia 14 hours after the last dose of plant extract and blood collected through the jugular vein into EDTA-tubes for biochemical analysis. The plasma was separated from whole blood by centrifugation at 3000 rpm for 10 min in a table centrifuge. The liver samples were quickly removed and rinsed in phosphate buffer saline (pH 7.0) homogenate was prepared as earlier described by Agbor.[19]

Biochemical Analysis

Plasma AST and ALT activities, cholesterol, triglyceride, glucose concentration were assayed using assay kits from Dialab Laboratories, Austria. The liver homogenate was used for the measurement of CAT[20] and SOD[21] activities, GSH,[22] MDA,[23] and protein[24] concentration.

Histopathology Study

Liver pieces were preserved in 10% formaldehyde solution. The pieces of liver processed and embedded in paraffin wax. Sections of about 4-6 microns were made and stained with hematoxylin and eosin and photographed.[25,26]

Statistical Analysis

The results were expressed as Mean ± SD. Statistical analysis and comparison between the groups was performed by one way analysis of variance (ANOVA) using SPSS version 10.0, followed by Dunnett's test. Difference between groups (with or without treatment) with a $P < 0.05$ was considered significant.

RESULTS

Plasma Biochemical Parameters

The effect of *M. lucida* on plasma biochemical parameters of experimental animals are depicted in Table 1. PCM administration induced a significant increase in plasma enzymes ALT and AST activity (76.7% and 634.3% respectively) compared to control rats. Co-administration of rats with aqueous extract of *M. lucida* remarkably ($P<0.05$) prevented PCM induced elevated plasma level of ALT (26.39%) and AST (46.76%) towards normal value respectively.

PCM also induced significant increase in the levels of total cholesterol and triglyceride (by 84.66% and 70.88% respectively) compared to control rats. Co-administration of rats with aqueous extract of *M. lucida* at the dose of 500 mg/kg body weight restored PCM induced elevated plasma level of cholesterol (22.24%) towards normal value. While there was no significant ($P>0.05$) restoration of triglyceride levels in animals co-treated with the plant extract. PCM administration did not have any significant effect on blood glucose concentration.

Hepatic Oxidative Stress Parameters

Oxidative stress markers in the liver of experimental animals are depicted in Table 2.

The levels of MDA as an index of lipid peroxidation in liver tissue of paracetamol treated rats were significantly ($P<0.05$) elevated (by 12.98%) when compared to control animals. However *M. lucida* stem bark extract presented a protective effect by keeping the MDA concentration (28.06% and 47.51% respectively) at the dose of 100 and 500 mg/kg body weight towards their normal. PCM administration equally had a significantly effect ($P<0.05$, by 64.94%) on glutathione (GSH) level in the liver compared to control group. Co-treatment with the

aqueous extract of *M. Lucida* had significantly prevented the depletion of the level of GSH. Restoration of reduced GSH level by 48% and 36% respectively at the dose of 100 and 500 mg/kg body weight by the extract towards their control value were observed. CAT activity was significantly (P<0.05) decline (58.6 %) in paracetamol treated group compared to the control. However, when *M. lucida* aqueous extract was co-administered at the doses of 100 and 500 mg/kg body weight a remarkable protection (37% and 31%, respectively) of CAT activity against PCM was observed. SOD activity also decreased in PCM treated rats when compared to control group. Administering *M. lucida* stem bark extract (100 and 500 mg/kg body wt) prevented this decrease in the concentration of SOD activity. In the oxidative stress parameter *M. lucida* portrayed an antioxidant potential by preventing the collapse of the antioxidant system of experimental animals intoxicated with PCM. This effect was dose dependent since the higher dose (500 mg/kg) was more effective than the lower dose (100 mg/kg) tested.

