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Protective Effects of Abrus precatorius Leaf Extract against Carbon Tetrachloride- Induced Liver Injury in Rats

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Abstract
The study was carried out to evaluate the protective effects of aqueous leaf extract of *Abrus precatorius* against carbon tetrachloride (CCl_4)- induced liver injury in rats. 100, 200 and 400 mg/kg b.wt. of *Abrus precatorius* extract was administered orally to separate groups of rats for 7days prior to CCl_4 administration. A significant decrease ($p<0.05$) was observed in both the groups treated with 200 and 400 mg/kg b.wt. of the leaf extract on the levels of the enzymes and non-enzyme markers of liver injury and lipid peroxidation as well as relative organ weight, with no significant changes in the Mean final body weight of the treated groups. This result shows that the leaf extract of *Abrus precatorius* contains phytochemical(s) that is (are) protective to the liver against CCl_4-induced injury in rats
Keywords: *Abrus precatorius,* carbon tetrachloride, hepatotoxicity, liver, rats.

1. Introduction

The liver is a highly specialized organ that regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions in humans(Maton et al 1993), it also plays a central role in transforming and clearing chemical agents and is susceptible to the toxicity from these agents. Liver damage is associated to membrane lipid peroxidation and cell necrosis (William and Burk 1990; Obidah et al 2010) which changes enzyme activity and finally induced hepatic injury. Other chemical agents, such as those used in laboratories (e.g. CCl_4) and industries, natural chemicals (e.g. microcystins) as well as herbal remedies can also induce hepatotoxicity.

More than 900 drugs have been implicated in causing liver injury (Jaeschke et al 2002) and a study in USA showed that drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Ostapowicz et al 2002).

Thus hepatotoxicity and drug-induced liver injury account for a substantial number of compound failures, highlighting the need that search for new drugs is gaining grounds thus the search for new and effective antioxidants in plants has given a new dimension to the antioxidant research (Guzdek and Nizankowska 1996).

The liver needs to be protected, using cheaper and affordable compounds with minimal health hazard. Medicinal plants are cheaper and more accessible to most of the population in the world, and despite the advances in Western medicine, traditional medicine has gained renewed interest in the health care services throughout the continents, as many diseases have been reported by traditional healers to have been treated with medicinal plants (Wakawa and Musa 2013). This could probably be due to the rapidly increasing awareness of the potential and curative abilities of alternative medicines, especially from the use of medicinal plants, therefore there is need to encourage the use of medicinal plants as potential sources of new drugs.

In recent times more attention has been paid to the protective effects of natural antioxidants against drug-induced toxicities (such as CCl_4) especially whenever free radical generation is involved (Wakawa and Hauwa 2013), and several plants and other animal products have been shown to protect the liver against such damages (Dahiru et at 2005; Hung et al 2006 Obidah et al 2010; Dahiru et at 2005; Hamad et al 2011; Khan and Alzohairy 2011; Al-Fartosi et al 2012). Also medicinal plants are used in preparations of herbal remedies and these herbal remedies which are perceived to be a cheaper means of treatment have often attained popularity for historic and cultural purposes (WHO 1999), thus a large number of the world's population depends on plants to treat many common ailments (Shri 2003).

A. precatorius Linn, is one of the medicinal plants that have received attention both in treatment of many diseases and use as a vegetable and artifacts in many cultures, and is widely found in Africa, India and many other parts of the world. The leaves have been employed to sweeten foods and certain medicines used for stomach complaints, to treat fevers, cough and cold (used as decoction). The leaves are casually chewed and the vine sometimes sold as a masticatory in Curacao (Morton 1981; Irvine 1961)

2. MATERIALS AND METHODS

2.1. Chemicals

Diagnostic kits for serum alanine aminotransferase (ALT) and aspartate amino transferase (AST), alkaline phosphatase (ALP) and billirubi were purchased from Randox Laboratories Ltd. and other chemicals and solvents were of highest grade commercially available.

2.2 *Abrus precatorius* leaves

Freshly harvested leaves of *A. precatorius* was used for the preparation of the crude extract. It was collected from an uncultivated farm land in Girei LGA of Adamawa State-Nigeria. It was authenticated in the plant science department of Modibbo Adama University of Technology Yola and given a voucher specimen number WH/APL015/06, it was dried under room temperature.

