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Reno-protective and Membrane Stabilizing Effect of Dioscorea bulbifera L. in CCL4 Induced Toxicity in Rats

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Abstract: The Hydroalcoholic extract of Dioscorea bulbifere L was evaluated for its reno-protective and membrane stabilizing effect against CCl4 induced toxicity in Rats. Animals were divided into four groups. First group was considered to be of normal, whereas second group meant for CCl4, third and fourth groups were kept for CCl4 and different doses of extract. The treatment was continued for 21 days. On 22nd day, animals were sacrificed and analyzed for the levels of various biochemical parameters such as Na + K + ATPase, Ca 2+ ATPase, Mg 2+ ATP ase, aspartate transaminase, alanine transaminase, alkaline phosphatase, acid phosphatase, catalase, glutathione–s-transferase, reduced glutathione and malondialdehyde. Alkaline phosphatase, acid phosphatase, aspartate transaminase and alanine transaminase were decreased significantly while antioxidants like catalase, glutathione-s-transferase and reduced glutathione were remained the other way. Membrane stabilizing enzymes like Na+, K+, ATPase, Ca2+ ATPase, Mg2+ and ATPase were increased significantly in treated animals. These observations suggest that hydroalocholic extract of Dioscorea bulbifera L at a dose of 400 mg/kg, b.wt as an effective dose for treating CCl4 intoxicated rats.

Keywords: CCl4, membrane stabilizing effect, Dioscorea bulbifera, reno-protection

INTRODUCTION

The kidney performs excretory homeostatic and endocrine functions. Kidney, skin, lung, gastrointestinal tract, salivary glands and liver are the major organs through which excretion takes place. Among the channels of excretion, kidneys are considered to be the chief. The kidney serves as the primary vehicle for excreting nitrogenous waste and other unnecessary substances from the body. Every day the kidneys filter several liters of fluid from the blood stream, allowing toxins, metabolic wastes, and excess ions to leave the body through urine, while returning only essentially needful substances to the blood. Failure of kidney to excrete waste products leads to accumulation of these products particularly nitrogenous substances. When kidney function is impaired, loss of control of

homeostasis mechanism occurs. It is causing ill health. This is called renal failure. There are two types of renal failure viz, acute and chronic[1].

An estimated 3.83 percent of adults aged 20 or more (7.7 million adults) have physiological evidence of chronic kidney disease having a moderately or severely reduced glomerular filtration rate[2]. A number of chemicals including various environmental toxicants and clinically useful drugs cause severe cellular changes in different organs of our body when exposed to a condition of more than optimum. Chronic exposure of CCl_4 at lower dose cause liver damage and necrosis of the renal tubular epithelium[3]. CCl_4 is converted to trichloromethylperoxy radical by cytochrome P_{450}[4]. These free radicals initiate the peroxidation of membrane leading to the generation of polyunsaturated fatty acids, which is in turn, covalently binds to microsomal lipids and proteins[5].

This phenomenon results in the generation of reactive oxygen species like superoxide anion $^{-}O_2$, H_2O_2 and the hydroxyl radical. Evidence suggests that the cell to scope has developed various enzymatic and nonenzymatic systems with the reactive oxygen species and other free radicals. However, when a condition of oxidative stress establishes, the defense capacities against reactive oxygen species becomes insufficient[6]. Thus, the administration of CCl_4 results in oxidative damage in the kidney[7]. The damages in kidney include glomerular necrosis and alteration in proximal and distal tubules progressing ultimately to detachment of the epithelial cells and tubular necrosis[8]. Majority of the world population in developing countries rely on herbal medicine. Currently 80 % of the world population depends on the plant-derived medicine for the first line of primary health care because of its lack of side effects[9].

