EFFECT OF *Azima tetracantha* ON LIVER MARKERS OF CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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Abstract : In this study protective activity of ethanol extract of *Azima tetracantha* leaves (ATL) was investigated using carbon tetrachloride (CCl_4)-intoxicated rat liver as the experimental model. In the present study, the elevated activity of ALT, AST, ACP, ALP, GGT, LDH, and content of Bilirubin were observed in the animals treated with CCl_4. Administration of *Azima tetracantha* leaf extract at the dose of 100, 200 and 400mg per kg body weight shows dose dependent decrease in the activity of liver marker enzymes. Among the various doses, 400mg/kgbw showed the significant effect that was observed when compared with other doses (100 and 200mg/kgbw). The ATL extract exhibited significant hepatoprotection against CCl_4 induced liver injury in rats through antioxidant potential and free radical scavenging activities.

Keywords: Carbon tetrachloride, *Azima tetracantha* leaves, Liver marker enzymes

INTRODCUTION

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world over including those in developed countries turning towards complementary and alternative medicine (CAM). Many traditional remedies employ herbal drugs for the treatment of liver ailments (Wolf, 1999). To the best of our knowledge, there is no scientific report available in support of the hepatoprotective activity of *Azima tetracantha* leaves extract. Hence, to justify the herbal claims we have evaluated the hepatoprotective effects of *Azima tetracantha* leaves on CCl_4 induced hepatotoxicity in rats. The hepatoprotective activity of *Azima tetracantha* leaves reported in this study would provide scientific evidence of its claimed medicinal properties.

It is well established that CCl_4 induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome *P*450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($^{\bullet}CCl_3$). Trichloromethyl free radical then combine with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethylperoxyl free radical leads to initiate the process of lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally, results in cell death (De Groot and Noll, 1986; Clawson, 1989; Reckengel *et al*., 1989). These result in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver damage (Recknagel and Glende, 1973; Reckengel *et al*., 1991; Wolf *et al*., 1980). MDA is a secondary product of lipid peroxidation is used as an indicator of tissue damage by series of chain reactions (Rayg and Husain, 2002). Hepatotoxic compounds like CCl_4 are known to cause marked elevation in serum enzyme activities.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately 3-4 months young (weighing approximately 140-160g) and 24-26 months old ones (weighing approximately 380-410g) were used in this study. They were healthy animals procured from Sri Venkateswara enterprises, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºC and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water *ad libitum*. They were acclimatized to the environment for 1 week prior to experimental use. The experiment was carried out according to the guidelines of the Committee (Ethical No: SAC/IAEC/BC/2016/Ph.D-005) for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals

Carbon tetrachloride (CCl_4), Nitro blue tetrazolium (NBT), ethylene diamine tetraacetic acid (EDTA), trichloro acetic acid (TCA), thiobarbituric acid (TBA), 1-chloro-2,4-dintiro benzene (CDNB), 5,5'-dithio-bis (2-nitrobenzoic acid), glutathione (reduced), glutathione (oxidized), Carbon tetrachloride and L-ascorbic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Plant Material

The fresh leaves of *A. tetracantha* were collected in the month of January 2015 at Melur, Thiruchirappalli District, Tamil Nadu, South India. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rabinat Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen (EP001) has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

Preparation of Plant Extract

The collected plant materials were washed, sliced and completely dried in a hot-air oven at 37°C. The dried materials was ground into make a fine powder and used for extraction. Three hundred grams (300g) of the powered plants were extracted with hexane, chloroform and ethanol using "Soxhlet Apparatus" for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The extract contains both polar and non-polar phytocomponents. For experiments 500mg/kg body weight of *Azima tetracantha* extract (ATE) was used. This effective dose was selected based on dose dependent studies of ATE carried out in our laboratory.

Experimental Design

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows.

Group I – Normal Rats

Group II – Negative control - Animals were administrated orally with CCl_4 (0.5 ml/150g of bw-v/v in olive oil) on 1st, 8th and 16th day.

