Evaluation of the antioxidant and hepatoprotective effect of *Solanum grandiflorum* Ruiz & Pav against CCl₄-induced oxidative stress in rats

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**ABSTRACT**

The present study was designed to demonstrate the antioxidant and hepatoprotective effect of *Solanum grandiflorum* (*S.grandiflorum*). In order to find in vitro antioxidant properties, *S.grandiflorum* extract was evaluated by Total phenolic content, DPPH and ABTS. In all of the tests, the extracts showed a potent antioxidant effect compared to ascorbic acid. The hepatoprotective activity of the extract was also demonstrated in vivo condition against CCl₄-induced liver damage, as evidenced by the decrease in the level of Aspartate transaminase (AST), alkaline phosphatase (ALP), Alanine transaminase (ALT), Lactate dehydrogenase (LDH) compared to CCl₄ treated rats, and Superoxide dismutase (SOD), Glutathione (GSH) and Glutathione-S-transferase (GST) levels were increased potentially due to treatment with the extract. The methanol extract of *S.grandiflorum* protected liver cells against histopathological changes produced by CCl₄ such as hepatocytic necrosis, fatty changes, vacuolation, etc. This study suggests that the presence of flavonoids and phenolics compound in the methanol extract of *S.grandiflorum*, which may be responsible to antioxidant and hepatoprotective activities. The results suggest that the leaves of *S.grandiflorum* are a source of natural antioxidants.

**Keywords:** Antioxidant; Carbon tetrachloride; Hepatoprotective; *Solanum grandiflorum*

**INTRODUCTION**

Formation of reactive oxygen species (ROS) is an unavoidable consequence in aerobic organisms during respiration. It has been shown that overproduction of unstable ROS leads to unwanted reactions with other groups or substances in the body, resulting in cell or tissue injury. In addition, numerous studies have revealed that uncontrolled lipid peroxidation is involved in the occurrence of many diseases, including Parkinson’s, arthritis, myocardial infarction, Alzheimer’s, cancer, cardiovascular disease, and liver damage (Qian, 2008; Blomhoff, 2005). Therefore, during the last few decades, human nutrition and biochemistry research have focused on antioxidants derived from foods that could prevent or diminish ROS-induced damage. Oxidative stress is a process where the physiological balance between pro-oxidants and antioxidants is disrupted in favor of the former, ensuing in potential damage for the organism (Halliwell, 1990). Liver damage is a widespread disease which can be caused by reactive oxygen species (ROS), and is characterized by a progression from steatosis to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Srivastava, 2010; Jin, 2005). Several compounds, such as carbon tetrachloride (CCl₄), acetaminophen, bromobenzene, ethanol, and polycyclic aromatic hydrocarbons have been implicated in the etiology of liver diseases (Adesanoye, 2010). CCl₄ is a classical hepatotoxin that causes rapid liver damage progressing from steatosis to centrilobular necrosis (Lin, 2008). Reductive dehalogenation of CCl₄ by the P450 enzyme system to the highly reactive trichloromethyl radical initiates the process of lipid peroxidation which is considered to be the most important mechanism in the pathogenesis of liver damage induced by CCl₄ (Demirdag, 2004). Dietary antioxidant intake may be an important strategy for inhibiting or delaying the oxidation of susceptible cellular substrates, and is thus relevant to disease prevention in many paradigms. Phenolic compounds such as flavonoids, phenolic acids, diterpenes and tannins have received attention for their high antioxidative activity (Rice-Evans, 1996). Due to the risks of synthetic antioxidants, there is a growing interest in the use of natural antioxidants to prevent oxidative stress-related liver pathologies (Dhanasekaran, 2009; Wang, 2004). A major defense mechanism involves antioxidant enzymes such as superoxide dismutase (SOD), and glutathione -s-Transferase (GST), which neutralize ROS in cells (Tsai, 2009). Additionally, carbon tetrachloride catabolised radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, causes the swelling and necrosis of hepatocytes and result to the release of cytosolic enzymes such as AST, ALT and ALP into the circulating blood (Singh, 1998; Xiong, 1998). Converging evidence from both experimental and epidemiological
studies has demonstrated that cereals, vegetables, and fruits contain a myriad of phenolic compounds.

