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Hepatoprotective and antioxidant effect of tender coconut water on carbon tetrachloride induced liver injury in rats

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Hepatoprotective and antioxidant effects of tender coconut water (TCW) were investigated in carbon tetrachloride (CCl_4)-intoxicated female rats. Liver damage was evidenced by the increased levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and decreased levels of serum proteins and by histopathological studies in CCl_4-intoxicated rats. Increased lipid peroxidation was evidenced by elevated levels of thiobarbituric acid reactive substance (TBARS) viz, malondialdehyde (MDA), hydroperoxides (HP) and conjugated dienes (CD), and also by significant decrease in antioxidant enzymes activities, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx) and glutathione reductase (GR) and also reduced glutathione (GSH) content in liver. On the other hand, CCl_4-intoxicated rats treated with TCW retained almost normal levels of these constituents. Decreased activities of antioxidant enzymes in CCl_4-intoxicated rats and their reversal of antioxidant enzyme activities in TCW treated rats, shows the effectiveness of TCW in combating CCl_4-induced oxidative stress. Hepatoprotective effect of TCW is also evidenced from the histopathological studies of liver, which did not show any fatty infiltration or necrosis, as observed in CCl_4-intoxicated rats.

Keywords: Hepatoprotective effect, antioxidant effect, tender coconut water, carbon tetrachloride-intoxication, rats

Liver injury induced by viruses, chemicals and drugs is a well recognized toxicological problem. The hepatotoxicity possibly results from a toxic intermediary that binds covalently to hepatocytes and causes a centrilobular hepatic necrosis. Alternate explanations of necrosis are lipid peroxidation and oxidation of thiol group[1]. One of the major causes of carbon tetrachloride (CCl_4)-induced hepatopathy is lipid peroxidation by its free radical derivative, $CCl_3^{\cdot}$ (ref. 2). Antioxidant activity or inhibition of generation of free radicals plays a crucial role in providing protection against such hepatic damage[3-7]. The tender coconut water (liquid endosperm) is one of the nutritious wholesome beverage that the nature has provided for the people of tropics. Numerous medicinal properties of tender coconut water (TCW) have been reported[8]. It is good for feeding infants suffering from intestinal disturbances, used as oral rehydration medium, contains organic compounds possessing growth-promoting properties and is useful in malnutrition (contains adequate potassium and glucose content, but is relatively deficient in sodium, chloride and bicarbonate[9] and is useful malnutrition. It is also effective in the treatment of kidney and urethral stones, urinary infections and an antidote for mineral poisoning[8]. It has also shown significant cardioprotective effect in rats (P Anurag & T Rajamohan, unpublished). The present investigation was undertaken to study hepato-protective and anti-peroxidative effects of TCW on CCl_4-induced hepatotoxicity in female rats.

Tender coconuts (West Coast Tall variety) of 5-6 months of maturity were obtained from the University Campus, Kariavattom, Kerala. The liquid endosperm obtained from tender coconut was stored at 0^oC.

Female albino rats (Sprague-Dawley strain, wt 150-180 g) housed in polypropylene cages and maintained in controlled temperature with alternate 12 hr periods of light and dark were fed with standard rat chow (Amrut Laboratory animal feed Maharashtra Chakan Oil Mills Ltd., Pune); food and water were provided *ad libitum.* They were given a week's time to get acclimatized with the laboratory conditions. The experimental protocol was approved by the Animal Ethics Committee of the Department of Biochemistry, University of Kerala. After 1 week, the body wt. of animals were recorded and they were divided into 3 groups of 8 animals each as follows: Group I; Normal control rats; Group II; CCl_4-treated control rats; and Group III, TCW pretreated rats intoxicated with CCl_4.

CCl_4 after dilution with groundnut oil in the ratio of 1:1 was administered orally by intragastric gavage, twice weekly at a dose of 0.1 ml/100 g body wt.