Group III – Animals were administrated orally with CCl_4 (0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th day) and treated with selected plant extract (100mg/ Kg BW) orally for 21 days.

Group IV - Animals were administrated orally with CCl_4 (0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th day) and treated with selected plant's extract (200mg/ Kg BW) orally for 21 days.

Group V –Animals were administrated orally with CCl_4 (0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th day) and treated with selected plant's extract (400mg/ Kg BW) orally for 21 days

Group VI –Animals were administrated orally with CCl_4 (0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th day) and treated with Silymarin (20mg/ Kg BW) orally for 21 days

Collection of Samples

After completing the experimental period, rats were killed by decapitation. Plasma was separated. The biochemical parameters were analyzed.

Biochemical parameters

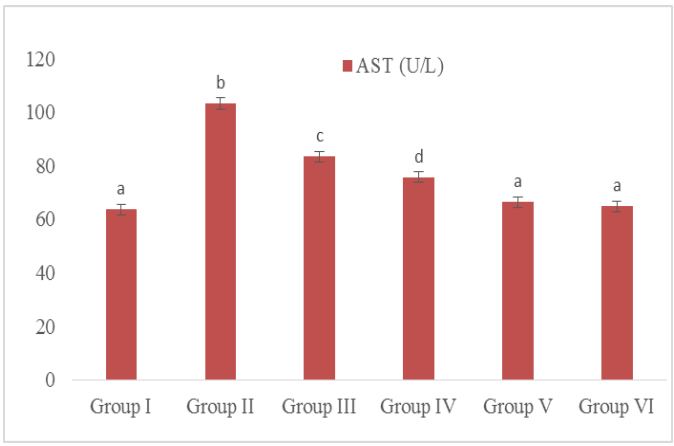
The serum AST, ALP and ALT levels were assayed using the method of King (1965). Acid phosphatase activity was measured by the method of Annon (1963). The serum γ GGT levels were assayed using the method of Rosalki and Rau (1972). The assay of lactate dehydrogenase according to the method of King (1965) and Bilirubin was estimated in serum by the method of Malloy (1937).

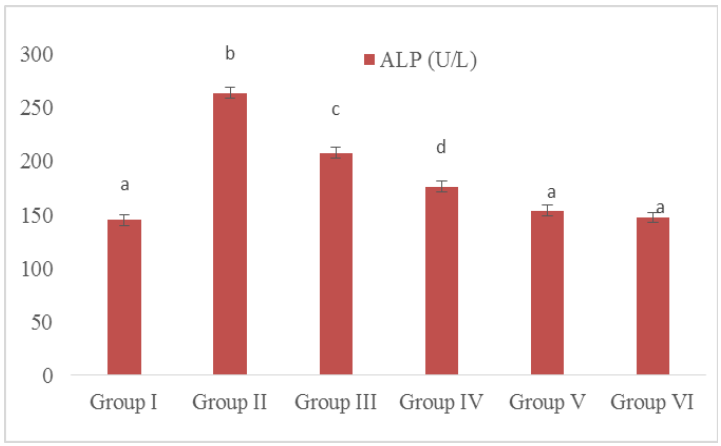
RESULTS AND DISCUSSION

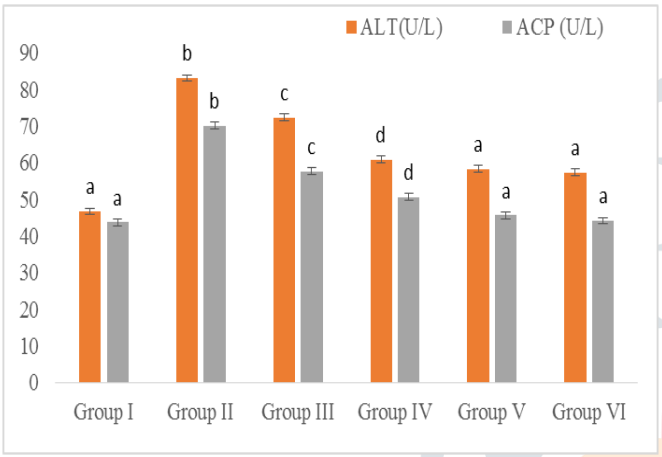
Effect of *Azima tetracantha* on liver markers in control and experimental rats