The leaves of *S. grandiflorum* was fractionated into petroleum ether, ethyl acetate and methanol fractions. Then each fractions were screened for their in vitro antioxidant activity using three methods as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test, ABTS (2,2’-azino-bis-3-ethylbenzthiazoline-6-sulfonic). The methanol fractions of *S.grandiflorum* was found to be the most active and was further investigated in hepatoprotective activity experiment against CCl₄-induced liver toxicity. Total phenol quantities of the methanol extracts were determined spectrophotometrically using Folin-Ciocalteau’s respectively.

In spite of tremendous strides in the modern medicine, there are not much drugs available for the treatment of liver diseases. There are a number of medicinal preparations recommended in the Indian traditional system of medicine “Ayurveda” for the treatment of liver diseases. There are scientific claims to offer significant relief as hepatoprotective (Rao, 2006).

*Solanaceae*, the nightshade or potato family of flowering plants include a number of important economic plants. Many members of the *Solanaceae* family are used by humans and are important sources of food, spice and medicine. Among the most important of these are the potato (*Solanum tuberosum*), eggplant (*S.melongena*), tomato (*Lycopersicon esculentum*), tobacco (*Nicotiana tabacum*) and *Solanum nigrum*. Members of the family are herbs, shrubs, trees, or sometimes vines. The flowers are usually actinomorphic. Flower shapes are typically rotate or tubular, with four or five petals that are usually fused. There are some species that are locally available in Mysore, Karnataka–India. Among these, we have selected *Solanum grandiflorum* species, which has shown potent antioxidant activity, but there is no further scientific information about this plant. The aim of the present study was to investigate the antioxidative activity and potential protective effects of the methanol extract of *S.grandiflorum* leaves in the CCl₄-induced hepatic damage in rats.

**MATERIALS AND METHODS**

**Chemicals and reagents**

DPPH (1,1-diphenyl-2-picryl hydazyl), ABTS (2,2’-azino-bis 3-ethylbenzthiazoline -6-sulfonic) were purchased from Sigma (St. Louis, MO,USA), TCA (Trichloro acetic acid), Thiobarbituric acid (TBA), pyrogallol, Sodium Citrate, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5’dithiobis(2-nitrobenzoic acid) (DTNB), hydrogen peroxide (H₂O₂), reduced glutathione (GSH), Ethylenediamine tetaacetic acid disodium salt (EDTA), Butylated hydroxy toluene (BHT), carbon tetrachloride (CCl₄) and other chemicals were obtained from Sisco Research Laboratories (SRL), Mumbai, India.

**Plant material**

The leaves of *S. grandiflorum* were collected from Mysore, Karnataka–India in March 2012 and were authenticated by a plant taxonomist. Healthy plants were screened and thoroughly washed to remove adhering dust and shade-dried. The dried plants were pulverized in a mechanical grinder and the coarse powder was used for further studies.

**Extraction procedure**

Dried and ground leaves were serially extracted with hexane, ethyl acetate and methanol using Soxhlet apparatus. The crude extract thus obtained from this extraction flask was transferred to flash evaporator for complete evaporation. The dried extracts were collected and stored in refrigerator for further studies.

**Total Phenolic Content Estimation**

The total phenolic content of the different extracts of *S. grandiflorum* was determined using the Folin-Ciocalteau's method.
Ciocalteau reagent method (Lister & Wilson, 2001). To 50 ml of each extract, 2.5 ml of Folin-Ciocalteau reagent (1/10 dilution) and 2 ml of 7.5% Na₂CO₃ (w/v) were added and mixed well. The blend was incubated at 45°C for 15 min. The absorbances of all samples were measured at 765nm with Na₂CO₃ solution (2 ml of 7.5% Na₂CO₃ in 2.55 ml of distilled water) as blank. The results were expressed as Gallic acid equivalence (GAE) in micrograms.

**Determination of antioxidant activity**

**DPHH radical free scavenging activity**

The DPPH assay was estimated according to the method described (Sultanova et al., 2001) with some modifications. The reaction mixture contains 5 µl of test samples (various extracts and compound dissolved in Methanol) and 95 µl of DPPH (300 µM) in Methanol. These reaction mixtures were taken in 96 well plate and incubated at 37 °C for 30 min, the absorbance was measured at 517 nm. Ascorbic acid was used as reference standard. The level of percentage inhibition of DPPH radical by the different extracts was calculated according to the following formula

\[ \% \text{ inhibition} = \frac{A_c - A}{A_c} \times 100 \]

Where \( A_c \) is the absorbance of the control and \( A \) is the absorbance of sample. Percentage scavenging was also evaluated in Ascorbic acid equivalence.

