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Full Length Research Paper

Hepatoprotective effect of the aqueous extract of leaves of *Acalypha racemosa* in carbon tetrachloride treated rats

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The effects of simultaneous treatment of CCl_4 (i.p) with 60 mg/kg (p.o) of aqueous extract of leaves of *Acalypha racemosa* on rat liver was evaluated. Analysis of serum ALT and AST activities with those of the concentrations of albumin, total protein, unconjugated and total bilirubin were carried out. The malondialdehyde (MDA) content of liver was determined to investigate a probable mechanism of action of the extract. Administration of CCl_4 alone to rats significantly increased total bilirubin concentration and the activities of ALT and AST ($p < 0.05$) in the serum while it significantly reduced ($p < 0.05$) serum total protein and albumin concentrations when compared with controls which received distilled water (p.o). Also it significantly increased ($p < 0.05$) liver MDA content when compared with control. However, simultaneous treatment of CCl_4 with 60 mg/kg of the aqueous extract significantly reversed ($p < 0.05$) these changes. Results of MDA content of liver homogenates suggest that a probable mechanism of action of the extract is antioxidation. Histopathological studies were carried out on the liver to confirm the observed changes

Key words: *Acalypha racemosa*, aqueous extract, hepatoprotective property, antioxidant effect, carbon tetrachloride, liver.

INTRODUCTION

Products of higher plant origin have been shown to be effective sources of chemotherapeutic agents without underlining side effect (Neetu and Meenakshi, 2003). The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs. Reactive oxygen species (ROS) have been implicated in more than 100 diseases (Ali et al., 2001). Experimental, clinical, and epidemiological studies have provided evidence in support of the role of ROS in the etiology of cancer (Ray et al., 2000). When produced in excess, ROS cause tissue injury. All aerobic organisms including humans have antioxidant defense mechanisms that protect against oxidative damage. However, the natural antioxidant defense mechanisms can be insufficient and hence dietary intake of antioxidant components is important and recommended (Duh, 1998).

A number of plants have been shown to possess hepatoprotective properties by improving the antioxidant status e.g. *Caesalpinia bonducella* (Gupta et al., 2005). However, there is still lack of scientific proofs to authenticate the hepatoprotective properties of some plants which are used traditionally to treat liver disorders. An example of such is *Acalypha racemosa* (Euphorbiaceae). A. racemosa is considered as an indigenous source of medicine exhibiting antibacterial activity (Musa et al., 2000). Also, traditional healing practitioners in Ilorin metropolis, Nigeria, use the decoction of A. racemosa leaves to treat liver disorders and other disease conditions which result in jaundice.

In our previous work, some of the secondary plant metabolites detected in the aqueous and methanolic extracts of the leaves of A.racemosa are flavonoids and phenolics (Iniaghe et al., 2008). Since it has been demonstrated that phenolic compounds are effective antioxidants and that flavonoids have potent antioxidant activities by scavenging hydroxyl radicals, superoxide anions and lipid peroxy radicals (Alan and Miller, 1996), it is reasonable to evaluate the antioxidant property of the

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leaves of this plant.

Thus this present investigation was undertaken to evaluate the hepatoprotective activities of aqueous extract of the leaves of A. racemosa against oxidative damage induced by CCl_4 in rats and to evaluate the possibility of extracts enhancing bilirubin clearance from the serum when its level is elevated.

MATERIALS AND METHODS

Animals

The fifteen albino rats (Rattus novergicus) used for this study were obtained from the Animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were maintained in standard conditions of temperature, relative humidity and light/night cycles. They were allowed free access to commercial pelleted rat chow (Bendel Feeds Ltd, Ewu, Nigeria) and water ad libitum.

Chemicals

2, 4-dinitrophennylhydrazine Bromocresol green, bilirubin and albumin reagent kit and all kits used for enzyme assay were obtained from Randox Company, United Kingdom. All other reagents used were of analytical grade and were prepared in all glass distilled water.

Plant material and extraction

The leaves of A. racemosa were obtained from Mount Olives Anglican Church, Pipeline Road, Tanke, Ilorin, Kwara State, Nigeria and were identified at the Herbarium section of the Department of Plant Biology of the University of Ilorin. Fifty grams of shade-dried leaves pulverized to powder was percolated in distilled water. The mixture was allowed to stand for 24 h and then filtered to obtain the aqueous extract. The filtrate was evaporated to dryness in a water bath at 60oC.

