Protective Effects of Aqueous and Ethanolic Extracts of the Leaf of *Cassia italica* in CCl_4-induced Liver Damage in Rats

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ABSTRACT

Renewed attention in recent decades to alternative medicines and natural therapies has stimulated a new wave of research interest in traditional practices. The plant kingdom has become a target for the search for new drugs and biologically active compounds. The present study was designed to evaluate the hepatoprotective effects of aqueous and ethanol extracts of *Cassia italica* leaves in carbon tetrachloride-induced hepatotoxicity in rats. Histopathology of the liver was also studied in CCl_4 induced liver damage in pretreated rats. Pre-treatment with 200mg/kg body weight of C*assia italica* leaf extract gave some measure of protection to the rats against CCl_4 liver damage. Serum and liver enzymes tested (AST, ALT, ALP and G-GT) were all significantly ($p \leq 0.05$) lowered when compared to with the negative control. Total bilirubin was reduced from 2.91 ± 0.03 in experimental control to 1.70 ± 0.06 with ethanol extract and to 1.41 ± 0.15 with aqueous extract. Cholesterol levels were significantly ($p \leq 0.001$) lowered from the elevated levels of the untreated group when compared to treated group (244.1 ± 4.76 to 127.2 ± 2.32 and 126.33 ± 2.01 with ethanol and aqueous extracts respectively). Albumin levels significantly ($p \leq 0.05$) increased with ethanol extract in serum of treated rats. Lipid peroxidation as assayed by thiobarbituric acid reactive substances was significantly reduced ($p \leq 0.05$). Histopathological studies also provided supportive evidence for the biochemical analysis.

Key words: CCl_4, liver, *Cassia italica*, extracts, histopathology

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INTRODUCTION

Nature has provided abundant plants for the wealth of all living creatures, which possess medicinal virtues with the most powerful cures for just about any disease (Mohammed *et al.*, 2006). Everyday medical researchers are pushing the frontier of nature's healing power by exploring healing medicine in the form of various plants, trees, herbs, animals and even insects. She has given us cures for diseases, and provides more than half of the compounds of the known drugs that form the core of western medicine.

A number of plants are traditionally used to treat liver diseases (Mukazayire *et al.*, 2012) Except for vaccines and inter-feron α-2b, which concern only viral infections, modern medicine is quite limited in preventing or treating hepatic diseases; the only drugs available are cholago-gues, choleretics, and drugs for cholesterolic lithiasis, N-acetylcysteine and flavolignanes obtained from *Silybum marianum.* This limitation of therapeutic options gives considerable interest to the search for plants traditionally used for these diseases (Evans, 2002).This study is aimed at the evaluation of C*assia italica* as one of the many medicinal plants used for the treatment of jaundice in herbal medicine in Adamawa State of Nigeria.

Cassia italica a member of the family *cesalpiniaceae* is a pereneal shrubby plant known as Eshriq. In Adamawa State of Nigeria, the leaves of *C.italic*a popularly known as **'ganyen shayi'** (tea leaf) or '**flesko**' is being widely used to treat liver dysfunctions and diabetes among other ailments by the traditional practitioners for many years. The leaf of *Cassia italica* is used in addition to treating liver injuries and diabetes mellitus treating/management of skin diseases, typhoid fever, hair treatment, as laxative and in management of termites. The flowers are use in treating malaria fever, the tender fruits are eaten by herdsmen and the seeds can be prepared into coffee-like tea.

The present study is intended to explore whether this herb can have protective effect on hepatocytes and to give an orientation to find hepatoprotective compounds that may be present in the extracts. This first step should eventually lead us to molecules responsible for the effect and further investigating mechanisms of action on the liver.

MATERIALS AND METHODS

Plant Materials

The leaves of *C. italica* were fetched from the vicinity of Modibbo Adama University of Technology, Yola and Yolde Pate a village in Yola South Local Government Area of Adamawa State, Nigeria and botanically identified by Briston Basiri of Plant. The leaves were air dried at room temperature and ground using a laboratory mortar and pestle followed by sieving using a 1mm endocoff sieve. The fine powdered sample was stored in a desiccator at room temperature until required.

Experimental Animals

Male Wistar strain albino rats weighing between 130+±10.38gm needed for this study was purchased from the animal unit of the Nigeria Institute for Trypanosomiasis Research (NITR), Vom. Plateau State, Nigeria. They were fed with standard rat diet and drinking water *ad libitum*.

Chemicals and Reagents

Reagents used were all of analytical grades.

Statistical Analysis

Numerical data obtained from the study were expressed as the mean value ±standard error of mean. Differences among means of control and tested group were determined using Statistical Package for social scientist (SSPS 11.0). A probability level of less than 5% ($p \leq 0.05$) was considered significant.