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Tender coconut water (6 ml/100 g body wt.) was given to each rat of Group III daily. Animals were fasted overnight on 29th day and sacrificed the next day after recording body wt. by decapitation. Blood was collected by incision of jugular vein. Serum was prepared. Liver was dissected out, blotted off blood, rinsed in phosphate buffer *(p*H 7.4*)* and immediately processed for biochemical estimations. Liver tissues were fixed in 10% neutral buffered formaldehyde for histopathological study, according to the procedure reported earlier[10].

Thiobarbituric acid reactive substance (TBARS), viz. malondialdehyde (MDA)[11] and hydroperoxide[12] and conjugated dienes[13] (CD) in lipid extracts were estimated. The marker enzymes SGOT (EC 2.6.1.1) and SGPT (EC 2.6.1.2) were estimated as described[14]. Superoxide dismutase (EC 1.15.1.11)[14], catalase (EC1.11.1.6)[15], glutathione peroxidase (GPx)[17], and glutathione reductase (GR)[18] were assayed. Reduced glutathione content was determined after deprotenisation as described[19]. Serum total protein and albumin were estimated by the method of Lowry *et. al*[20].

Statistical analysis was carried out using student's *'t'* test.[21] The results are presented as the mean $\pm$ SE. Significance was accepted at $P \leq 0.05$ level.

Carbon tetrachloride (CCl_4) caused elevation in SGOT and SGPT levels in the serum and also lead to liver necrosis and fatty liver, while CCl_4-treated rats given TCW showed decreased activities of these enzymes (Fig. 1A). Elevated levels of TBARS, conjugated dienes and hydroperoxides were observed in liver of CCl_4-treated rats, compared to normal rats

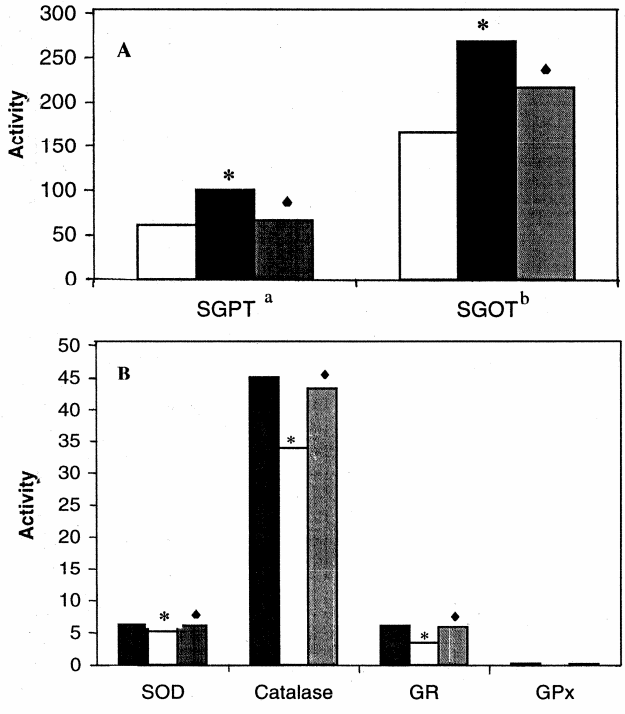

Fig. 1 — (**A**): Activities of marker enzymes in serum; (**B**): Activities of antioxidant enzymes in rat liver [Group I, control; group II, CCl_4-treated; group III, CCl_4+TCW [*Results are significantly different from group I; ♦Indicate that the results are significantly different from group II; [a]SGPT, μ moles of pyruvate liberated/min/mg protein; [b]SGOT, μ moles of oxalo acetate liberated /min/mg protein]

(Table 1), while CCl_4-intoxicated TCW administered rats showed a near normal level. GSH content in liver was decreased in CCl_4-treated control but increased in CCl_4-intoxicated TCW administered rats (Table 1).

