Hepatoprotective Effect of Aqueous Extract of Water Leaf (Talinum Triangulare) on Carbon tetrachloride (CCL4) Induced Liver Damage in Wistar Rats

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Abstract
The experiment was designed to study the hepatoprotective effects of aqueous water leaf extract on the liver following Carbon tetrachloride (CCl4) induced damage in wistar rats. Twenty-five wistar rats were divided into five groups of five (5) rats each. The first group served as control, group B rats were given 0.5 ml/kg of CCl4, groups C, D, and E, received 200mg/kg, 400mg/kg and 600mg/kg of the aqueous extract of Talinium triangulare respectively and 0.5ml/kg of CCl4 i.p concurrently once daily for seven days. Our findings reveal a dose dependent suppression of oxidative damage in the liver cells following the administration of aqueous extracts of T. triangulare at high doses (P<0.05). There are reductions in the serum hepatic marker enzymes while tissue histology showed some improvement in the state of the injured liver cells. These suggest that diets supplemented with T. triangulare will improve hepatoprotection against oxidative liver damage.

INTRODUCTION
The prevalence of hepatopathies in the world indicates an increased incidence rate with severe consequences on the liver. The pathogenesis of liver fibrosis is not clear, but there is no doubt that reactive oxygen species (ROS) plays an important role in the pathological changes in the liver in alcoholic and toxic liver disease (Poli and Parola, 1997). The peroxidation of unsaturated fatty acids in the biological membrane gives rise to a disruption of membranes’ integrity and function which is implicated in serious pathological changes (Halliwell, 1987). Several endogenous protective mechanisms have been evolved to limit ROS and the damage caused by them (Sies, 1993). However, this protection may not be complete, hence, ROS becomes excessive.

Many natural agents are known to possess antioxidant properties and have been proposed in the treatment and prevention liver diseases or damage induced by oxidative stress (Sieber, 1997). There are also increasing evidence for the hepatoprotective activities of hydroxyl and polyhydroxyl- organic compounds found in vegetables, fruits and some herbs,(Bass, 1999). Preliminary phytochemical studies on Talinium triangulare (water leaf) found mostly in the southern part of Nigeria reveals the presence of Omega -3 fatty acid and high levels of nutritionally important vitamins (such as C, E ,and betacarotene), minerals (such as calcium, magnesium and potassium) and soluble fibres (pectin) all of which contributes to its highly elevated antioxidant values (Ezekwe et al ,2004). Other phytochemicals present in the plant are saponins, phytic acid, tannic acid and oxalate (Akindahunsi and Salawu, 2005).

The current study was designed to examine the hepatoprotective effect of aqueous extract of the leaves of Talinium triangulare against CCl4- induced hepatotoxicity in the Wistar rats animal model.

MATERIALS AND METHODS
T. triangulare were bought from the general market, Ilorin and were authenticated at the Department of Plant Biology, University of Ilorin. The leaves were washed in distilled water, shack dried and pulverized in 100grams of powdered material was soaked in 100mls of distilled water (1:10w/v), poured in a conical flask, agitated vigorously for 5 minutes and kept for 12hours. The mixture was filtered and evaporated to dryness at room temperature. The resultant powder was stored in the refrigerator for the study.
ANIMALS AND ADMINISTRATION

Twenty five male wistar rats weighing between 150- 200g were obtained from the animal house unit of the Department of Anatomy, University of Ilorin. The animals were maintained under standard laboratory conditions with commercial pellet diet and water given ad libitum. The animals were randomly divided into five groups of five rats each. Group A was kept on normal diet and served as control. Group B received distilled water orally and CCl4 (0.5ml/kg;i.p,) once daily for seven days. Group C received extract concentration 200mg/kg, orally and 0.5ml/kg of CCl4 intraperitoneally concurrently, for seven days. Group D received 400mg/kg of the aqueous extract orally and 0.5 ml/kg of CCl4 intraperitoneally concurrently for seven days. Group E received 600 mg/kg of the aqueous extract orally and 0.5 ml/kg of CCl4 intraperitoneally concurrently for seven days. The procedure was carried out as approved by the Animal Ethics Committee of the University of Ilorin for use of animals in experiments.

The animals were anaesthetized on the 8th day and blood was collected by direct cardiac puncture. Serum was obtained by centrifugation at 3000rpm for 10minutes and was analyzed for the activities of marker enzymes alkaline phosphate (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using standard assay kits (Randox Lab Ltd. UK.). The livers were removed, fixed in 10% formalin and processed for paraffin embedding using the standard micro technique (Galozhger and Kocloff, 1971). Sections of the liver (5µm) were stained with alum haematoxylin and eosin and observed with the light microscope for histopathological changes.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the student t-test and thereafter one-way analysis of variance was determined. The level of significance was set at p<0.05. The result are presented as the mean ± SEM.

RESULTS

Administration of CCl4 for seven days led to significant (p<0.05) increases in the serum alkaline phosphates, alanine aminotransferase and aspartate aminotransferase which were significantly reduced in the groups treated with higher concentration of the extract. Histopathological examination of the liver section of the rats in the CCl4 treated group revealed an intense distortion of the hepatic architecture. The groups treated with 600mg/kg of the extract showed signs of protection against toxicity evident from reduced i, fatty degeneration, ii, necrosis and iii, vacuoles. The signs of hepatoprotection were not evident in the groups treated with the low doses.

DISCUSSION

Carbon tetrachloride (CCl4) is one of the most frequently
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used hepatotoxins in experimental studies of liver diseases (Uma and Rao, 2005). The hepatotoxic effects of CCl4 are largely due to its active metabolites, trichloromethyl radical. These activated radicals bind to micromolecules and reduce lipids peroxidative degradation of polyunsaturated fatty acids. This leads to the formation of lipid peroxides which in turn gives products like malonylaldehyde that cause damage to membranes (Ulicna et al 2003). This lipid peroxidative degradation of biomembranes is one of the main causes of hepatotoxicity by CCl4. this is evidenced by a rise in the serum marker enzymes namely alkaline phosphates (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). T. triangulare administration at 200mg/kg concentration did not significantly reduce the levels of ALP, AST and ALT but at higher concentrations, it significantly reduced these levels at which levels of the marker enzymes fell, indicating hepatoprotective activity.

In the study, an elevation in the levels of the end products of lipids peroxidation of the liver of rats treated with CCL4 was observed. The increase in ALP, AST and ALT suggests enhanced lipid peroxidation giving rise to liver damage and failure of the antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with T. triangulare reversed the changes (p<0.05) at high concentrations, hence it is possible that the mechanism of hepatoprotection of T. triangulare is due to its antioxidant effect. Histological studies showed minimal reduction in steatosis and degeneration of the liver tissue with treatment. This suggests that physiologic recovery preceded obvious histological changes. These results suggest that diets supplemented with T. triangulare will improve hepatoprotection against oxidative liver damage.
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