Table 1: Effects of aqueous extract of *M. lucida* stem bark on biochemical markers in PCM induced hepatotoxicity

Groups	AST (IU/l)	ALT (IU/l)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Glucose (mg/dl)
Control	70.59 ± 23.50	34.32 ± 1.67	104.07 ± 27.44	73.65 ± 12.89	103.04 ± 1.57
PCM (2.5g/kg)	518.37 ± 8.25[a]	60.66 ± 6.41[a]	192.71 ± 20.32[a]	125.86 ± 17.10[a]	92.68 ± 50
PCM (2.5g/kg) + *M. lucida* (100 mg/Kg)	270.75 ± 28.58[a,b]	44.65 ± 5.01[b]	154.85 ± 10.60[a]	114.82 ± 22.20[a]	91.76 ± 7.40
PCM (2.5g/kg) + *M. lucida* (500 mg/Kg)	64.47 ± 9.65[b]	34.78 ± 8.33[b]	149.9 ± 17.20[a,b]	116.96 ± 16.10[a]	99.35 ± 5.64

Values are expressed as mean ± SD for five animals in each group. Level of significance: **a** P< 0.05 compared to the control group; **b** P<0.05 compared to PCM treatment.

Table 2: Effects of aqueous extract of *M. Lucida* stem bark on oxidative stress markers in liver tissue in PCM induced hepatotoxicity

Groups	MDA (nM/mg protein)	CAT (U/min/mg protein)	SOD (U/min/mg protein)	GSH (µmol/ mg proteins)
Control	345.5 ± 11.37	196.48 ± 5.27	27.83 ± 4.61	1.94 ± 0.79
PCM (2.5g/kg)	390.36 ± 9.41[a]	81.8 ± 0.33[a]	20.88 ± 1.57[a]	0.68 ± 0.14[a]
M. lucida (100 mg/kg) + PCM (2.5g/kg)	280.8 ± 19.18[a, b]	97.33 ± 8.27[a, b]	23.54 ± 2.84	1.94 ± 0.50[b]
M. lucida (500 mg/kg) + PCM (2.5g/kg)	205.13 ± 7.70[a, b]	127.14 ± 3.65[a, b]	26.04 ± 2.07[b]	2.69 ± 0.39[a, b]

Values are expressed as mean ± SD for five animals in each group. Level of significance: **a** p< 0.05 compared to the control group; **b** p<0.05 compared to PCM treatment.

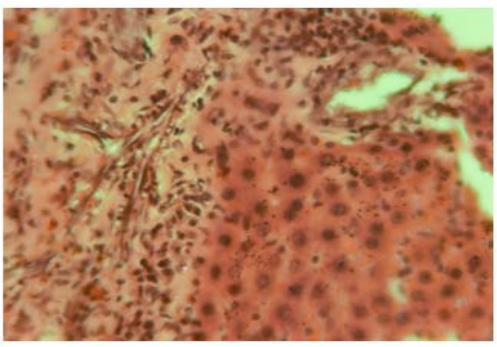

Figure 1: Control Rat Liver H & E x400: Normal architecture of liver

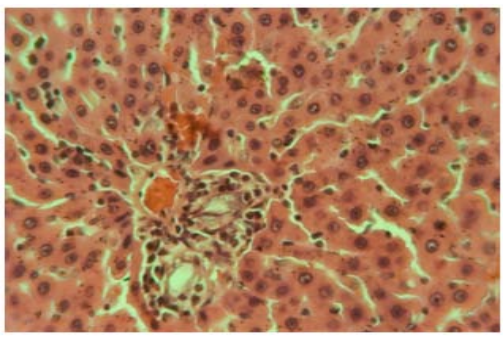

Figure 2: PCM treated Rat Liver H & E x400: periportal inflammation, lymphocytic infiltration and sinusoidal dilatations