Fig 1 and 2 represents the effect of *Azima tetracantha* on liver markers in control and experimental rats. In the present study, the elevated activity of ALT, AST, ACP, ALP, GGT, LDH, and content of Bilirubin were observed in the animals treated with CCl_4. Administration of *Azima tetracantha* leaf extract at the dose of 100, 200 and 400mg per kg body weight shows dose dependent decreased in the activity of liver marker enzymes. Among the various doses, 400mg/kgbw showed the significant effect that was observed when compared with other doses (100 and 200mg/kgbw). The ATE extract exhibited significant hepatoprotection against oxidative stress induced liver injury by CCl_4 in rats through antioxidant potential and free radical scavenging activities.

Ample experimental and epidemiological studies support the involvement of oxidative stress in the pathogenesis and progression of several chronic diseases (Tewari *et al*., 2000). It is now known that oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as reactive oxygen species (ROS). The high reactivity of ROS may trigger a host of disorders in body resulting in tissue damage and necrosis in many instances (Prasad Varier *et al*., 1999). It has been hypothesized that one of the principal causes CCl_4- induced liver injury is LPO by free radical derivatives of CCl_4. Thus the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 –induced hepatopathy (Castro *et al*., 1974). CCl_4 mediated oxidative stress was taken here as the experimental model for hepatotoxicity and oxidative stress.







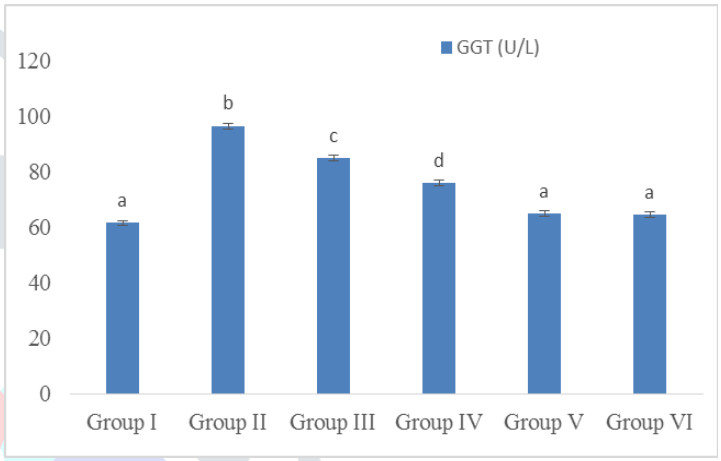
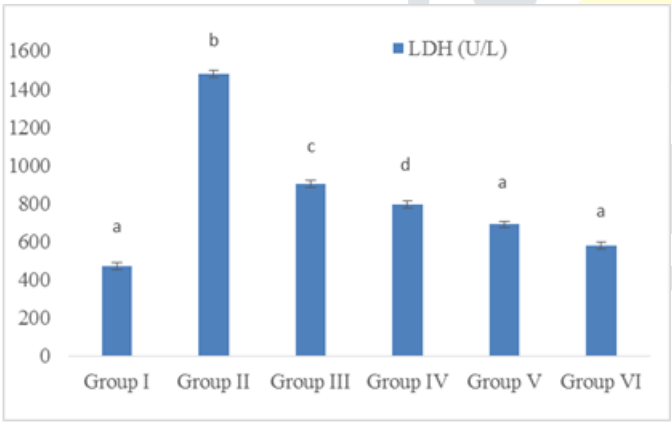