2.3 *Drug preparation*

The freshly dried leaves of *A. precatorius* was grounded into fine powdered form using laboratory mortar and pestle and electric blender. 150mg of the powdered leaf was weighed into a beaker and mixed with 400ml of distilled water and allow to stand for 12hr with continues shacking at time interval. The mixture was then filtered using Whatman filter paper No.4 and the filtrate was concentrated using water bath a 50^{o}C. It was then stored under frozen condition until use.

2.4. Breeding of Animals

A total number of twenty five (25) male albino rats weighing between 90-150kg were purchased from the animal farm, National Veterinary Research Institute Vom, Jos Plateau state-Nigeria. They were housed in cages at room temperature under 12/12 night/dark and were fed with pelleted standard laboratory feed (Vital Feeds, Grand cereals and oil mills Jos) and water *ad libitum.* They were allowed to stay for 7days to acclimatize before the commencement of the work.

2.5. Experimental protocol

The rats were randomly divided into five (5) groups of five (5) rats per group 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 100mg/kg b.wt. Leaf extract + **CCl_4** +diet/water
Group 4: (treated). Rats were given 200mg/kg b.wt. Leaf extract + **CCl_4** +diet/water
Group 5: (treated). Rats were given 400mg/kg b.wt. Leaf extract + **CCl_4** +diet/water

Groups 3,4 and 5 were pre-treated with the aqueous leaf extract of ***A. Precatorius*** for 7days prior to **CCl_4** administration.

The **CCl_4** was dissolved in olive oil and administered intraperitoneally (1:1) 2mL/kg b. wt. to induce liver injury

2.6. Blood and tissue Collection

All the rats from the various groups were sacrificed 48hr after the **CCl_4** administration using standard laboratory procedures and then blood samples were collected via ocular vein into clean containers and allow to stand for 10min. It was then centrifuged at 3000rpm for 15min to obtain serum. The serum was then separated for the estimation of liver marker enzymes (transaminases and alkaline phosphatase) and total bilirubin.

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

2.7. Statistical analysis

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

3. RESULTS

The result of the effect of pretreatment against CCl_4-induced liver damage in rats with aqueous leaf extract of *Abrus precatorius* was examined on serum ALT, AST, ALP and TB levels as shown in Table 1

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

Group	ALT (µ/L)	AST (µ/L)	ALP (µ/L)	TB (mg/dL)
1	21.67 ± 2.76	39.33 ± 4.37	218.4 ± 11.5	14.80 ± 0.69
2	37.50 ± 8.57*	60.06 ± 9.35*	245.6 ± 13.62*	22.30 ± 0.12*
3	35.73 ± 3.21	58.83 ± 7.25	239.2 ± 28.70	19.88 ± 0.84
4	30.65 ± 2.65	52.56 ± 1.32**	232.2 ± 15.60**	19.65 ± 0.65
5	25.50 ± 2.10**	47.33 ± 1.24**	226.5 ± 37.90**	16.82 ± 0.87**

Results are Mean ± SEM (n= 5), *significantly higher than control group ($p<0.05$)
**significantly lower than group induced with CCl_4

The result in Table 1 showed a significant increase ($p<0.05$) in the serum levels of ALT, AST and ALP in group 2 (CCl_4 group) as compared to group 1 (control). However there was an observed significant decrease ($p<0.05$) in the 200 and 400 **mg/kg b.wt. extract treated groups prior to the** CCl_4 administration as compared to group 2 which was observed to be dose dependent.

The result also show a significant increase ($p<0.05$) in the level of bilirubin concentration in group 2 (CCl_4 group) as compare to group 1 rats. However the pretreated group (400 **mg/kg b.wt. extract) showed a** corresponding decrease in the level of the bilirubin concentration as compared to group 2.