Preparation of Extract

100g of the powdered sample/leaf was extracted by adding 500ml 70% ethanol and with water. The mixture was left overnight at room temperature on a shaker. The extract was decanted and the fibrous residue rinsed exhaustively. The extract and the risings were pooled together and filtered through whatman No. 1 filter paper and the filtrate freeze dried using a freeze dryer *(Adzu et al., 2003*). Water was used to reconstitute the solid extract to a desired concentration for the study.

Experimental Design

A total of 42 rats were used for this study. The rats were divided randomly in 7 groups.

- Group 1: Served as control
- Group 2: was administered intraperitonealy CCl_4 2ml/kg that was dissolved in olive oil to induce liver damage.
- Group 3 & 4: Were administered 200mg/kg of aqueous and ethanolic extract of *C.italica* for 14 day orally before CCl_4 induction.

Twenty four hours after the experimental period, rats in all the groups were sacrificed under a mild anaesthesia; blood & liver tissue were collected for the estimation of biochemical and histological analysis respectively.

Hepatoprotective effect of the extracts

Rats were pretreated with 200mg/Kg body weight of the extracts for fourteen (14) days before induction with 2ml/kg body weight of 1:2 carbon tetrachloride in olive oil. Twenty four hours after the experimental period, rats in all the groups were sacrificed under a mild anaesthesia; blood & liver tissue were collected for the estimation of biochemical and histological analysis respectively.

Histopathological studies: A section of liver tissue from each animal was removed after sacrificing the animal, placed in 10% formalin solution and processed by paraffin technique. Section of 5µm thicknesses were cut and stained by haematoxylin and eosin for histopathological examination and later the microscopic slides were photographed.

Biochemical estimation: Diagnostic kits were employed in the analysis of most of the biochemical parameters that were determined. AST, ALT (Reitman and Frankel, 1957) ALP (Deutsche Gesellschaft fur Klinische Chemie (Rec. GSCC DGKC), 1972) GGT (Rosalki *et al.*, 1970). Serum total and direct bilirubin concentrations (Malloy and Everlyn, 1937) ALB, TSP **(**Reinhold, 1953), Cholesterol (Zak *et al.*, 1953), catalase (Sinha, 1972) and TBARS (Ohawa *et al.*, 1979).

RESULTS

Table 1, presents the results of some non enzyme biochemical indices of hepatic damage in pre-treatment with 200mg/kg body weight *Cassia italica* leaf extracts (ethanol and aqueous). Pretreatment with 200mg/kg of aqueous and ethanol extracts of C. *italica* brought about significantly ($p \leq 0.05$) difference in the levels of bilirubin and cholesterol and at the same time maintained the levels of protein significantly when compared to the CCl_4 treated group.

Table 1: Effect of pretreatment with aqueous and ethanol extracts of *C italica* leaf on serum enzymes of rats administered CCl_4 (2ml/kg body weight)

Treatment	AST (U/l)	ALT (U/l)	ALP (U/l)	GGT (U/l)
Normal	40.10 ± 0.91	28.45 ± 1.04	103.55 ± 2.17	78.78 ± 2.95
CCl_4 Control	99.70 ± 2.51	48.03 ± 1.43	132.0 ± 7.47	107.51 ± 1.37
E E + CCl_4	53.5 ± 0.96*	40.5 ± 3.37*	102.5 ± 0.87*	98.25± 1.11*
Aq E + CCl_4	66.25 ± 0.91*	42.75 ± 2.56*	96.30 ± 1.88*	93.0 ± 1.68*

Values are means of six determinations ± SEM;
*Significantly lower compared to values obtained for group treated with CCl_4 only ($p<0.05$)
E E – ethanolic extract
Aq E – aqueous extract

Table 2: Effect of pretreatment with aqueous and ethanol extracts of *C italica* leaf on some serum non-enzyme biochemical indices of rats administered CCl_4 (2ml/kg body weight)

Treatment	TB (mg/dl)	CHOL (mg/dl)	ALB (mg/dl)	TSP (mg/dl)
Normal	0.78 ± 0.10	71.47 ± 1.81	3.47 ± 0.17	56.20 ± 2.78
CCl_4 Control	2.91 ± 0.03	168.53 ± 4.81	2.44 ± 0.09	48.03 ± 1.25
E E + CCl_4	1.70 ± 0.06**	127.2 ± 2.32**	2.88 ± 0.09	53.35 ± 0.58*
Aq E + CCl_4	1.41 ± 0.15**	126.33 ± 2.01**	2.53 ± 0.03	47.03 ± 2.43

Values are means of six determinations ± SEM:
** Significantly lower compared to values obtained for group treated with CCl_4 only ($p<0.01$)
* Significantly higher compared to values of group treated with CCl_4 alone ($p<0.05$)

Pre-treating the rats with 200mg/kg of aqueous and ethanol extracts of C. *italica* significantly ($p \leq 0.05$) lowered the serum levels of lipid peroxidation as measured by thiobarbituric acid reactive substances and at the same time raised/enhanced the levels of catalase significantly when compared with rats induced with CCl_4 (table 3).