Table 1 — Concentration of lipid peroxidation content and reduced glutathione (GSH) in the liver tissue (m*M*/100 g)
[Values are mean ± SEM of 8 animals in each group]

Groups	MDA	Hydroperoxides	Conjugated dienes	GSH
I (Normal control rats)	1.135±0.0333	28.10±0.562	22.69±0.454	502.0±10.04
II (CCl_4-treated rats)	1.680±0.034[a]	48.30±0.966[a]	37.03±0.741[a]	460.3±10.58
III (TCW fed rats treated with CCl_4)	1.147±0.023[b]	33.86±0.677[b]	24.75±0.495[b]	490.0±9.8[b]

[a]$P<0.05$ as compared to group I;[b]$P<0.05$ as compared to group II

Table 2 — Concentration of total protein, albumin, globulin and A/G ratio in plasma
[Values are mean ± SEM of 8 animals in each group]

Group	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio (g/dl)
I	4.625 ± 0.093	1.863± 0.0373	2.762± 0.055	0.675± 0.0135
II	2.425± 0.049[a]	1.025± 0.0205[a]	2.4± 0.048[a]	0.732 ± 0.01[a]
III	4.375 ± 0.088[b]	1.623± 0.0325[b]	2.752 ± 0.055[b]	0.589± 0.0118[b]

[a]$P<0.05$ as compared to group I; [b]$P<0.05$ as compared to group II

Similarly total protein, albumin, globulin and A/G ratio were significantly decreased in CCl_4-treated rats, but showed increased level in TCW treated CCl_4-intoxicated group (Table 2).

SOD, catalase, GPx and GR activities were significantly decreased in CCl_4-treated rats compared to normal controls. However, activities of these enzymes were a near normal in CCl_4 treated rats fed TCW (Fig. B). Rats treated with CCl_4 showed central lobule necrosis with lymphocytic and fatty infiltration (Fig. 2B). The sinusoids were also dilated, while CCl_4-treated rats given TCW had normal liver lobule with no fatty changes or necrosis (Fig. 2C).

Most of the mammals have an effective mechanism to prevent and neutralize the free radical- induced damage, which is accomplished by a set of endogenous enzymes, such as SOD, catalase, GPx and GR. As the balance between reactive oxygen species production and antioxidant defenses is lost, the 'oxidative stress' results, which through a series of events deregulates the cellular functions, leading to various pathological conditions[22]. An antioxidant compound might contribute partial or total alleviation of such damage.

The administration of CCl_4 in rats increased the levels of SGOT, SGPT, MDA, conjugated dienes and hydroperoxides resulting liver necrosis, fatty liver, and excessive formation of free radicals. It also activates lipid peroxidation, leading to hepatic damage. However, significant decline in the levels of these constituents in the liver of CCl_4-intoxicated rats given TCW indicates the hepatoprotective and antioxidant nature of TCW.

GSH, a major non-protein thiol in living organisms, plays a crucial role in coordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system can lead to serious consequences. Decline in GSH content in liver of CCl_4-intoxicated rats, and the subsequent reversal to near normal level of GSH in the liver of CCl_4-intoxicated rats given TCW from a declined level in CCl_4-intoxicated rats demonstrates the anti-lipid peroxidative effect of TCW. The possible mechanisms underlying the hepatoprotective properties of TCW include prevention of GSH depletion[23] and destruction of free radicals[24].

SOD, catalase, GPx and GR constitute a mutually supportive team of defense against reactive oxygen species. SOD, a metallo-protein is the first enzyme involved in the antioxidant defense by lowering the steady-state level of $O_2^{-\cdot}$. Catalase, a hemoprotein, localized in the peroxisomes or micro-peroxisomes catalyzes the decomposition of H_2O_2 to water and oxygen and thus protects the cell from oxidative