Dioscorea bulbifera is a large genus of annual twining herbs, distributed throughout the moist tropics of world and extending into warm temperate regions. About 50 species are found in India. A large number among them occur in the wild state. Few are cultivated for their edible tubers. Dioscorea species are distributed nearly throughout India except in the dry north – western regions. They are found growing at elevations of 8000-15000 ft. In himalayas. Some important Dioscorea species are *D. Prazeri, D. alata, D. oppositifolia, D. pentaphylla, D. bulbifera, D. triphylla, D.aculeate, D. purpurea, D. Sativa* and *D. globosa*[10]. Most of them are found in N.S. Himalayas, U.P., Darjeeling, Sikkim, Assam, Bengal, Orissa, Bihar, Chotanagpur, M.P., Bombay and Madras are used to reduce swellings, have anthelmintic property also used in leprosy and piles.Whereas the tuber of *Dioscorea bulbifera* is used in diarrhoea, dysentery, piles, as a tonic, alternative, aphrodiasic, stomachic, anthelmintic, improves appetite, dyspepsia, leucoderma, bronchitis and applied to ulcer. It is common throughout India ascending up to 6,000 fit in the Himalayas. In its wild state, it is extremely bitter.

Extract of *Dioscorea bulbifera* is immunomodulatory. Immunomodulating properties are seen late in addition to atherosclerotic and anti-obesity[11]and significantly reduces serum lipid peroxidation, lowers serum triglycerides, phospholipids and increases HDL levels**.** It is used for preparing a formulation consisting of the biological active ingredient to the reduction of atherogenic

lipoproteins so as to obtain the moderate property of this part in reducing the atherogenic lipoproteins and enhancing general body resistance against oxidative injury[12-13]. Effect of *Dioscorea bulbifera* against CCl_4 induced toxicity has not yet been studied. Renoprotective and membrane stabilizing effect of *Dioscorea bulbifera* in CCl_4 induced toxicity has been evaluated here.

MATERIAL AND METHODS

Preparation of *Dioscorea bulbifera* extract

The tubers of *Dioscorea bulbifera* were collected from different areas of Tamilnadu. They were authentified at Rabinot herbarium, Trichy and Botanical survey of India, Coimbatore, and Tamilnadu. They were shade dried and coarsely powdered. The extract of the same was made with ethanol using soxhlet apparatus. The extract was concentrated *invaccuo*. The brown colored semisolid extract was used for the following study.

Experimental animals

Healthy Wistar albino rats weighing 180 – 200g were obtained from the SASTRA animal house, Tanjore, Tamilnadu. They were maintained in controlled temperature (23 ±3°C) and humidity 60-65% with 12hr dark and light cycle at CARISM Animal house, SASTRA Thanjore. The animals were fed with commercial diet (Tetragon chemie pvt. Ltd., Doddaballapur, Bangalore).The study was permitted by the Institutional Animal Ethical Committee with Reg No. 817/04/ac/CPCSEA.

Treatment of animals

Animals were randomly divided into 4 groups with 8 animals in each. Group 1 Normal distilled water, 0.3 ml, p.o. Group 2 (Control) received 30% CCl_4 in liquid paraffin (1 ml/kg body weight, i.p) Group 3 and 4 received 30 % CCl_4 in liquid paraffin (1 ml/kg body weight, i.p) and *Dioscorea bulbifera* extract at the dose of 150 and 250 mg/kg, p.o, respectively. Treatment duration was 21 days and the dose of CCl_4 was administered after every 72 hr[14]. The overnight fasted animals were sacrificed 24 h after the last injection of CCl_4 and blood was collected in tubes containing 10 % EDTA as anticoagulant. The organs were excised and they were washed in ice-cold saline and then, homogenized. 10% kidney homogenate was prepared in 0.1M HCl buffer pH 7.4.

In homogenate and plasma various biochemical parameters like Aspartate transaminase, alanine transaminase[15], alkaline phosphatease, acid phosphatase[16] were analyzed. Erythrocyte was isolated by Quist[17] method and various membrane stabilizing enzymes like Na^+ K^+ ATPase[18], Ca^{2+} ATPase , Mg^{2+} ATPase[19] were analyzed, Enzymatic antioxidants like catalase[20], in organs and glutathione-s-transferase[21] in plasma and organs were estimated. Nonenzymatic antioxidant like reduced glutathione[22] in plasma and various other organs was estimated. Lipid peroxidation was evaluated by estimating thio barbituric acid reactive substances[23] in plasma and various other organs.