Table 3: Effect of pretreatment with aqueous and ethanolic extracts of *C. italica* leaf on serum TBARS and Catalase of rats administered 2ml/kg body weight of CCl_4

	TBARS (nmol/L)	Catalase
Normal	105. 12 ± 1.16	40. 83 ± 7. 53
CCl_4 control	230. 5 ± 11. 51	21. 73 ± 1. 71
EE + CCl_4	128. 6 ± 7. 55**	34. 71 ± 3. 2*
AqE + CCl_4	151. 3 ± 2. 96**	27. 80 ± 1. 05

Lipid peroxidation as assayed by thiobabituric acid reactive substances (TBARS) and catalase activity were analysed at the end of treatment period.
Values are means of six determinations ± SEM:

** Significantly lower compared to values obtained for group treated with CCl_4 only ($p<0.01$)
* Significantly higher compared to values obtained for rats treated with CCl_4 only ($p<0.05$)

The use of medicinal plants among different cultural groups is a long- standing tradition. The preventive measures and treatments vary according to customs and beliefs. Today many people make good use of medicinal herbs.

Liver disease and toxicity is common, especially with many drug treatments. Liver injury induced by CCl_4 is the best characterised system of xenobiotic induced hepatotoxicity and is a commonly used model for the screening of anti-hepatotoxic/hepatoprotective activity of drugs (Brautbar & Williams, 2002). Administration of CCl_4 orally causes acute liver damage that mimics natural causes (Kamble *et al.,* 2008, Fallah *et al.,* 2012). It mediates changes in liver functions and ultimately leads to destruction of hepatocellular membrane. Cytochrome P_{450} activates CCl_4 to form various free radicals which are involved in pathogenesis of liver damage in chain reactions resulting in peroxidation of lipids, covalently binding to macromolecules, disruption of metabolic mechanisms in mitochondria, decrease levels of phospholipids, increase triglycerides levels, inhibition of calcium pump of microsomes thus leading to liver necrosis (Kamble *et al.,* 2008).

AST & ALT are the most commonly used biochemical markers of liver injuries (Shih *et al., 2005).* Levels of all marker enzymes of liver injury increased significantly in the negative control group after CCl_4 administration ($P< 0.001$) as compared to normal control group (Table 1). Pretreatment with *Cassia italica* leaf extracts caused significant decrease in the activities of these enzymes. The increase in activities of the liver marker enzymes such as AST, ALT and GGT in the serum of CCl_4 induced rats indicated damage to hepatic cells (Wolf, 1999). The increase in serum levels of AST and ALT have been attributed to the damaged structural

integrity of the liver. This is because they are cytoplasmic in their location and are released into circulation after cellular damage (Hwang *et al.*, 2007). The results of this study demonstrated that pre treatment with aqueous and ethanolic extracts of *Cassia italica* leaves significantly ($p < 0.05$) caused a decrease in most of the biochemical parameters tested in comparison to carbontetrachloride control. The extracts protected the hepatocytes from the CCl_4-induced injuries. The stabilization of transaminases denotes the renewal of the normal hepatic activity (Galati *et al*, 2005). This work tallies with the effects of *Moringa oleifera* leaf used in treatment of liver damage (Shahjahan *et al.*, 2004; Nadro *et al.*, 2006).

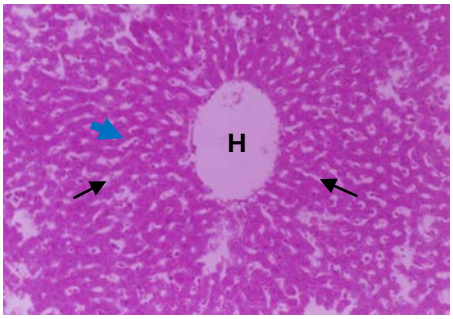

Fig. 1 Photomicrograph of rat liver showing normal hepatocytes (arrow blue), sinusoids (arrow) and normal centralobular area (H) H&E x 200.

