ISSN 1996-3351

Asian Journal of Biological Sciences

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Asian Journal of Biological Sciences 6 (4): 234-238, 2013
ISSN 1996-3351 / DOI: 10.3923/ajbs.2013.234.238

Protective Effect of *Erythrina senegalensis* (DC) Leaf Extract on Carbon Tetrachloride-induced Liver Injury in Rats

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ABSTRACT

The aqueous leaf extract of *Erythrina senegalensis* (ES) was evaluated for its protective activity against carbon tetrachloride (CCl_4)-induced liver injury. A 100, 200 and 400 mg kg^{-1} b.wt. of the ES leaf extract was administered to different groups of rats for 7 days prior to the CCl_4 administration. A significant ($p<0.05$) decrease was observed in both the groups pretreated with 200 and 400 mg kg^{-1} b.wt. of the leaf extract on the levels of the enzymes and non enzyme markers of tissue damage, lipid peroxidation and relative organ weights and this is shown to be dose dependant when compared to rats that were given CCl_4 only. These results showed that ES possess hepatoprotective principle (s) that was (were) able to prevent the toxicity of CCl_4 against the liver of rats.

Key words: *Erythrina senegalensis*, leaf, carbon tetrachloride, hepatotoxicity and protection

INTRODUCTION

Hepatotoxicity resulting from exposure to environmental chemicals is a major global public health problem and a number of hepatotoxicants have been documented (Timbrell, 1991). CCl_4 is a chemical model commonly used for animal experiment to induce reactive oxygen formation and depletion of glutathione, this may reduce antioxidant enzymes as well as the substrate to induce oxidative stress (Dahiru *et al*., 2010). The liver damage is associated with membrane lipid peroxidation and cell necrosis (Williams and Burk, 1990; Obidah *et al*., 2010) which change enzyme activity and finally induce hepatic injury. ES (DC) is a tropical plant of the family fabaceae. The plant has shown to exhibited medicinal potentials in many separate studies (Linuwa *et al*., 1994; Togola *et al*., 2005; Mann *et al*., 2008) and the leaf is also used as vegetable. Some diseases that has been reported by traditional healers to have been treated by the plant include amenorrhea, malaria, jaundice, ulcer, diarrhea, gastrointestinal disorder sterility and wound and body pain (such as chest, back and abdominal pains) (Togola *et al*., 2005).

This study was designed to evaluate the effect of pretreatment with the leaf extract of ES on CCl_4-induced liver injury in rats.

MATERIALS AND METHODS

Plant: ES leaf was collected from uncultivated from farmland in Girei Local Govt. Area of Adamawa state and authenticated in plant sciences Department of Modibbo Adama University of technology yola and given a voucher specimen number WH/ESS015/05. The leaf was dried at room temperature.

Preparation of aqueous extract: Freshly plucked leafs of ES were dried at room temperature and ground to powdered form with laboratory mortar and pestle. The powder was sieved and 150 mg was weighed and mixed with 400 mL of distilled water and allow to stand for 6-12 h with continues shaking at time interval. The mixture was then filtered with Whatman filter paper No. 4. The filtrate was then evaporated using rotary evaporator at room temperature (>50°C).

Animals: Thirty male rats weighing 110-150 g were purchased from the animal house of Biochemistry Department of University of Jos, plateau state Nigeria. The rats were then house in cages at room temperature under 12/12 light/dark and were fed with pelleted standard laboratory feed (Vital feed, Grand cereal and oil Mills Jos) and water *ad libitum*. They were allowed to stand for a period of 7 days to acclimatized before the commencement of the study.

Experimental design: The rats were divided into 5 groups (1-5) o f 6 rats each and were given the extract as follows:

Group 1: (Control)
Group 2: Rats were given single dose of CCl_4+diet/water
Group 3 (treated): Rats were given 100 mg kg^{-1} b.wt. of ES leaf extract+CCl_4+diet/water
Group 4 (treated): Rats were given 200 mg kg^{-1} b.wt. of ES leaf extract+CCl_4+diet/water
Group 5 (treated): Rats were given 400 mg kg^{-1} b.wt. of ES leaf extract+CCl_4+diet/water

Groups 3, 4 and 5 were pretreated with aqueous leaf extract of ES for 7 days prior to CCl_4 administration. The $CCl_4$was dissolved in olive oil and administered intraperitonealy (1:1) 2 mL kg^{-1} b.wt. to induce liver injury.