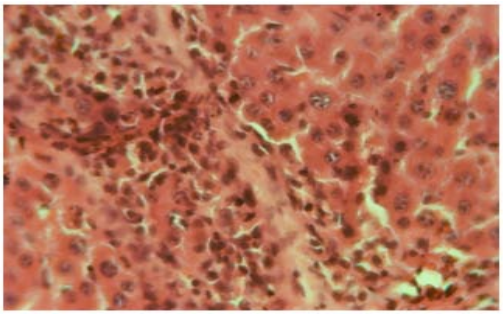

Figure 3: PCM + M. lucida extract 100 mg/kg body weight, H & E x400: Near normal appearance of hepatocytes

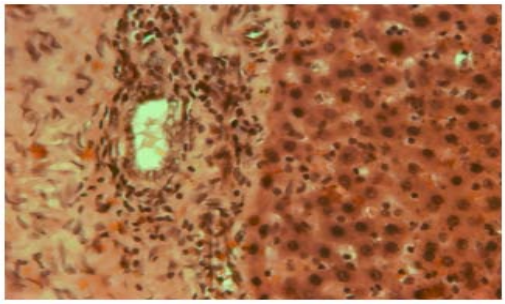

Figure 4: PCM + M. lucida extract 500 mg/kg body weight, H & E x400: near normal appearance of hepatocytes (no sinusoidal dilatations)

Histopathology

Histological profile of animals is depicted in Figure 1,2,3,4. Histological profile of control animals showed normal hepatocytes (Fig. 1). The section of the liver of the PCM treated group exhibited periportal inflammation, lymphocytic infiltration around portal vein and sinusoidal dilatations (Fig. 2). The liver section of animals treated with PCM and the extract at the doses of 100 mg/kg body weight showed normal hepatic architecture with few sinusoidal dilatations (Fig. 3). The liver section of the animals treated with PCM and the extract at the doses of 500 mg/kg body wt showed normal hepatic cords and absence of sinusoidal dilatations (Fig. 4).

DISCUSSION

PCM an effective and over the counter antipyretic and analgesic agent, is safe at therapeutic doses though at higher doses it may produce hepatic damage or necrosis in rodents and man.[27] Protection against PCM-induced toxicity has been used as a reliable test for screening hepatoprotective agents.[28] Metabolism of PCM takes place in the liver producing the excretable glucuronide and sulphate conjugates.[29,30] However, an alternative rout of metabolism by hepatic cytochrome P-450 that produces a highly reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI) occur at a minimal rate and has been associated with PCM toxicity.

The minimal amount of NAPQI produced is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid. However, in overdose of PCM the rate of NAPQI formation exceeds the rate of detoxification by GSH and hence NAPQI accumulates resulting to oxidation of macromolecules such as lipid or – SH group of protein and alters membrane structure and homeostasis of calcium.[31] The alterations of membrane structure render it porous and some liver substances leach out of the tissue into the circulating blood resulting to their increases which are detectable in the plasma. Some of the substances that leached from the liver tissue include AST, ALT, Cholesterol and triglyceride. Other chemicals/drugs used for induction of liver damage in similar studies include CCl4, methotrexate, cadmium and sodium fluoride.[5,32-35]

Elevated activities of ALT and AST enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver.[36] AST and ALT (transaminases) play vital role in the conversion of amino acids to keto acids.[37] This hepatotoxicity model has been found to be of great value in assessing clinical and experimental liver damage[38] and explains why an increase in their activities and concentrations were observed in the present study in groups of animals treated with PCM overdose. Earlier studies have also reported PCM induced elevation in the activity of ALT and AST.[5,39-42] Treatment with aqueous extract of *M. lucida* stem bark at doses of 100 and 500 mg/kg significantly reduced the elevated levels of the AST and ALT towards the respective normal value indicating stabilization of plasma membrane as well as repair of hepatic tissue damage induced by PCM. These changes may be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells.[42] PCM administration may also cause impairment in lipoprotein metabolism[43] and also alterations in cholesterol metabolism. The levels of cholesterol and triglyceride were significantly increased in PCM treated rats, when compared to control, and *M. lucida* treated rats. Elevation of triglycerides level during PCM intoxication could be due to increased availability of free fatty acids, decreased hepatic release of lipoprotein and increased esterification of free fatty acids. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics and aging.