Experimental design

The rats were randomly divided into three groups (n = 5) as follows:

(i) Control received orally 1 ml of distilled water daily.
(ii) Group A received 1.5 ml/kg body weight (i.p.) CCl_4 daily.
(iii) Group B received 1.5 ml/kg body weight (i.p.) CCl_4 and 60 mg/kg body weight of aqueous extract of A. racemosa leaves (orally) simultaneously.

After the experimental period of seven days, animals were sacrificed and venous blood was collected into sample bottles containing no anticoagulant as earlier reported (Adebayo et al., 2003). The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 rpm for 5 min (Ogbu and Okechukwu, 2001) . The clear serum was removed by pipetting which was used for the assay of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and the determination of concentrations of serum total protein, albumin, unconjugated bilirubin and total biliburin.

A section of the liver for each rat in each group was preserved in 10% formalin for histopathological studies while the remaining was homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v) and centrifuged at 4000 rpm for 10 min. The supernatant was used for the estimation of malondialdehhyde (MDA) content and liver total protein concentration.

Biochemical studies

The determination of serum albumin concentration was done using the method described by Gowenlock et al. (1988) while that of serum bilirubin concentration was done using the method described by Malloy and Evelyn (1937). Liver malondialdehyde content determination was done by measuring the concentration of thiobarbituric acid reacting substrate (TBARS) in liver using the method of Olusin (2002). Total protein concentration was estimated in the serum and liver using the Biuret method (Plummer, 1978). The activities of serum aminotransferases (ALT & AST) were assayed basically by the method of Reitman and Frankel (1957). The method of Baker and Silverton (1985) was employed for the histopathological studies.

Statistical analysis

The statistical analysis was carried out by one way Analysis of variance and Duncan Multiple Range test. P values < 0.05 were considered significant.

RESULTS

Histopathological studies of the liver of control animals showed normal histology (Figure 1a). The Group to which CCl_4 was simultaneously administered with 60 mg/kg body weight of aqueous extract of A. racemosa leaves separately also showed normal liver histology (Figure 1c). For animals to which CCl_4 only was administered, inflammation of hepatic cells was observed (Figure 1b).

Administration of CCl_4 alone to rats caused a significant increase ($p < 0.05$) in serum unconjugated bilirubin and serum total biliirubin (Table 1) when compared with controls. Simultaneous administration of 60 mg/kg aqueous extract with CCl_4 to rats significantly reduced ($p < 0.05$) the level of unconjugated biliirubin in serum to the range of the control value.

A significant decrease ($p < 0.05$) in concentration of

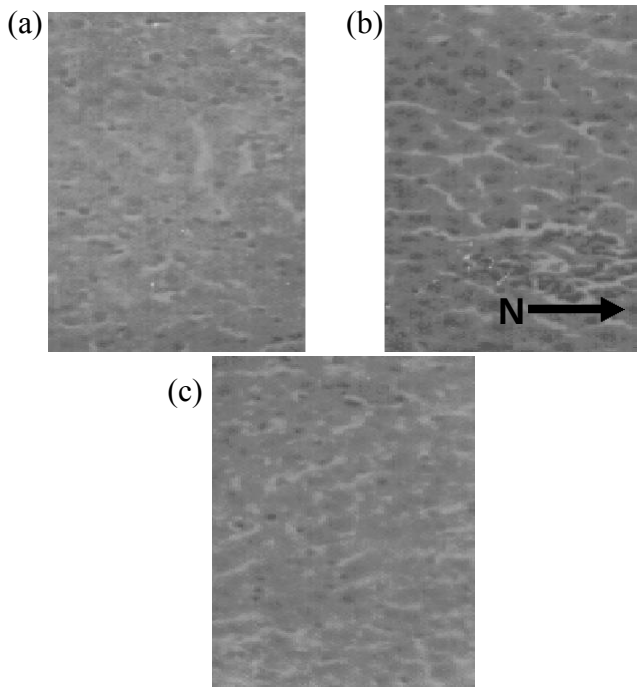

Figure 1. Photomicrographs of the liver of experimental rats (Magnification: x400). N = neutrophils at the site of inflamemation. a: Control (Distilled water only) b: CCl_4 (1.5 ml/kg) only c. CCl_4 + 60 mg/kg aqueous extract

albumin in serum of rats treated with CCl_4 only when compared with control was observed (Table 2). The change effected by the administration of CCl4 only was significantly reversed ($p < 0.05$) by the simultaneous administration of CCl4 with 60 mg/kg body weight of aqueous extract.