Collection of samples (serum and liver): Rats from all the different groups were sacrificed 48 h after CCl_4 administration and blood samples were collected via ocular vein and allowed to stand for 7-10 min, it was then centrifuged at 300 rpm for 15 min to obtain serum. This was separated for the estimation of transaminases, alkaline phosphatase and bilirubin.

Liver of the rats were quickly excised, weighed and used for the determination of lipid peroxidation. The hepatic lipid peroxidation was determine as thiobarbituric acid reactive substance (TBARS) and expressed as the amount of malondialdehyde (MDA) (Uchiyama and Mihara, 1978).

Statistical analysis: The data generated was subjected to statistical analysis and the result expressed as Mean+SEM. Student t-test was used to determine the statistical difference between 2 mean values at 95% level of confidence ($p<0.05$).

RESULTS

The result of the pretreatment with the aqueous leaf extract of ES on transaminases, alkaline phosphatase and Total Bilirubin (TB) levels in CCl_4 induced liver injury is shown in Table 1.

The result above demonstrate a trend of increased levels of ALT, AST and ALP significantly ($p<0.05$) in group 2 (group that was administered CCl_4 alone) as compared to the control group (group 1). There was however an observed significant ($p<0.05$) decreased in the groups pretreated with 200 and 400 mg kg^{-1} b.wt. of ES leaf extract for the period 7 days prior to the CCl_4 administered on the levels of the marker enzymes as compared to group ii.

Table 1: Effects of pretreatment with ES aqueous leaf extract against CCl_4 induced liver damage on enzyme and non enzyme markers of liver damage

Group	ALT (μ L^{-1})	AST (μ L^{-1})	ALP (μ L^{-1})	TB (mg dL^{-1})
Control	28.25±2.14	48.14±0.97	58.41±20.7	0.39±0.13
CCl_4	71.40±6.73*	118.42±3.19*	124.62±28.4*	1.84±0.20*
100 mg kg^{-1} b.wt.±ES±CCl_4	42.40±1.66	95.99±3.98	102.49±19.3	0.96±0.15
200 mg kg^{-1} b.wt.±ES±CCl_4	41.67±1.33	72.06±0.80**	86.04±11.9	0.69±0.13**
400 mg kg^{-1} b.wt.±ES±CCl_4	33.20±2.77**	58.46±0.95**	65.84±22.1**	0.51±0.12**

Results are Mean±SEM. (n = 6), *Significantly higher than control group ($p<0.05$), **Significantly lower than group given CCl_4 only

Table 2: Effects of pretreatment with ES aqueous leaf extract against CCl_4 induced liver damage in liver lipid peroxidation

Group	MDA (mmoles mg^{-1} protein)
Control	35.45±4.22
CCl_4	61.52±3.68*
100 mg kg^{-1} b.wt.±ES±CCl_4	58.22±3.72
200 mg kg^{-1} b.wt.±ES±CCl_4	56.56±4.15
400 mg kg^{-1} b.wt.±ES±CCl_4	45.62±4.36**

Results are Mean±SEM. (n = 6), *Significantly higher than control group ($p<0.05$), **Significantly lower than group given CCl_4 only

Table 3: Effects of pretreatment with ES aqueous leaf extract against CCl_4 induced liver damage on relative organ weight

Group	Mean final body weight (g)	Relative organ weight(g/100 g b.wt.)
Control	223.45±5.61	1.45±0.17
CCl_4	214.25±6.17	2.27±0.13
100 mg kg^{-1} b.wt.±ES±CCl_4	224.65±4.98	2.29±0.14
200 mg kg^{-1} b.wt.±ES±CCl_4	220.53±5.78	2.19±0.12
400 mg kg^{-1} b.wt.±ES±CCl_4	221.34±5.89	1.97±0.13**

Results are Mean±SEM. (n = 6), **Significantly lower than group given CCl_4 only

Also the decrease in the pretreated groups was observer to be dose dependent as group 4 (400 mg kg^{-1} b.wt.) indices of the tissue damage was observed to be decreased significantly ($p<0.05$) than group 3 and 4 (100 and 200 mg kg^{-1} b.wt., respectively).