RESULTS

In CCl_4 induced renal toxic rats serum alanine transaminase, aspartate transaminase, alkaline phosphatase, acid phosphatase and total protein were found to be increased significantly ($p<0.05$, Table I). But on treating animals with extract though significant difference has not been observed at a dose of 200 mg/kg b.wt., the drug can decrease all the above said enzymes significantly ($p<0.05$, Table 1). In kidney homogenate also similar results were observed and the values are mentioned in the Table 1.

Table 1. Levels of aspartate transaminase, alanine transaminase, alkaline phosphatease, acid phosphatase and total protein in plasma of CCl_4 intoxicated and rats treated with extract of *Dioscorea bulbifera*. (Values are mean ± SE)

Treatment	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	ACP (IU/L)	Total protein (g/dl)
Normal	21.8 ± 2.04	9.4 ± 0.1	396.0 ± 10.8	99.0 ± 2.9	4.6 ± 0.1
Control (CCl_4)	58.8 ± 0.85 *	17.7 ± 2.17 *	1094.0 ± 11.7 *	512.0 ± 9.0 *	2.8 ± 0.3 *
DB 200mg/ kg	39.5 ± 3.8	13.4 ± 1.1	717.3 ± 12.2	277.4 ± 1.06	4.2 ± 0.6
DB 400mg/ kg	23.8 ± 2.6 *	10.3 ± 1.3 *	425.0 ± 4.95 *	121.0 ± 5.8 *	5.6 ± 0.6 *

Note - * - $p<0.05$

In CCl_4 induced renal toxic rats' serum and kidney malondialdehyde level was found to be increased significantly ($p<0.05$, Table II). But on treating animals with extract even at lower concentration 200 mg/kg b.wt., the malondialdehyde level was found to be decreased significantly ($p<0.05$, Table 2).

Table 2. Levels of thiobarbituric acid, glutathione, glutathione-s-transferase in plasma of CCl_4 intoxicated and rats treated with extract of *Dioscorea bulbifera* (Values are mean ± SE)

Treatment	TBARS (nanomoles of MDA /mg of protein)	GSH (µg of GSH/mg of protein)	GST (nanomoles of CDNB-GSH conjugate formed/min/mg of protein)
Normal	0.22 ± 0.01	2.13 ± 0.02	2.15 ± 0.02
Control (CCl_4)	1.3 ± 0.02 *	1.38 ± 0.01 *	0.07± 0.001 *
DB 200mg/ kg	0.39 ± 0.08 *	1.79 ± 0.02	1.98 ± 0.01 *
DB 400mg/ kg	0.21 ± 0.02 *	2.18 ± 0.02 *	2.00 ± 0.01 *

Note * - $p<0.05$

In CCl_4 induced renal toxic rats serum reduced glutathione and glutathione –s-transferase level was found to be decreased significantly ($p<0.05$, Table II). But on treating animals with extract at 400 mg/kg b.wt, reduced glutathione was found to be increased significantly ($p<0.05$, Table 2). Glutathione-s-transferase level was found to be increased significantly even on treating animals with lower dose like 200 mg/kg b.wt. ($p<0.05$, Table 3).

Table 3. Levels of thiobarbiuric acid, catalase, glutathione, glutathione-s-transferase in kidney tissue of CCl_4 intoxicated and rats treated with extract of *Dioscorea bulbifera* (Values are mean ± SEM)

Treatment	TBARS (nanomoles of MDA /mg of protein)	GSH (µg of GSH/mg of protein)	GST (nanomoles of CDNB-GSH conjugate formed/min/mg of protein)	Catalase (µmoles of H_2O_2 used /min/mg of protein)
Normal	0.37 ± 0.02	4.43 ± 0.19	6.21 ± 0.02	196.0 ± 12.5
Control (CCl_4)	1.5 ± 0.1 *	2.07 ± 0.09 *	3.65 ± 0.19 *	45.6 ± 2.5 *
DB 200mg/ kg	0.9 ± 0.03	4.18 ± 0.17 *	5.67 ± 0.42	80.2 ± 4..39
DB 400mg/ kg	0.4 ± 0.02 *	5.15 ± 0.29 *	6.33 ± 0.4 *	142.3 ± 14.4 *

Note * - $p<0.05$

In CCl_4 induced renal toxic rats, reduced glutathione, glutathione-s-transferase and catalase level in kidney was found to be decreased significantly ($p<0.05$, Table II). But on treating animals with extract, enzyme level was found to be increased significantly ($p<0.05$, Table II) and return back to normal. Similarly levels of membrane stabilizing enzymes were found to be decreased significantly ($p<0.05$, Table IV) in CCl_4 intoxicated rats. On treating animals with *Dioscorea bulbifera* level of those enzymes were found to be reversed and return to normal (Table 4).