Treatment of rats with CCl_4 alone significantly reduced ($p < 0.05$) the concentration of total protein in both the liver and the serum when compared with control (Table 2). Simultaneous administration of rats with CCl_4 with 60 mg/kg body weight of aqueous extract significantly reversed the change ($p < 0.05$).

A significant increase ($p < 0.05$) in MDA content, an end product of lipid peroxidation, in the liver of rats treated with CCl_4 only was observed when compared with control (Table 3). Simultaneous administration of CCl_4 with the aqueous extract of A. racemosa significantly reduced ($p < 0.05$) the MDA content in the liver when compared with that of rats administered CCl_4 only, thus restoring it back to the range of control (Table 3).

Table 3 also shows the effect of the aqueous extract of A. racemosa leaves on the activities of serum AST and ALT in rats. Hepatotoxicity was evidenced by a significant increase ($p < 0.05$) in the activities of serum AST and ALT in the group treated with only CCl_4 when compared with control. Simultaneous administration of the extract with CCl_4 significantly reduced ($p < 0.05$) the activities of these enzymes in serum when compared with the group treated with CCl_4 only.

DISCUSSION

The effects of the aqueous extracts of leaves of A. racemosa on carbon tetrachloride - induced hepatotoxicity in albino rats were evaluated. For animals to which CCl_4 only was administered, inflammation (signified by the appearance of neutrophils) and an early phase of necrosis of hepatic cells were observed (Figure 1b). Neutrophils are characteristic of inflammation in the early stages. They are the first to appear in an infected area (Guyton and Hall, 2000). An attempt has been made to explain the mechanism leading to the infiltration of inflammatory cells into liver and liver cell necrosis resulting from CCl_4 poisoning. After CCl_4 poisoning, intracellular adhesion molecule 1 (ICAM 1) in the liver, tissue increases (especially in sinusoidal endothelial cells and hepatocytes) (Neubauer, 1998). Also leukocyte function antigen 1 (LFA-1) around vessel wall increases, thus leukocyte function antigen 1 and ICAM 1 accumulate at necrotic areas increasing inflammatory cells and inducing hepatotoxicity (Neubauer, 1998). Due to the fact that no inflammation was observed in those rats to which CCl_4 and aqueous extract of leaves of A. racemosa were simultaneously administered (Figure 1c), it therefore suggests that the seven-day administration of the aqueous extract of A. racemosa leaves was effective in reversing the hepatotoxicity caused by the administration of CCl_4. Bilirubin is the main bile pigment that is formed from the breakdown of heme in the red blood cells. It is transported to the liver where it is secreted by the liver into the bile. Conjugation of bilirubin is a prerequisite for its excretion into the bile (Nelson and Cox, 2000). Malfunctioning of the liver was evidenced by the significant increase ($p < 0.05$) in the level of unconjugated bilirubin in the serum of the group treated with only CCl_4 when compared with control (Table 1). Increase in the level of unconjugated bilirubin in the blood may result from a defect in the function of the liver to conjugate the bilirubin being produced (Marks and Dennis, 1996). The significant reduction ($p < 0.05$) of unconjugated bilirubin level in the serum when CCl_4 was simultaneously administered with the aqueous extract when compared with the administration of CCl_4 alone indicates that the conjugating function of the liver was improved. The reduction of the unconjugated bilirubin level by the aqueous extract suggest that the extracts may activate the constitutive androstane receptor (CAR) which is a key regulator in bilirubin clearance in the liver (Moore et al., 2004). The primary function of CAR in the bilirubi clearance pathway is to direct a coordinate response to elevated levels of bilirubin by increasing the hepatic expression of each component of the pathway (Moore et al., 2004).

The ability of simultaneous administration of CCl_4 with aqueous extract to significantly reduce ($p < 0.05$) the level of serum total bilirubin when compared with that of the CCl_4 - treated group suggests the potential of the is extract in clearing bilirubin from the serum when its level elevated (Table 1) (Gupta et al., 2005).

Table 1. Effects of aqueous extract of leaves of *A. racemosa* on the level of serum bilirubin in experimental rats.

Groups	Serum unconjugated bilirubin concentration (µmol/L)	Serum total bilirubin concentration (µmol/L)
Control: Distilled water only	4.50±0.13 [a]	7.40 ± 0.24[a]
CCl_4 (1.5 ml/kg) only	8.50±0.25[b]	8.10 ± 0.25 [b]
CCl_4 + 60 mg/kg aqueous extract	6.00±0.50[c]	7.1±0.70[a]

Values are mean ± S.D of 5 determinations. Values with different alphabet superscripts are significantly different at P < 0.05.