**ABTS radical scavenging assay**

For ABTS assay, the method of (Re et al., 1999) was adopted with some modification. The stock solutions included 7 mM ABTS solution and 2.45 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12-16 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS⁺ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (10 µl) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm for 6 min using the spectrophotometer. The ABTS⁺ scavenging capacity of the extract was compared with that of ascorbic acid and percentage inhibition calculated as ABTS radical scavenging activity.

\[ \% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \]

Where Abs control is the absorbance of ABTS radical + methanol, Abs sample is the absorbance of ABTS radical + sample extract/standard.

**In vivo studies**

**Animals and treatment**

Albino Wistar rats weighing 180 -200g were used for the study. Appropriate guidelines of the local animal ethics committee were followed for the animal experiments. Animals were allowed free access to standard dry pellet diet and water. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment.

**Experimental design**

Methanol extract (ME) was dissolved in sterile distilled water. Two different concentration of ME was administered orally. Animals were divided into five groups comprising six rats in each group as follow:

- **Group I** was administered sterile distilled water, served as positive control.
- **Group II** was administered CCl₄ (1 ml/kg b.w.)
- **Group III** was administered ME 100 mg/kg b.w. + CCl₄
- **Group IV** was administered ME 200 mg/kg b.w. + CCl₄
- **Group V** was administered ME 200 mg/kg b.w. only

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>AST (u/l)</th>
<th>ALT (u/l)</th>
<th>ALP (u/l)</th>
<th>LDH (u/l)</th>
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</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>233.4±7.2ᵃ</td>
<td>74.3±3.5ᵇ</td>
<td>124.6±6.4ᵇ</td>
<td>1254.8±95.6ᵇ</td>
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<td>Group II</td>
<td>CCl₄ (1 ml/kg b.w.)</td>
<td>687.3±15.4ᵈ</td>
<td>219.4±2.6ᵈ</td>
<td>232.6±6.3ᵈ</td>
<td>1863±143.8ᵈ</td>
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<tr>
<td>Group III</td>
<td>ME 100 mg/kg b.w. + CCl₄</td>
<td>574.4±16.5ᵇ</td>
<td>163.4±4.6ᵇ</td>
<td>198.5±7.5ᵇ</td>
<td>1552.4±152.8ᵇ</td>
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<tr>
<td>Group IV</td>
<td>ME 200 mg/kg b.w. + CCl₄</td>
<td>424.6±15.2ᵇ</td>
<td>136.4±2.3ᵇ</td>
<td>164.5±6.4ᵇ</td>
<td>1367.6±76.3ᵇ</td>
</tr>
<tr>
<td>Group V</td>
<td>ME 200 mg/kg b.w. only</td>
<td>317.6±12.4ᵇ</td>
<td>96.3±2.4ᵇ</td>
<td>112.3±8.3ᵇ</td>
<td>1295.6±68.7ᵇ</td>
</tr>
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Table 1: Serum level of AST, ALT, ALP, and LDH

Data are expressed as means ± SE with different suffix letters differ significantly (p < 0.05, n=6). AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: serum alkaline phosphatase, LDH: Lactate dehydrogenase.
Group II: was administered CCl₄ served as negative control.

Group III: was administered 100 mg/kg b.w of ME orally for 7 days + single oral dose of CCl₄ on the seventh day (1 ml/kg b.w)

Group IV: was administered 200 mg/kg b.w of ME orally for 7 days + single oral dose of CCl₄ on the seventh day (1 ml/kg b.w)

Group V: was administered 200 mg/kg b.w of ME orally for 7 days.

On the seventh day, the animals of all groups except control group were administered simultaneously with CCl₄ in olive oil as carrier (1 ml/kg b.w) after 1h of administration of the ME. After 16-18 h of CCl₄ administration, animals were sacrificed by anesthesia. Blood was collected from heart puncture and serum used for estimation of blood marker enzymes. The liver perfused with saline were dissected out and processed immediately for histopathology and biochemical assays.

**In vivo assays**

Estimation of serum biochemical parameters

The collected blood was allowed to clot and serum was separated at 2500 rpm for 15 min and the biochemical parameters like serum enzymes: aspartate aminotransferase (AST), serum glutamate pyruvate transaminase (ALT), serum alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were assessed by commercial kit methods.