Table 4. Levels of Na^+ K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase in erythrocytes of CCl_4 intoxicated and rats treated with extract of *Dioscorea bulbifera* (Values are mean ± SEM)

Treatment	Na^+K^+ATPase (nanomoles of P liberated/min/mg of protein)	Mg^{2+}ATPase (nanomoles of P liberated/min/mg of protein)	Ca^{2+} ATPase (nanomoles of P liberated/min/mg of protein)
Normal	10.6 ± 0.2	10.6 ± 0.04	7.2 ± 0.2
Control (CCl_4)	5.6 ± 0.3 *	6.9 ± 0.4*	3.5 ± 002*
DB 200mg/ kg	8.0 ± 0.1*	8.5 ± 0.2	4.3 ± 0.06
DB 400mg/ kg	9.8 ± 0.2*	10.4 ± 0.5*	8.0 ± 0.3*

Statistics

Values are Mean ± SE of 6 animals and the significant difference was calculated using student's't' test using SPSS software version 11.0.

DISCUSSION

Our research was focused on the membrane stabilizing effect of *Dioscorea bulbifera* which was evaluated by assessing the biochemical parameters such as aspartate transaminase, alanine transaminase, alkaline phosphatease, acid phosphatase, Na^+ K^+ ATPase, Ca^{2+} ATP ase, Mg^{2+} ATP ase, catalase, glutathione-s-transferase, reduced glutathione and thiobarbituric acid reactive substances in experimentally CCl_4 intoxicated rats. Basu *et al*[24] have explained that increased free radical production and lipid peroxidation have been proposed as a major

cellular mechanism involved in CCl_4intoxicity. Slater *et al*[25] have shown that free radical or reactive oxygen species such as hydroxyl radical, peroxy radical and hydrogen peroxide are produced during lipid peroxidation.

The level of thiobarbituric acid reactive substances was significantly ($p<0.05$, Table 2) elevated in CCl_4 intoxicated group. On treating CCl_4 intoxicated animals with different doses of extract, Malondialdehyde level was decreased significantly ($p<0.05$, Table 2). This may be due to the anti-lipid peroxidation activity of extract. Anti-lipid peroxidative Effect of *Dioscorea bulbifera* at a dose of 400 mg/kg b.wt was also observed in kidney tissue. The effect on kidney is mentioned in Table 2. Catalase is one of the important enzymes in the supportive team of defence against reactive oxygen species, which is present in most cells and catalyses the decomposition of H_2O_2 to H_2O and O_2[26]. Catalase plays a vital role in scavenging of radicals. The inhibition of catalase activity may be due to the enhanced production of O_2 and peroxyl radical during the chronic administration of CCl_4[27].

Our present study revealed that the activity of catalase in renal tissue was significantly ($p<0.05$, Table 2) lowered in CCl_4 intoxicated rats. Decreased activity of catalase in CCl_4 treated rats may increase their susceptibility to oxidative injury. Administration of *Dioscorea bulbifera* tubers extract increases the activity of catalase ($p<0.05$, Table 2). This may be due to the increasing synthesis of catalase by extract. This increased catalase may scavenge the produced peroxy radical.

Reduced glutathione is a tripeptide. It contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamateside chain. Glutathione, an antioxidant, protects cells from toxins such as free radicals[28]. Glutathione constitutes the first line of defence against free radical. It performs much function. Reduced glutathione can react with singlet oxygen, superoxide and hydroxyl radical and therefore, function directly as a free radical scavenger. Reduced glutathione may stabilize membrane structure by removing acyl peroxide formed by lipid peroxidation reaction[29].

In CCl_4 intoxicated rats glutathione level was decreased significantly ($p<0.05$, Table 2,4) in both plasma and renal tissue. This may be due to the utilization of reduced glutathione to scavenge the produced free radicals. On treating animals with *Dioscorea bulbifera,* reduced glutathione level was increased significantly ($p<0.05$, Table 2) against disease control animals. These results also reveal that *Dioscorea bulbifera* extract can increase the synthesis of reduced glutathione. Glutathione-s-transferase acts like peroxidase and removes the stable peroxide from the system, resulting in the reduction of peroxide induced damage[30].Significantly decreased ($p<0.05$, Table 2) activity of GST in both plasma and renal tissue was seen in CCl_4 intoxicated arts. But, the reconstitution of the level of GST activity was seen in rats treated with *Dioscorea bulbifera* extract. These results also revealed the antioxidant activity of *Dioscorea bulbifera.*

Necrosis in kidney by CCl_4 usually associated with elevated level of plasma enzymes, the indicator of cellular leakage and loss of functional integrity of the cell membrane in kidney[31]. Aspartate transaminase, alanine transaminase and

alkaline phosphatase are ubiquitously distributed in the body tissue including the heart, liver, kidney and muscle.

From the present study it was observed that aspartate and alanine transaminase and alkaline phosphatase activity was increased significantly ($p<0.05$, Table 1) in plasma and decreased significantly in renal tissue ($p<0.05$, Table 1) in CCl_4 intoxicated rats as reported[32]. This may be due to the membrane damage caused by trichloroperoxy radical, a free radical formed from CCl_4. On treating animals with different doses of extract, release of enzymes decreased dose dependently and significant difference in both plasma and tissue ($p<0.05$, Table 1) was observed at a dose of 250 mg/kg b.wt. Such protective effect of *Dioscorea bulbifera* confirms its antioxidant activity and thereby, protection of membrane.

Acid phosphatase is frequently employed as a marker enzyme to assess the lysosomal changes in-vivo because it is a localized almost exclusively in the particulars and its release parallels that of lysosomal hydrolysis[33]. A significant increase ($p<0.05$, Table 1) in plasma and decrease ($p<0.05$, Table 1) in kidney acid phosphatase was observed in CCl_4 intoxicated rats. This may be due to the damage of lysosomal membrane caused by CCl_4. But on treating with *Dioscorea bulbifera* extract at a dose of 250 mg/kg b.wt, significant reduction ($p<0.05$, Table 1) in plasma and an escalation ($p<0.05$, Table 1) of acid phosphatase in kidney tissue was observed. ATPases are lipid dependent as well as thiol dependent membrane bound enzymes. Enhanced susceptibility to lipid peroxidation of membrane can lead to thiol formation. Thereby, there is a change in membrane function. The ionic concentration of hemolymph in crustaceans is maintained by active absorption of sodium chloride from the surrounding medium.

Enzymes like $Na^+ K^+$ ATP ase, Mg^{2+} ATP ase and Ca^{2+} ATP ase are responsible for the ionic regulation of hemolymp[34].Membrane bound $Na^+ K^+$ ATPase is an important enzyme utilizing the energy of ATP hydrolysis for transport of several cations. The inhibition of this enzyme produces an addition in intracellular calcium and a lowering in intracellular magnesium[35]. The plasma membrane Na^+K^+, and ATPase are concerned with the maintenance of low concentration of Na^+ and consequently of cellular water content. Decreased activity of $Na^+ K^+$ ATPase can lead to a deduction in sodium efflux thereby alter the membrane permeability[36]. Ca^{2+}ATP ase regulates the calcium pump activity. Lower Ca^{2+} ATP ase activity has been reported during oxidative stress due to hydroperoxides[37]. Mg^{2+} ATP ase is the major ATP ase for the plasma membrane for the amino phospholipids translocase activity of the plasma membrane[38].

Conclusion

Tuber extract of *Dioscorea bulbifera* was found to exhibit antioxidant activity at a dose of 250 mg/kg b.wt. Moreover it prevent the renal damage caused by CCl_4. Membrane stabilizing effect of *Dioscorea bulbifera* at a dose of 250 mg/kg b.wt was also evaluated. The mechanism of action of *Dioscorea bulbifera* in membrane stabilizing effect and the phyto constituents responsible for this protection should be evaluated.

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