Table 2. Effects of aqueous extract of leaves of *A. racemosa* on the levels of serum and liver protein in experimental rats.

Groups	Serum albumin concentration (g/L)	Serum total protein concentration (g/L)	Liver total protein concentration (mg/L)
Control: Distilled water only	26.0±1.0[a]	46.0±3.0 [a]	4.57±0.16[a]
CCl_4 (1.5 ml/kg) only	18.5±0.9[b]	34.0±3.2 [b]	3.65±0.11[b]
CCl_4 + 60 mg/kg aqueous extract	23.0±0.3[c]	45.0±2.5 [a]	4.59±0.16[a]

Values are mean ± S.D of 5 determinations. Values with different alphabet superscripts are significantly different at P < 0.05.

Table 3. Effects of aqueous extract of leaves of *A. racemosa* on liver MDA content and serum AST and ALT activities in experimental rats.

Groups	Liver MDA content (µmol/L)	Serum AST activity (U/L)	Serum ALT activity (U/L)
Control: Distilled water only	45.00±10.50[a]	36.5±0.05[a]	27.1±0.06[a]
CCl_4 (1.5 ml/kg) only	80.00±4.00[b]	90.0±0.10[b]	89.4±0.01[b]
CCl_4 + 60 mg/kg aqueous extract	50.00±2.00[a]	64.1±0.20[c]	34.1±0.18[c]

Values are mean ± S.D of 5 determinations. Values with different alphabet superscripts are significantly different at P < 0.05.

Since the results obtained for the serum albumin, serum total protein and liver protein concentrations followed the same trend (Table 2), it thus implicates the same mechanism by which the extract exerts its effect on these three parameters. The administration of CCl_4 alone may adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins such as albumin in the liver. Simultaneous administration of CCl_4 to rats with 60 mg/kg body weight aqueous extract significantly reversed ($p < 0.05$) these changes may be by increasing protein synthesis. This indicates the hepatoprotective activity of A. racemosa leaves against damage by CCl_4. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates regeneration of cells (Awang, 1993).

Hepatotoxicity was also evidenced by a significant increase ($p < 0.05$) in activities of serum AST and ALT in the group treated with only CCl_4 when compared with controls (Table 3). This may be as a result of leakage from the cells through peroxidative damage of the membrane. This is evidenced by the increase in liver MDA content after the administration of CCl_4 only (Table 3). MDA level is used to determine early liver oxidative stress (Tokyay et al., 1999). It is also used to investigate the oxidative damage of proteins and lipid peroxidation of the membrane and lipoproteins as possible pathogenic mechanism for liver injury (Kocic et al., 1998). The heaptotoxic effects of the metabolism of CCl_4 are due to its active metabolite, trichloromethyl radical (Johnson et al., 2002). The activated radical binds covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum which are rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides which in turn gives product such as malondialdehyde (MDA) that cause damage to the membranes. This lipid peroxidative degradation of biomolecules is one principal cause of hepatotoxicity of CCl_4 (Cotran et al., 1994). The increased liver MDA contents in rats treated with CCl_4 only suggest that the natural antioxidant defense mechanism to scavenge excessive free radicals has been compromised. Increased serum bilirubin level in this group could be looked upon as a com-

pensatory/retaliatory phenomenon in response to cellular peroxidative changes (Pratibha et al., 2004). This is because bilirubin functions in vivo as a powerful antioxidant, anti-mutagen, and an endogenous tissue protector (Pratibha et al., 2004). This thus shows a positive correlation between MDA content and serum bilirubin level which supports the report of Pratibha et al. (2004).

However, simultaneous administration of the aqueous extract with CCl_4 significantly reduced ($p < 0.05$) the activities of these enzymes in the serum when compared with the group treated with CCl_4 only. This also indicates hepatoprotection.

These findings all point to the fact that the aqueous extract of A. racemosa leaves is hepatoprotective against CCl_4 induced liver damage. Hence it is possible that a probable mechanism of hepatoprotection of A. racemosa leaf extract against CCl_4 induced damage is the antioxidant activity. The antioxidant activity of the extracts may be attributed to the presence of phenolics and flavornoids (Iniaghe et al., 2008).

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