**Lipid peroxidation (LPO)**

This assay is used to determine thiobarbituric acid reactive substances (TBARS) level as described by (Ohkawa, 1979) with some modification. 1 ml of tissue homogenate supernatant (10% w/v in 1.15 % KCl solution) was added to 1.5 ml of 20% Trichloro acetic acid (TCA) and 1.5 ml of 0.6% thiobarbituric acid(TBA) and were incubated at 95°C for 30 minutes. The samples were allowed to cool at room temperature. Then 2mL of butanol were added, vortexed, and centrifuged at 2000g for 15 minutes. The absorbance of 3 ml of the colored layer was measured at 532nm spectrophotometrically using 1,1,3,3-tetraethoxypropane as a standard.

**Glutathione (GSH) activity**

Hepatic GSH level was determined by the method of (Ellman, 1959) with slight modification. Briefly, liver tissue was homogenate in 10 % TCA and 10mM EDTA in (ratio in 1:1, 10% w/v). The samples were centrifuged at 5000 rpm for 5 min at 4 °C. Supernatant (100 µl) was taken in a tube and 50 µl of Ellman’s reagent (DTNB solution) was added to it in final concentration of 1ml in Tris-HCl ph 8.2. Finally, the yellow color developed was read at 412 nm.

**Superoxide dismutase (SOD) and Glutathione-S-transferase (GST)**

The liver was perfused with cold saline to completely remove all the red blood cells. Then it was suspended in 10% (w/v) ice-cold 50 mM phosphate buffer (pH 7.4) cut into small pieces, and the required quantity was weighed and homogenized using a Teflon homogenizer. The homogenate was centrifuged at 10,000g for 20 min at 4°C to remove the cell debris, unbroken cells, nuclei, erythrocytes and mitochondria. The supernatant was used for the estimation of superoxide dismutase (SOD), Glutathione-S-transferase (GST). Superoxide dismutase (SOD) activity was measured using pyrogallol (2 mM) autoxidation in Tris buffer (Marklund, 1974). Glutathione-S-transferase (GST) activity was assayed by the method of (Warholm, 1985) in phosphate buffer (0.1 M, pH 7.6) containing glutathione (0.5 mM) and CDNB (0.5 mM) and change in the absorbance at 344 nm was monitored in a UV–visible spectrophotometer.

**Histopathological studies**

Pieces of liver tissues were excised, washed with normal saline and processed separately for histopathological observation. Initially the liver tissues were fixed in 10% buffered neutral formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H–E) dye. The sections were examined microscopically for histopathology

| Table 2: Tissue homogenate level of SOD, GSH, LPO and GST |
|---|---|---|---|---|
| Group | Treatment | SOD | GSH | LPO |
| Group I | Control | 2.26±0.38<sup>a</sup> | 13.46±1.45<sup>b</sup> | 4.09±0.64<sup>b</sup> | 137.58±12.43<sup>b</sup> |
| Group II | CCl₄(1 ml/kg b.w.) | 0.43±0.39<sup>a</sup> | 10.63±1.73<sup>b</sup> | 5.85±0.83<sup>b</sup> | 116.56±14.03<sup>b</sup> |
| Group III | ME 100 mg/kg b.w. + CCl₄ | 0.67±0.25<sup>a</sup> | 14.54±1.84<sup>b</sup> | 4.85±0.59<sup>b</sup> | 147.52±10.54<sup>b</sup> |
| Group IV | ME 200 mg/kg b.w. + CCl₄ | 1.45±0.47<sup>a</sup> | 15.04±1.65<sup>b</sup> | 4.47±0.62<sup>b</sup> | 174.85±12.32<sup>b</sup> |
| Group V | ME 200 mg/kg b.w. only | 2.94±0.36<sup>a</sup> | 16.43±2.13<sup>b</sup> | 3.86±0.73<sup>b</sup> | 168.54±13.65<sup>b</sup> |

Data are expressed as means ± SE with different suffix letters differ significantly (p < 0.05, n=6). Values are expressed as SOD, superoxide dismutase(unit/min/mg protein); GSH, glutathione (µg/mg protein); LPO, lipid peroxidation (nmol of malonaldehyde (MDA) formed/mg protein/h); GST, glutathione-s-transferase (µmole CDNB conjugate/min/mg protein).
changes, including cell necrosis, fatty change and vacuolation.