Fig 1 Effect of *Azima tetracantha* on liver markers in control and experimental rats



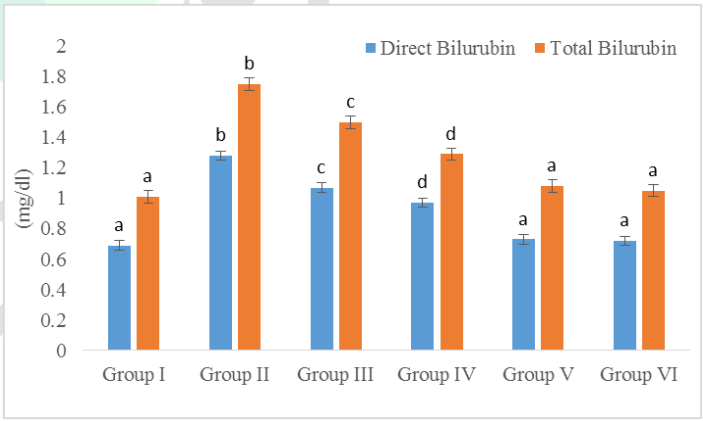


Fig 2 Effect of *Azima tetracantha* on liver markers in control and experimental rats

The diagnosis of organ disease / damage is aided by measurement of a number of non-functional serum enzymes characteristic of that tissue or organ. The amount of enzyme released depends on the degree of cellular damage, the intracellular concentration of the enzymes and the mass of affected tissue. The concentration of the enzymes released reflects the severity of the damage. In the assessment of liver damage by carbon tetrachloride, the determination of enzyme activities such as aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) is largely used. Serum activities of AST, ALT and alkaline phosphatase (ALP) are the most frequently utilized indicators of hepatocellular injury. Necrosis or membrane damage releases the enzymes into circulation; and therefore, they can be measured in serum. ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver (Wolf, 1999). The mechanism by which alkaline phosphatase reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions and the other hypothesis is that the damaged liver fails to excrete alkaline phosphatase made in the bone, intestine and the liver (Thapa and Walia, 2007). Serum total protein, albumin and bilirubin levels, on other hand, are related to the function of hepatic cells i.e they reveal the functional status of the hepatic cells. Decreased levels of total protein and albumin are indicative of the failure of the biosynthetic function of the hepatocyte, while increased levels of bilirubin indicate defective hepatocellular uptake, conjugation and excretion of bilirubin due to the failure of hepatic cell function (Crawford, 2004).

ALT, AST, ACP, ALP, GGT and LDH are enzymes normally present in the liver, heart, muscles and blood cells. They are basically located within hepatocytes. So when liver cells are damaged or die transaminases are released into blood stream, where they can be measured. They are therefore of index of liver injury (Vasudha *et al*., 2006). Free bilirubin is not water-soluble and must be bound to albumin to facilitate transport to the liver. Total serum bilirubin concentrations indicate the functional transport capacity of the liver. The degree of increase in serum bilirubin values has prognostic significance of liver injuries (Dickson *et al*., 1989). Administration of *Azima tetracantha* leaf extract to CCl_4 intoxicated rats restored the activities of ALT, AST, ACP, ALP, GGT, LDH, and content of Bilirubin offering the maximum hepatoprotection with respect to different liver marker enzymes. This confirms the liver protective activity of *Azima tetracantha* leaf extract. Further, *Azima tetracantha* leaf has significantly increased the level of liver protein, which indicates hepatoprotective activity.

CONCLUSION

All the variables tested as ALT, AST, ACP, ALP, GGT, LDH, and content of Bilirubin recorded a significant alteration observed in CCl_4 treated rats. However treatment with Azima tetracantha leaf extract restored the level to near normal was observed. The potential hepatoprotective activity of *Azima tetracantha* leaf extract is due to the presence of phytochemical constitution present in plant. Some of these phytochemical such as flavonoids and polyphenolic compounds have possessed hepatoprotective activity.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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