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

Group	MDA (mmoles/mg)
1	38.67 ± 2.76
2	61.50 ± 8.57*
3	58.73 ± 3.21
4	45.65 ± 2.65**
5	42.50 ± 2.10**

Results are Mean ± SEM (n= 5), *significantly higher than control group ($p<0.05$)
**significantly lower than group induced with CCl_4

The result in Table shows the effect of pretreatment with the leaf extract on lipid peroxidation. It showed a significant increase in the MDA levels in group 2 as compared to group 1 (control) with a corresponding decrease in the pretreated groups (3 and 4)

Table 3: Effects of pretreatment with 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)
1	232.67 ± 2.76	1.63 ± 4.37
2	221.50 ± 8.57	2.28 ± 9.35*
3	234.73 ± 3.21	2.19 ± 7.25
4	231.65 ± 2.65	2.21 ± 1.32
5	235.50 ± 2.10	2.16 ± 1.24**

Results are Mean ± SEM (n= 5), *significantly higher than control group ($p<0.05$)
**significantly lower than group induced with CCl_4

The result in Table 3 showed a relative increase in organ weight in the CCl_4 group as compared to group 1 and a relative increase in the mean final weight of the pretreated groups as compared to the CCl_4 group. planning is barely seen. As the gap in the literature is addressed, this paper intends to formulate a holonic model called Workforce Sizing Plan (WOZIP), which is particularly suitable for job-shop production.

4. DISCUSSION

Hepatotoxicity resulting from exposure to environmental chemicals is now a major global public health problem. CCl_4 is a chemical model commonly used in laboratories for animal studies to induce reactive oxygen formation and depletion of gluthathione, this may reduce antioxidant enzymes as well as the substrate to induce stress (Dahiru et al 2010). The liver damage is associated with membrane lipid peroxidation and cell necrosis (William and Burk 1990; Obidah et al 2010) which changes enzyme activity and finally induce hepatic injury (Wakawa and Musa 013).

The magnitude of hepatic damage is usually assessed by the measure of the levels of the cytosolic transaminases in circulation (Perez and Solis 2009) and from the result obtained in this study, hepatotoxicity was observed in the rats treated with CCl_4 as shown by the increase in the levels of the serum marker enzymes of hepatic injury (transaminases), this could be attributed to the damage caused by CCl_4 on the structural integrity of the liver (Dahiru et al 2005; Galati et al 2005; Dahiru et al 2010; Perez and Solis 2009) being cytosolic enzymes and are released into circulation after damage. These enzyms play a significant role in diagnosis of

diseases, investigations and assessment of drugs or plant extracts for safety and toxicity (Adeoye and Oyedepo 2004), and the increase in serum concentration reflects the cytosolic release that might have been as a result of the necrotic and degenerative response of hepatocytes as a result of the CCl_4 administration.

Several studies have provided a considerable support for evidencing the protective effects of medicinal plants (Dahiru et at 2005; Dahiru et al 2010; Hung et al 2006; Obidah et al 2010) and camel milk on liver damage (Hamad et al 2011; Khan and Alzohairy 2011; Al-Fartosi et al. 2012). Also, these studies declared that the protective effect of camel milk against CCl_4-induced oxidative stress in the rat is due to its antioxidant properties.

From the result obtained in this study, it was observed that pretreatment with the extract of *A. Precatorius* presented a relative difference in the concentration of the marker enzymes as compared to the CCl_4 treated group.

The difference in the results was also observed on the bilirubin concentration in pretreated groups as compared to the CCl_4 group which tend to show an elevated concentration, which is an index of jaundice (a condition indicating liver injury), possibly due to either increase production or decrease uptake by the liver, decrease conjugation and secretion from the liver or blockage of bile duct which might be as a result of CCl_4 administration.

The result of the study also presented a relative difference in the levels of MDA in the pretreated groups as compared to the CCl_4 group, which was observed to be elevated after CCl_4 administration, a toxicant that is known to increase lipid peroxidation. There was also an observed difference in the relative organ weight, a condition that normally reflect the pathological state of the liver.

The findings in this study revealed that the leaves of *A. Precatorius* provided a protection to the liver against hepatotoxicity due to CCl_4 administration, as demonstrated by the significant difference in the levels of the marker enzymes, the non enzyme marker, bilirubin, as well as the decrease liver susceptibility to lipid peroxidation and invariably oxidative stress as seen in the marked levels of MDA in the pretreated groups as compared to the CCl_4 induced liver damage group.

Thus the leaves of *A. Precatorius* can be said to possess phytochemicals that have the capability of protecting the liver against CCl_4 induced hepatotoxicity as well as cellular degeneration and fatty liver development.

5. Conclusion

The findings of this study suggest that the leaves of *A. Precatorius* could block or minimized adipogenesis to a greater extend thus having a potent hepatoprotective agent against CCl_4 induced liver damage in rats.

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