Statistical analysis
All results are expressed as mean±SE. One-way analysis of variance (ANOVA) followed by multiple comparisons with the Tukey post hoc test was used to compare different parameters between the groups. A p value <0.05 was considered significant.

RESULTS
Generally, total phenol contents of plant extracts are considered to be correlated with their antioxidant activity as phenolics are strong antioxidants. Therefore, total phenol contents in the methanol extract was determined and it was found to contain quite well amount of total phenol as gallic acid equivalent (Figure 4).

DPPH and ABTS assay is widely used as a free radical to evaluate the antioxidant activity of natural compounds. Figure 2,3 demonstrate the antioxidant activity of three different fractions (Hexane, Ethyl acetate, Methanol) of *S. grandiflorum* using DPPH and ABTS. These fractions at different doses showed free radical scavenging activity in dose dependent manner. Among all of the fractions, the methanol fraction of *S. grandiflorum* had the highest activity in both tests. The methanol extract fraction exhibited 85.66 % scavenging effect against DPPH, respectively, where it showed 90.33 % scavenging effect in ABTS.

Serum marker enzymes
The effects of methanol extract of *S. grandiflorum* leaf at two dose levels (100 and 200 mg/kg, b.w.) on serum marker enzymes in CCl₄-induced hepatic injury are shown in (Table 1). These results revealed a significant elevation in serum of AST, ALT, ALP and LDH activity in the CCl₄-treated group compared with the normal control. Administration of methanol extract of *S. grandiflorum* leaf at two different dose levels attenuated the increased levels of the serum enzymes, produced by CCl₄.

Antioxidant enzymes
The effects of methanol extract of *S. grandiflorum* leaf at two dose levels (100 and 200 mg/kg, b.w.) on liver antioxidant enzymes in CCl₄-induced hepatic injury are shown in (Table 2). The level of SOD, GSH and GST decreased substantially in CCl₄-treated group when compared with normal control group. In rats which received CCl₄ with methanol extract (group III and group IV), there was a dramatic increase in SOD, GSH and GST activity compared to the CCl₄-treated group.

Lipid peroxidation was increased in CCl₄ group, as indicated by elevated MDA levels, when compared with the normal control group. Treatment with *S. grandiflo-
rum methanol extract 100mg and 200mg/kg significantly decreased the MDA levels (Table 2).

Histopathological evaluation

The CCl$_4$ induced histopathological changes in the liver with significant degeneration and necrosis of hepatocytes in the centrilobular region and with perivenular inflammatory infiltrates. Histological changes, including increased degeneration, necrosis, hepatitis and portal triaditis were evaluated by hematoxylin and eosin stain in rat liver. In normal control animals (Group I), liver sections showed normal hepatic cells with well-preserved cytoplastom, prominent nucleus and nucleolus, and central vein (Figure 4A). The liver sections of animals treated with CCl$_4$ (Group II) showed a moderate degree of centrilobular necrosis, and mild degree of infiltration of leukocytes, bile duct proliferation, mitosis, and calcification (Figure 4B). Treatment with methanol extract (Group III and Group IV) ameliorated the CCl$_4$-induced liver injury and the typical histological changes were markedly alleviated in the liver sections (Figure 4C, 1D). Rats treated with only methanol extract (Group V) showed normal architecture of hepatocytes (Figure. 4E).

DISCUSSION

Free radicals have been a subject of significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many workers. It has been proved that free radicals play an important role in the pathogenesis of certain diseases and aging. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing, the development of complications associated with diseases (Ross et al., 1982; Sunil and Ignacimuthu, 2011). Many synthetic antioxidant components have shown toxic and/or mutagenic effects. Hence, attention has been given to naturally occurring antioxidants. Numerous plant constituents have shown free radical scavenging or antioxidant activity (Arumoa and Cuppett, 1997). Flavonoids and other phenolic compounds (hydroxyl cinnamic derivatives, catechines, etc.) of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (Formica and Regelson, 1995). Polyphenols are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties (Nitin et al., 2010; Sunil and Ignacimuthu, 2011) which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Our study revealed the antioxidant property of *S.grandiflorum* leaf extract. We believe that this was due to the presence of good amount of phenolics as estimated by Folin–Ciocalteau method.