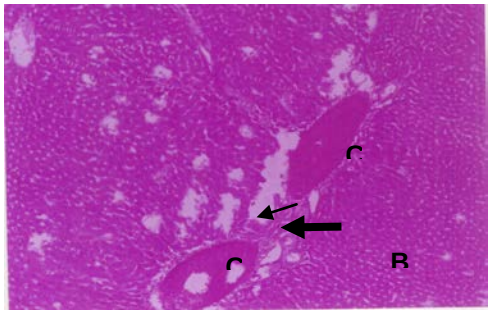

Fig. 2 Photomicrograph of rat liver treated with CCl4 showing severe congestion and thrombus (C), fibrous connective tissues (thick arrow) and fatty infiltration (thin arrow) H & E x200

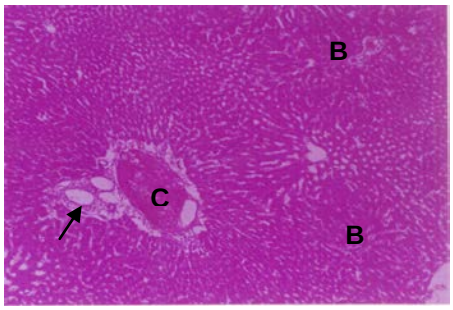

Fig. 3 Photomicrograph of rat liver pre-treated with 200 mg/kg bw aqueous extract of *Cassia italica* and later with CCl_4, showing severe congestion and thrombus (C), Fatty infiltration (thin arrow) and cloudy swelling (B) H & E x200.

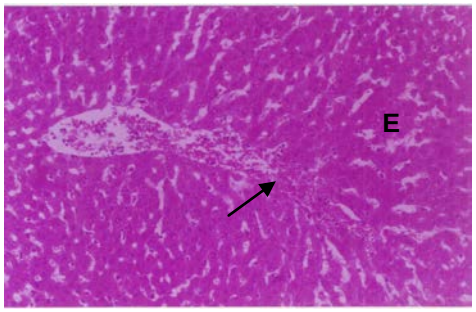

Fig. 4 Photomicrograph of rat liver pre-treated with mg/kg bw ethanolic extract of *Cassia italica* and later with CCl4, showing mild thrombus at the centrilobular area (D), interstitial hemorrhage (arrow) and hepatic necrosis (E).H & E x 200

The liver not only synthesizes the protein for its needs but produces numerous export proteins. Among the latter, serum albumin is the most important (Podolsky and Isselbacher, 1991; Khorshid *et al.*, 2008). In this experiment, CCl_4 induced liver damage in rats which is indicated by the decrease in levels of albumin/proteins. The results of this study demonstrated that pretreating rats with the extracts (aqueous and ethanolic) effectively protected the rats against CCl_4 – induced hepatotoxicity as evidenced by the increase in contents of both hepatic protein and serum albumin (Koneri *et al.*, 2008). Both aqueous and ethanolic extracts of *Cassia italica* clearly remitted the decrease of protein contents in the liver and albumin content in the serum. Thus it is shown to ameliorate the decline of liver synthetic functions caused by CCl_4 induced liver damage. The decrease in total serum protein (TSP) observed in CCl_4 treated rats (Table 2) may be associated with the decrease in the number of hepatocytes which in turn, may

have led to the decreased hepatic capacity to synthesize protein (Shahjahan *et al.*, 2004) but the restoration of the level of TSP after administration of *Cassia italica* extracts is a reflection of the hepatoprotective nature of this plant.

A number of plants have been shown to possess hepatoprotective property by improving antioxidant status (Shahjahan *et al.*, 2004). *Cassia italica* leaf was investigated for hepatoprotective activities. Serum activities of Pre-treating the rats with 200mg/kg of aqueous and ethanol extracts of C. *italica* significantly ($p \leq 0.05$) lowered the serum levels of lipid peroxidation as measured by thiobarbituric acid reactive substances and at the same time raised/enhanced the levels of catalase significantly when compared with rats induced with CCl_4 (table 3). Catalase (CAT) is a hemeprotein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water and oxygen and thus, protecting the cell from oxidative damage by H_2O_2 and OH. In this study, decline in the activities of this enzyme in CCl_4- administered rats revealed that lipid peroxidation and oxidative stress elicited by CCl_4 – intoxication have been decreased due to the effect of extracts of *C. italica* leaf.

Histological examination

Histological examination of the liver sections revealed that the normal liver architecture (fig. 1) was disturbed by hepatotoxin intoxication (fig. 2). In the sections obtained from the rats treated with aqueous and ethanolic leaf extracts of *Cassia italica* and intoxicated with CCl_4, the normal cellular architecture was retained to some extent, thereby confirming the protective effect of the extract. The rats intoxicated with CCl_4 (fig. 2) and subsequently treated with aqueous and ethanolic extracts of *Cassia italica* leaf (200 mg/ kg body weight), appeared to have less damage compared to untreated group indicating some form of restoration to the CCl_4 - induced liver injury (fig. 3 and 4). The results obtained indicate that the plant may be useful in the treatment of jaundice.

Conclusion

Although the extract chemical compound/s responsible for protective effect of leaf extracts still remain/s speculative, experimental evidence obtained in the present study indicates that aqueous and ethanolic extracts from *C. italica* possess to some extend protective properties. This observation lends pharmacological support to the report of folkoric uses of the plant leaves in the management and/or control of jaundice in some parts of the North East of Nigeria.

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