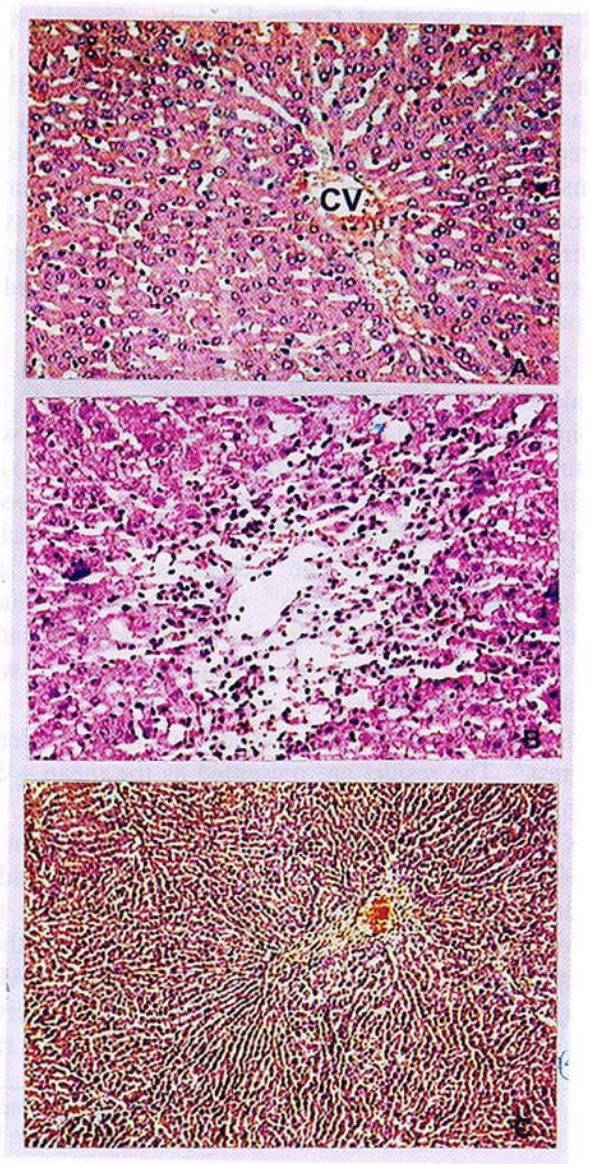

Fig. 2 — Cross section of normal and experimental liver or rats stained by haemetoxylin-eosin x 100 [(**A**), Control; (**B**), CCl_4-treated; (**C**), CCl_4 +TCW; **A:** showing central vein (CV) and cords of hepatocytes, liver cells are spherical vascular nuclei, the sinosides are narrow and regular; **B:** CV dilated and congested. Hepatocytes show fatty vacuole around CV, sinosides are dilated; **C:** showing normal lobule, no fatty change and necrosis are seen, mild atrophy of hepatocytes is indicating by prominent sinosides

damage by H_2O_2 and .OH. GPx catalyzes the destruction of H_2O_2 and lipid hydroperoxides by reaction with reduced glutathione (GSH) to form glutathione disulfide (GSH) and the reduction product of hydroperoxide. In our study, the decline in the activities of these enzymes in CCl_4-administered animals and their reversal to near normalcy in CCl_4-intoxicated rats given TCW indicate that lipid peroxidation and oxidative stress elicited by CCl_4-intoxication is nullified due to the effect of TCW. This observation is in agreement with, hepatoprotective and antioxidant activities reported in *Boehmeria nivea*[25].

Histopathological studies show that CCl_4-intoxicated rats cause fatty infiltration and necrosis in liver (Fig. 2B), similar to as reported earlier[26]. On the other hand, no fatty infiltration or necrosis was observed and the structure, shape and size of liver was almost normal, after the treatment with TCW (Fig. 2C), indicating that TCW possesses antioxidant property, which might have contributed to the hepatoprotective effect.

The major constituents present in TCW are: sugars, minerals, free amino acids, certain B vitamins and vitamin C. TCW is also a rich source of potassium (300 mg/100 ml), which is reported to lower the blood pressure[27] and a free amino acid, L-arginine (30 mg/dl), which significantly reduce the free radical generation[28]. Recently, L-arginine has also shown significant antioxidant activity[29]. Also, the administration of ascorbic acid, a constituent of TCW (15 mg/100 ml) significantly reduces lipid peroxidation in rats[30]. In addition, several minor constituents, growth factors etc. present in TCW, may also have beneficial effects.

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