GSH is an intracellular reductant and plays major role in catalysis, metabolism and transport. Glutathione an important cytosolic antioxidant protecting against reactive oxygen species (ROS). It protects cells against free radicals, peroxides and other toxic compounds. Glutathione reductase uses NADPH to maintain the reduced state of cellular GSH which is important in its antioxidant function.[33] Therefore, a significant depletion in glutathione levels may lead to a reduction in efficiency

of the antioxidant enzyme defense system giving an upper hand to ROS[44] which may have several metabolic effects. For example, liver injury included by consuming alcohol or by taking drugs like acetaminophen, lung injury by smoking and muscle injury by intense physical activity,[45] which are all known to be correlated with low tissue levels of GSH. This has led to considerable interest on compounds that have the ability of stimulating GSH synthesis or those that work as antioxidants.[46] From this point of view, exogenous aqueous extract of *M. lucida* stem bark supplementation may provide a means to recover reduced GSH levels and to prevent tissue disorders and injuries. The present study, projects aqueous extract of *M. lucida* stem bark effectiveness against depletion of GSH by PCM in experimental rats.

Lipid peroxidation is a complex process mediated through free radical mechanism and is implicated in many pathological conditions. Under normal conditions low concentrations of lipid peroxides are seen in cells. However there is an increase in its concentration in pathological conditions.[5] MDA is a stable metabolite of lipid peroxidation cascade.[47,48] Hence, MDA is usually used as a marker of oxidative stress and membrane (lipid layer) damage.[49] In the present study PCM administration led to increased MDA concentration in rat liver. The increase in MDA level in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms. Treatment with aqueous extract of *M. lucida* stem bark significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection of aqueous extract of *M. lucida* stem bark is due to its antioxidant effect.

Biological systems protect themselves against the damaging effects of reactive oxygen species by several means. These include free radical scavengers and reaction chain terminators; enzymes such as SOD and CAT.[50] The enzymes SOD and CAT are major antioxidant defense systems of the body which protect the cell membrane and other cellular constituents against oxidative damage by reactive oxygen species (ROS).[51] Increase in SOD, CAT and GRX activities in the cardiac and hepatic tissues could be due to the likely supportive interactions amongst these enzymes which help provide a defense mechanism against free radicals. SOD will scavenge superoxide anions which if allowed to accumulate will inhibit the activity of CAT. [52] The product of the dismutation activity of SOD is hydrogen peroxide which is a substrate for CAT.

Thus, a decrease in SOD activity leads to a decrease in CAT activity for hydrogen peroxide degradation. In the present study PCM administration induced a collapse of the liver antioxidant defense system by inducing a decrease in the antioxidant enzymes activities. Similar results on PCM induced collapse of the antioxidant defense had earlier been reported.[53-56] This effect of PCM was well tolerated by experimental animals receiving *M. lucida* hence, preventing the collapse of the antioxidant enzymes (SOD and CAT). The observed increase of SOD activity suggests that the aqueous extract of *M. lucida* stem bark have an efficient protective mechanism in response to oxidative stress and may be associated with decreased oxidative stress and free radical-mediated tissue damage.

In the present study, liver sections of the rats intoxicated with PCM showed periportal inflammation, lymphocytic infiltration and sinusoidal dilation. Nithianantham[41] have reported the induction of periportal inflammation, lymphocytic infiltration and sinusoidal dilation which are indicative of hepatic tissue damage. The damage of liver tissue integrity was effectively inhibited by aqueous extract of *M. lucida* stem bark indicating pronounced protection of hepatocytes in paracetamol induced hepatic damage.

CONCLUSION

This study has demonstrated that aqueous extract of *M. lucida* stem bark is a potent hepatoprotective agent against paracetamol induced damage in rats which may be related to its antioxidant and free radical scavenging mechanism.

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