There is a significant ($p<0.05$) increase in bilirubin concentration in group administered CCl_4 alone as compared to control (group i). However pretreatment with 200 and 400 mg kg^{-1} b.wt. of ES leaf extract prior to CCl_4 administration results in significant ($p<0.05$) decrease in the level of bilirubin concentration as observed in groups iv and v as compared to group administered CCl_4 alone.

Table 2 shows the effects of pretreatment of with ES leaf extract on lipid peroxidation. The levels of MDA in the group treated with CCl_4 alone increased significantly ($p<0.05$) when compared to the control group, however the is a significant ($p<0.05$) decreased as observed in the pretreated groups and it dose dependent manner.

From Table 3, the result shows that pretreatment with the aqueous leaf extract resulted in a significant ($p<0.05$) increase in the relative organ weight when compared to normal. The Mean final body weight is not however affected by either the CCl_4 or the extract administration.

DISCUSSION

Hepatotoxicity was observed in rats treated with CCl_4 alone, this is an experimental model widely used for hepatoprotective drug screening, as shown by the increased serum levels of the transaminases, this may reflect the cytosolic release of liver marker enzymes into serum that results

from the necrotic and degenerative response of hepatocytes (Chawla, 1999) following the CCl_4 administration. The marker enzymes assay plays a significant role in diagnosis of diseases, investigation and assessment of drugs or plant extract for safety and toxicity (Adeoyo and Oyedepo, 2004).

CCl_4 undergoes a biotransformation by hepatic microsomal cytochrome P_{450} to produce a metabolite trichloromethyl free radicals, this hepatotoxic metabolite can react with protein and lipid in the membrane of the cells or organelle leading to necroses of hepatocytes and as a result of the injury, the altered permeability of the membrane causes the enzymes from the cells to be released into the circulation (Drotman and Lawhorn, 1978). The observed significant increase in the serum levels of ALP activity and total bilirubin concentration further demonstrate hepatoxicity in the rats.

Pretreatment with leaf extract of *E. senegalensis* protect the organ against CCl_4 induced hepatotoxicity as demonstrated by significant decrease in the levels of the liver marker enzymes and other biochemical indices.

The magnitude of hepatic damage is usually assessed by measuring the level of release of cytosolic transaminases (AST, A LT) in circulation (Perez Gutierrez and Solis, 2009). Thus, the observed increase in the levels of these transaminases in group 2 could be attributed to the damage caused by CCl_4 on the structural integrity of the liver, as reported by other researchers (Dahiru *et al*., 2005, 2010; Galati *et al*., 2005; Perez Gutierrez and Solis, 2009). Since they are located in the cytoplasm and are released into circulation after damage.

Increase in bilirubin concentration is an index of jaundice, a condition of liver injury, possibly due to increase production or decrease uptake by the liver, decreased conjugation and decreased secretion from the liver or blockage of bile duct (Bun *et al*., 2006). This was observed in group 2 after CCl_4 administration as an indication of hepatoxicity.

There was an observed significant elevation in the levels of MDA in the CCl_4-treated group. The elevated levels could be seen to be elicited by the administration of a toxicant, CCl_4 which is known to increase lipid peroxidation. However pretreatment with *E. senegalensis* leaf extract indicated an effective protection of the animals against CCl_4 induced liver lipid peroxidation. Consumption of the extract may be seen to decrease liver organ susceptibility to lipid peroxidation and invariably oxidative stress.

Also pretreatment with the leaf extract protect the liver against change in relative organ weight which normally reflects the pathological state of the organ. The relative weights were observed to consistent with the serum levels of the marker enzymes and MDA.

These findings further demonstrated that from the result presented above and earlier reports by other researchers that CCl_4 causes mononuclear cell infiltration, necrosis and degeneration of hepatocytes (Hung *et al*., 2006; Liu *et al*., 2006), the leaf extract of *E. senegalensis* have the capability of protecting the liver against CCl_4 induced hepatoxicity as well as cellular degeneration and fatty liver development. Thus the leaf extract could be seen to block or minimize to a greater extend adipogenesis.

CONCLUSION

From the results obtained in this study, it is clear that the leaf extract of *E. senegalensis* has a potent hepatoprotective agent against CCl_4 induced liver damage in rats.

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