In this study two different free radicals were used to assay the free radical scavenging activity of the extract and fractions of *S.grandiflorum* namely, DPPH$^+$ and ABTS$^{**}$. The observed differential scavenging activities of the extracts against DPPH$^+$ and ABTS$^{**}$ radicals may be referred to the different mechanisms of the radical antioxidant reactions in the two assays. In the DPPH test, the extracts reduced the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The method was based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction (Brand-Williams et al., 1995). MD formulation exhibited good DPPH free radical and ABTS scavenging activity which confirm antioxidant activities which might be due to presence of phenolic compounds. It was reported that flavonoids are phenolic compounds that exert multiple biological effects on cellular system (Hazra et al., 2008).

The liver is one of the vital organs in our body responsible for detoxification of toxic chemicals and drugs. Thus, it is the target organ for all toxic chemicals. Carbon tetrachloride (CCl$_4$) has been extensively used in animal models to investigate chemical induced hepatic injury. The trichloromethyl free radical (CCl$_3$•), an active metabolite of CCl$_4$, is mainly associated with CCl$_4$-induced hepatic damage (Johnston, 1998). These radicals are suggested to react with sulfhydryl groups of glutathione and protein thiols. The covalent binding of these radicals to sulfhydryl-containing proteins in cells will initiate a chain of events leading to membrane lipid peroxidation and cell necrosis (Reckengel, 1989; Muriel, 2001). Administration of CCl$_4$ markedly raised the serum level of enzymes such as AST, ALT, ALP and LDH in rats. This increase in the serum AST, ALT, ALP and LDH enzyme levels in CCl$_4$-treated animals indicates hepatic cell damage (Wolf, 1999). However, pre-treatment of the rats with Methanol extract of *S.grandiflorum* at 100 and 200 mg/kg b.w for 7 days prior to CCl$_4$ administration resulted in a significant decrease in serum AST, ALT, and LDH activities. With respect to the histological examination, pre-treatment with Methanol extract (Group III and Group IV) suppressed the acute hepatic damage and was consistent with an improvement in the serum biological parameters of hepatotoxicity.

Reactive oxygen species (ROS), such as superoxide anions and H$_2$O$_2$, are produced in cells during normal aerobic metabolism. The intracellular concentration of ROS is a consequence of both their production and removal by various antioxidants. The antioxidant activity and the inhibition of free radical generation are important in terms of protecting the liver from CCl$_4$-induced damage. Generally, lipid peroxides or reactive oxygen species easily inactivate an antioxidant enzyme such as SOD which results in decreased activities of these enzymes in CCl$_4$ toxicity. SOD is an extremely effective antioxidant enzyme, which is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to H$_2$O$_2$ (Reiter, 2000).

Methanol extract of *S.grandiflorum* potentially increased the levels of SOD in treated rats when clean.
pared with CCl₄ alone treated rats. ME exhibits preventive effect in CCl₄ induced hepatotoxicity by reducing the levels of oxidative injuries on hepatocytes. GST is important markers in protecting the liver from damage of lipid peroxidation. When trichloromethyl appeared as a highly reactive metabolite of CCl₄, GST would immediately increase in high level in blood, promoting the combination of free radicals and cell proteins (Sugiyma, 2006). GSH is a major non-protein thiol in living organisms, which plays a central role in coordinating the body’s antioxidant defence processes. Perturbation of GSH status of a biological system has been reported to lead to serious consequences (Ozer, 2008; Janero, 1990).

Lipid peroxidation is a major parameter, which can be included as a marker of oxidative damage. The biochemical mechanisms involved in the development of CCl₄-mediated hepatotoxicity have long been investigated. MDA is widely used as a marker of lipid peroxidation (Mansour, 2000). Rats treated with CCl₄ showed a striking increase in MDA levels compared with the normal control group. Pre-treatment with different doses of ME (100 & 200 mg/kg b.w) produced potential reductions in CCl₄-induced MDA elevations.

According to microscopic evaluations, severe liver damage induced by CCl₄ was markedly reduced by the administration of ME of S. grandiflorum, which was in good correlation with the results of hepatic antioxidant enzyme activities and hepatic lipid peroxidation.

CONCLUSION

In the present study, Methanol extract of S. grandiflorum restored the elevated serum enzyme levels and decreased liver antioxidant parameters, suggesting that it has hepatoprotective, curative, and antioxidant capacities in CCl₄-intoxicated rats. However, the protective, curative and antioxidant qualities of S. grandiflorum need to be confirmed using larger number of animals, by characterizing the active ingredient(s) of this plant as well as its mechanism(s) of action.

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DECLARATION OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES


