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Research Article

ANTIOXIDANT AND ANTIHEPATOTOXIC ACTIVITIES OF ETHANOLIC EXTRACT OF *SOLANUM TORVUM*

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ABSTRACT

In the present investigation, the antioxidant and antihepatotoxic activities of the crude ethanolic extract of *Solanum torvum* (STE) was studied.The total antioxidant activity of herbal ethanolic extract was investigated in linoleic acid emulsion system.Total phenolic and flavonoid content of the extract were determined by a colorimetric method. The ethanolic extract of *S.torvum* (STE) also showed potent antihepatoxic activity against carbon tetrachloride –induced acute toxicity in rat liver. The extract at a dose level of 200 mg/kg body weight was administered to rats orally once daily for 14 days. The degree of liver protection was determined by estimating the levels of serum marker enzymes such as ALT, AST, ALP, ACP and LDH. The biochemical parameters like total protein, total bilirubin, total cholesterol, triglycerides and urea were also estimated. Silymarin at a dose level of 50 mg/kg was used as standard. The results revealed that *S.torvum* extract (STE) has notable inhibitory activity on peroxides formation in linoleic acid emulsion system in a dose-dependent manner. There was marked elevation of serum marker enzyme levels in CCl_4 treated rats, which were restored towards normalization in these drug treated animals. The biochemical parameters were also restored towards normal levels. The results of this study strongly indicate that the hepatoprotective effect of the plant extract is possibly related to its marked antioxidant activity.

Keywords: Hepatoprotective, marker enzymes, carbon tetra chloride.

INTRODUCTION

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism[1].Hepatic damage is associated with distortion of these metabolic functions[2]. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages[3]. Carbon tetra chloride is toxic to the liver and its toxicity is dose dependent and time of exposure[4]. In the liver, CCl_4 is metabolized in to the highly reactive trichloromethyl radical. The free radical generated would lead to auto oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and cause functional and morphological changes in the cell membrane by inducing lipid peroxidation. Lipid per oxidation also generates some oxidation reaction products which can react with biomolecules and exert cytotoxic and genotoxic effect. High levels of lipid peroxides have been found in the serum of patients suffering from liver disease, diabetes, vascular disorders and tumors[5].In situations of increased free radical generation, the reinforcement of endogenous antioxidants via intake of dietary antioxidants may be of particular importance in attenuating the cumulative effects of oxidatively damaged molecules. 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[6].In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders[7].In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

With the above scenario, the ethanolic extract of *Solanum torvum* (STE) fruit extract was subjected to various assays in order to evaluate their antioxidant and antihepatotoxic activities. *Solanum torvum* a member of the family Solanaceae and its fruits are commonly called as" night berries"which are used as a common vegetable by south Indian people. This plant is used for its haemostatic properties[8].Recent investigations demonstrated that *Solanum torvum* has antimicrobial activity [8,9]. There have been several reports regarding the chemical constituent of this plant which include documental steroidal compounds[10].Since no literature is currently available to substantiate the Hepatoprotective and antioxidant properties of *S.torvum,* the present study was designed to provide scientific evidence of the claimed ethnopharmacological properties.

MATERIALS AND METHODS

Chemicals

All routine chemicals were obtained from SD Fine Chemicals Mumbai. CCl_4 was obtained from Merck Ltd, India. Standard Silymarin was obtained from Ranbaxy (India) Ltd, New Delhi. All the chemicals used were of analytical grade.

Collection of Plant Material

The fruits of *Solanum torvum* were collected from Koyambedu market and were authenticated by Dr. Sankaranarayanan, Assistant Director, Dept of Research and Development,Sairam siddha Medical College and research centre, Chennai. India. The voucher specimen is also available in herbarium file of the same centre.

Preparation of extract

The fruits of *Solanum torvum* were washed thoroughly in tap water, shade dried and powdered. The powder was passed through 40-mesh sieve and exhaustively extracted with 90% (v/v) ethanol in Soxhlet apparatus at 60^{o}C. The extract was evaporated under pressure till all the solvent had been removed and further removal of water was carried out by freeze drying to give an extract sample with yield of 24.56% (w/w).The extract was stored in refrigerator and a weighed amount of the extract was dissolved in 2% (v/v) aqueous Tween- 80 and used for the present investigation.

Animals

Adult albino male rats of Wister strain weighing 150 - 175 g were used in the pharmacological and toxicological studies.The inbred animals were taken from animal house in Central Leather Research Institute, Adyar,Chennai, India.The animals were maintained in well ventilated room temperature with natural 12 ± 1 h day–night cycle in the propylene cages. They were fed balanced rodent pellet diet from Poultry Research Station Nandam, Chennai, India and tap water *ad libitum* was provided throughout the experimental period. The animals were sheltered for one week and prior to the experiment they were acclimatized to laboratory temperature. The protocol was approved by Animal Ethics Committee constituted for the purpose as per CPCSEA Guideline.

Acute Toxicity studies

Acute toxicity studies were conducted with the plant extract in Wister albino rats by staircase method[11].First group served as normal control. The animals were subjected to overnight fasting prior to the experiment and were administered with single dose of the extract dissolved in 2% aqueous Tween 80 and observed for mortality for 48 hours. Based on the short-term toxicity, the dose administered to the next animal was determined as per OECD guideline 423.All the animals were also observed for further 14 days.LD_{50} doses were selected for the evaluation of hepatoprotective activity.

Assay of antioxidant activities

Total antioxidant activity

The total antioxidant activity of the extract was measured by use of a linoleic acid system by the method of Mitsuda, et al. (1996)[12]. The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 emulsifier and 50 ml of phosphate buffer (0.2 M, pH 7.0). The mixture was then homogenized.A 0.5 ml of different concentration of the extract and standard sample (in ethanol) was mixed with linoleic acid emulsion (2.5 ml, 0.2 M, pH 7.0) and phosphate buffer (2 ml, 0.2 M, pH 7.0). The reaction mixture was incubated at 37°C in the dark to accelerate the per oxidation process. The levels of per oxidation were determined according to the thiocyanate method by sequentially adding ethanol (5 ml, 75%), ammonium thiocyanate (0.1 ml, 30%), sample solution (0.1 ml) and ferrous chloride (0.1 ml, 20 mM $FeCl_2$ in 3.5% HCl).Vitamin E was used as positive control. After mixing for 3 min, the peroxide values were determined by reading the absorbance at 500 nm.

Determination of total phenolic content

Total phenolic content in the lyophilized extract was determined with the Folin–Ciocalteu's reagent (FCR) according to a published method of Slinkard and Singleton (1977)[13]. 100 mg of the sample dissolved in 0.5 ml of water was mixed with 2.5 ml FCR (diluted 1:10, v/v) followed by 2 ml of Na_2CO_3 (7.5%, v/v) solution. The absorbance was then measured at 765 nm after incubation at 30°C for 90 min. Results were expressed as gallic acid equivalents (mg gallic acid/g dried extract).

Determination of total flavonoid content

The total flavonoid content of the ethanolic extract was determined by a colorimetric method as described in the literature of Zhishen *et al.* (1999)[14]. The 100 mg sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a $NaNO_2$ solution (15%).After 6 min, 0.15 ml of an $AlCl_3$ solution (10%) was added and allowed to stand for 6 min, and then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm versus prepared water blank. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Assay of Hepatoprotective activity

Carbon tetra chloride induced hepatotoxicity in rats was used as a model to determine the hepatoprotective activity of the *Solanum torvum* ethanolic extract. The rats were divided into four groups with six animals in each group and were given dose schedule as follows: Group I: Animals were given a single administration of 0.5 ml vehicle (2% v/v aqueous Tween 80) po for 14 days. This group served as control. Group II, III and IV: Animals were given a single dose of CCl_4 (2ml/kg, po for 7 days) according to the method of Shivaipandey *et al*[15]. Group III: Animals were pre treated with plant extract (200 mg/kg, po for 7 days) and simultaneously received the same during CCl_4 treatment for next 7 days. Group IV: Animals were pre treated with Silymarin (50mg/kg, po for 7 days) and received the same along with CCl_4 treatment for next 7 days .This group served as positive control

On the 15th day, the animals were sacrificed by cervical decapitation and various biochemical parameters were analyzed.

Biochemical analysis

At the end of the experimental period, animals were sacrificed by cervical decapitation under light ether anesthesia and blood was collected, serum was separated by centrifuging at 3,000 rpm for 10 min. The serum was used for the assay of marker enzymes, such as alanine amino transferase (ALT)[16], aspartate amino transferase (AST)[16], alkaline phosphatase (ALP)[17], acid phosphatase (ACP)[17] and lactate dehydrogenase (LDH)[18]. The biochemical parameters such as total protein [19], total cholesterol [20], total bilirubin[21], triglycerides[22] and urea[23] were also estimated. All the enzymatic and biochemical assays were read at specific wavelength using Shimadzu spectrophotometer, UV-1601 model.

Statistical Analysis

Values reported are mean ± S.E. The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's 't' test. P values <0.05 were considered as significant[24].

RESULTS

Acute toxicity studies

In the acute toxicity studies death was recorded during the treatment period in treated groups receiving 2000mg/kg po of plant extract orally. The animals showed changes in general behavior and other physiological activities like giddiness, sniffing, aggressiveness, tachypnoea, and finally convulsion. From the above toxicity studies the ED_{50} dose of the STE was calculated and it was fixed as 200 mg/kg body weight.

Assay of antioxidant activities

Total antioxidant activity

The total antioxidant activity of *S.torvum extract* was measured using ferric thiocyanate test which determines the amount of peroxide produced at the initial stage of lipid peroxidation.

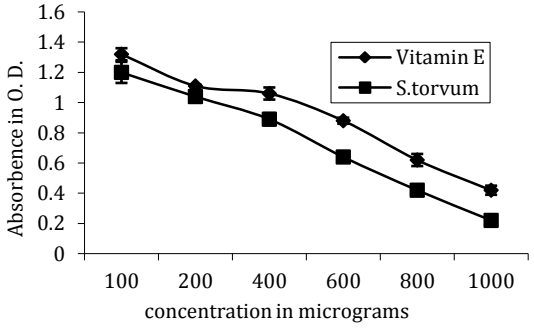

Fig 1:Total antioxidant activity of different concentrations of ethanolic extract of *S.torvum* and Vitamin E in linoleic acid emulsion determined by thiocyanate method. Each value represents mean ± SEM (n=3).

Lower absorbance indicates a higher level of antioxidant activity. Figure-1 shows the changes in the absorbance under the influence of different concentrations of the extract (100 – 1000 µgm/ml) at 37°C compared to vitamin E as a positive control. According to this Figure the extent of inhibition of lipid oxidation is moderate at low (100µg/ml) doses of extract. However, at higher concentrations (800 and 1000µg/ml), the plant extract suppressed lipid oxidation to a considerable extent.

Total phenolic and flavonoid contents

The antioxidant activity of *S. torvum* extract is probably due to its phenolic contents. Flavonoids are a class of secondary plant phenolics with powerful antioxidant properties. Therefore, it would

be valuable to determine the total phenolic and flavonoid content of the plant extracts. The extracts were investigated regarding their composition by different colorimetric techniques, such as the content of total phenolic compounds by the Folin– Ciocalteu's assay and flavonoids by $AlCl_3$ reagent. Total phenolic and Flavonoid contents of each gram of dried extract were estimated to be equivalent to 54.8 mg gallic acid and 29.8 mg catechin. The antioxidant activity of the plant extract is probably due to its phenolic content and the secondary plant phenolics the flavonoids.

CCl_4-induced Hepatotoxicity

A significant increase in the serum enzyme levels were seen in the Group II CCl_4 intoxicated animals (Table-1). These enzymes were brought back to near normal levels in drug (200 mg/kg body weight) pretreated Group III animals *(P*<0.001).All the parameters were under normal limits in the silymarin treated group, which acted as a positive control. The biochemical parameters such as serum bilirubin and urea levels were also lowered significantly in Group III *S.torvum* extract treated animals (*P*<0.001), when compared with the CCl_4 intoxicated Group II animals which had an increased level of total bilirubin and urea respectively (Table-2).

TABLE1: Effect of STE and Silymarin on various enzymatic parameters in CCl_4 intoxicated rats

Groups	AST(U/L)	ALT (U/L)	ALP (IU/L)	ACP (K.A Units)	LDH (U/L)
I (Control)	46.15 ± 1.10	46.00 ± 1.03	148.36±0.25	4.11±0.23	145.90±1.87
II(Toxicant) a	143.79±4.50***	145.50±1.08***	172.68±0.64**	12.25±1.06a***	435.38±1.84***
III(STE+CCl_4treated) b	92.11±2.45 *	92.86±3.04*	151.17±0.21 *	9.15±0.41*	324.98 ±0.22**
VI(Positive control) c	76.92± 3.60 NS	78.16±0.54*	121.28±1.00*	6.70±0.20*	240.71±2.94*

Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's `t' test. Comparison between: a– Group I and Group II, b– Group II vs Groups III, IV, and V and c - Group III vs Group VI. P Values: * <0.05, ** <0.01, * <0.001 NS-Non significant.**

Table2: Effect of STE and Silymarin on various Biochemical parameters in CCl_4 intoxicated Rats

Groups	Total protein (g/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	Urea (mg/dl)	Bilirubin (mg/dl)
I (Control)	7.80±0.090	122.12±1.44	146.98±1.62	19.00 ±1.50	0.95±0.04
II(Toxicant) a	5.25±0.18*	115.33±2.90*	125.50±2.10**	45.00±2.4***	2.47±0.09 ***
III(STE+CCl_4treated) b	6.80±0.04*	132.16±1.12**	186.00±0.6**	39.40±1.34*	1.76±0.78*
VI(Positive control) c	6.28±0.32NS	139.06±3.1 c*	148.80±1.49*	32.33±2.40 *	1.46±0.04 *

Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's `t' test. Comparison between: a– Group I and Group II, b– Group II vs Groups III, IV, and V and c - Group III vs Group VI. P Values: * <0.05, ** <0.01, * <0.001 NS-Non significant.**

Whereas there was a significant increase in total protein, total cholesterol and triglyceride levels in the CCl_4 intoxicated and plant drug treated animals (*P*<0.001) when compared with to CCl_4 intoxicated animals. Group comparison between Group III and Group VI showed no significant variation in these parameters indicating that the plant extract had effects similar to silymarin, which was the positive control in this study.

DISCUSSION

Free radicals have been implicated in many disease conditions, the important ones being superoxide radical, hydroxy radical, peroxyl radical and singlet oxygen. Although there are some synthetic antioxidant compounds such as BHT and butylated hydroxyanisole (BHA), which are commonly used in foods processing, it has been reported that these synthetic antioxidants are not devoid of biological side effects and their consumption may lead to carcinogenicity and causes liver damages[25].Therefore, the development of alternative antioxidants mainly from natural sources has attracted considerable attention. It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. The *S.torvum* extract at higher concentrations inhibits the lipid oxidation indicating that it has considerable quantities of phytochemicals responsible for the antioxidant activity. Arthan *et,al* have reported the presence of isoflavonoid sulfate and steroidal glycosides with antiviral activity.These chemicals namely torvanol A , and a steroidal glycoside, torvoside H may be responsible for the total antioxidant activity observed in our present investigation[26].

The antioxidant activity of the plant extracts is probably due to its phenolic contents. It is well known that phenolic compounds are constituents of many plants, and they have attracted a great deal of public and scientific interest because of their health promoting effects as antioxidants. The total phenolic and flavonoid contents of *S.torvum* was determined and expressed in terms of gallic acid and catechin equivalents. The presence of significant quantities of phenol and flavonoid in the plant extracts are considered as the major contributors towards the antioxidant activity observed in our present study.

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[27].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 combines with cellular lipids and proteins in presence of oxygen to form trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethylperoxyl free radical elicits lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally, results in cell death[28].Assessment of liver damage can be made by estimating the activities of serum enzymes ALT, AST, ALP, LDH and ACP which are originally present in higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage[29]. The elevated level of these marker enzymes observed in the Group II CCl_4 treated rats in the present study correspond to the extensive liver damage induced by the toxin. The reduced concentrations of ALT and AST as a result of plant extract administration observed during the present study may probably be due in part to the presence of catechins in the extract. The tendency of these marker enzymes to return towards near normalcy in Group III (drug treated) rats was a clear manifestation of anti-hepatotoxic effect of S.torvum extract. The results were found comparable to silymarin. Silymarin contains three flavonoids and is isolated from milk thistle *Silybum marinum*. It is used as hepatoprotective against experimental hepatotoxicity of various chemicals including CCl_4[30].

In the present study it was noted that the administration of CCl_4 decreased the levels of total protein, total cholesterol, and triglycerides. These parameters were brought back to normal levels in Group III S. torvum extract treated animals. Drug treatment showed a protection against the injurious effects of CCl_4 that may result from the interference with cytochrome P450, resulting in the hindrance to the formation of hepatotoxic free radicals. The site-specific oxidative damage in some susceptible amino acids of proteins is now regarded as the major cause of metabolic

dysfunction during pathogenesis. Attainment of near normalcy in protein, cholesterol, and triglycerides levels in CCl_4 intoxicated and plant extract treated rats confirms the hepatoprotective effect of the plant. The marked elevation of bilirubin and urea level in the serum of group II CCl_4 intoxicated group rats were significantly decreased in group III ethanolic extract of *s.torvum* pretreated animals.Bilirubin is the conventional indicator of liver diseases[31].

Bilirubin is an endogenous organic anion binds reversibly to albumin and it is transported to the liver, conjugates with the glucouronic acid and excreted in the bile.Hepatobiliary disease is indicated when the total bilirubin exceeds the upper limit of normal[32].The bilirubin lowering ability of the extract in drug pretreated rats further indicate the hepatoprotective nature of our plant drug on hepatocytes, when compared with rats administered CCl_4 alone.

CONCLUSION

On the basis of the results obtained in the present investigation it can be concluded that the ethanolic extract of *S.torvum* extract hepatoprotective activity and may serve as a useful adjuvant in several clinical conditions associated with liver damage. Possible mechanism that may be responsible for the protection of CCl_4 induced liver damage by the plant extract may be that it could act as a free radical scavenger intercepting those radicals involved in CCl_4 metabolism by microsomal enzymes. By trapping oxygen related free radicals the extract could hinder their interaction with polyunsaturated fatty acids and would abolish the enhancement of lipid peroxidative processes. Flavonoids and glycosides are known strong antioxidants. Antioxidant principles from herbal resources are multifaceted in their effects and provide enormous scope in correcting the imbalance through regular intake of a proper diet.

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