HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF DECALEPS HAMILTONII AGAINST ACETAMINOPHEN-INDUCED HEPATIC INJURY IN RATS

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ABSTRACT

This study was undertaken to investigate the protective effect of methanol extract of Decaleps hamiltonii on acetaminophen-induced hepatotoxicity in rats. Hepatotoxicity was induced by administering an oral dose of acetaminophen (2 g/kg b.wt) to rats for 10 days resulting in significantly elevated levels of hepatic marker enzymes in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin and a significantly decreased serum levels of total protein were noted, compared to controls. In the liver, significantly elevated levels of lipid peroxidation (LPO), and lowered levels of antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD) and non-enzymatic antioxidants like reduced glutathione (GSH) and ascorbic acid were observed following acetaminophen administration. When rats with acetaminophen-induced hepatotoxicity were treated with the extract of Decaleps hamiltonii, the serum ALT, AST, ALP, bilirubin and total protein levels reverted to near normal, while the hepatic concentration of CAT, SOD, GSH and ascorbic acid were significantly increased and that of LPO significantly lowered, when compared to acetaminophen -induced rats. Histopathological studies confirmed the hepatoprotective effect conferred by the extract of Decaleps hamiltonii. These results reveal that a methanolic extract of Decaleps hamiltonii is able to significantly alleviate the hepatotoxicity induced by acetaminophen in the rat.

Keywords: Acetaminophen; Hepatotoxicity; Decaleps hamiltonii; Free radical. Antioxidants

INTRODUCTION

Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Liver damage is the widespread pathology which in most cases involves oxidative stress and is characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages. Free radical initiated auto oxidation of cellular membrane lipids can lead to cellular necrosis and is now accepted to be important in connection with a variety of pathological conditions. Reactive oxygen species (ROS), from both endogenous and exogenous, are implicated in the etiology of several degenerative diseases, such as coronary artery diseases, stroke, rheumatoid arthritis, diabetes and cancer1. It is well known that, free radicals are the reactive species derived from them cause damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. In order to protect the tissues from damage caused by ROS, organisms possess enzymatic and non-enzymatic antioxidant systems2.

Mammalian cells possess elaborate defense mechanisms for radical detoxification. Key metabolic steps are super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which destroy toxic peroxides. Some non-enzymatic molecules including thioredoxin, thiols and disulfide bonding play important roles in antioxidant defense systems. Some of these compounds are obtained from food such as α- tocopherol, β- carotene and ascorbic acid and such micronutrient elements as zinc and selenium. High consumption of fruits and vegetables is associated with low risk for these diseases, which is attributed to the antioxidant vitamins and other phytochemicals. There is a great deal of interest in edible plants that contain antioxidants and health promoting phytocompounds, in view of their health implications.

Treating liver diseases with herbal drugs has a long tradition in India, China and Japan. About 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess liver protective activity. About 600 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the globe. Around 40 patented polyherbal formulations representing a variety of combination of 93 Indian herbs from 44 families are available in Indian market. Some herbal preparations exist as standardized extracts with major known ingredients or even pure compounds.
MATERIALS AND METHODS

Preparation of the Decalepis hamiltonii extract

The root of Decalepis hamiltonii were collected from local market in Chennai, India and the plant material was identified and authenticated taxonomically at Plant Anatomy and Research Center, Chennai, India. The dried roots were powdered to 40 meshes, the powder was defatted with petroleum ether (60°–80°C) and then extracted with 90% methanol using Soxhlet apparatus. The residue was filtered and concentrated to a dry mass by vacuum distillation; the filtrate thus obtained was used as Decalepis hamiltonii extract.

Experimental design

Healthy adult male albino rats of body weight ranging from 120–150 g were housed in polypropylene cages under controlled conditions of temperature (25±2°C) with a 12-h/12-h day-night cycle, during which they had free access to food and water ad libitum and fed standard pellet diet (obtained from Sai-Durga feeds and foods, Bangalore, India). Animals were maintained per national guidelines and protocols approved by the Institutional Animal Ethical Committee, proposal number being JSSCP/IAEC/p cog/06/2010-2011.

The animals were divided into five groups of six rats each:

Group 1: Control rats received only olive oil orally (vehicle) (2g/kg bw)
Group 2: Rats received acetaminophen (2g/kg bw orally after every 72 hours for 10 days).
Group 3: Rats received acetaminophen orally + 100mg of methanolic extract of Decalepis hamiltonii/kg bw orally for 10 days.
Group 4: Rats received acetaminophen orally + 200mg of methanolic extract of Decalepis hamiltonii/kg bw orally for 10 days.
Group 5: Rats received acetaminophen orally + 400mg of methanolic extract of Decalepis hamiltonii/kg bw orally for 10 days.

At the end of the experimental period, all the animals were killed by cervical decapitation. From each animal, blood samples were collected and the hepatic tissue was excised. All the samples were stored at -80°C until analysis.

Preparation of serum and hepatic tissue samples for analysis

From each blood sample, serum was separated by centrifugation at 2500 rpm for 10 minutes for various biochemical estimations. Prior to biochemical analysis, each hepatic tissue (100 mg/ml buffer) was homogenized in 50 mM phosphate buffer (pH 7.0); the homogenate was then centrifuged at 10,000 rpm for 15 min and the supernatant obtained was used for biochemical analysis. All liver parameters level, the tissue homogenate (in 0.1 M phosphate buffer pH 7.4) was centrifuged at 2000 rpm for 10 min at 4°C. The supernatant was centrifuged at 10,000 rpm for 15 min and the supernatant obtained was used for biochemical analysis. All liver parameters were expressed as activity per mg protein. The protein concentration in each fraction was determined by the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard.

Biochemical analysis

To assess the membrane damage, the activities of liver marker enzymes like Alkaline phosphatase (ALP) by king’s method 196522, and the liver marker enzymes were expressed as activity per mg protein. The protein concentration in each fraction was determined by the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard.

Determination of Lipid peroxidation in hepatic tissue

The extent of lipid peroxidation was assayed by analysis of thiobarbituric reactive substance by Ohkawa et al method24. TBARS in tissues was estimated by the method of Ohkawa et al. To 0.5 ml tissue homogenate, 0.5 ml saline and 1.0 ml of 10% TCA were added, mixed well and centrifuged at 3000 rpm for 20 min. To 1.0 ml of the protein-free supernatant, 0.25 ml of thiobarbituric acid (TBA) reagent was added; the contents were mixed and heated for 1 h at 95°C. The tubes were cooled to room temperature under running water and absorption measured at 532 nm. The levels of lipid peroxides were ex-pressed as nmol of thiobarbituric acid reactive substances (TBARS)/mg protein.

Determination of activities of antioxidant enzymes

The antioxidant enzymes occurring in the liver tissue of the rats were assayed.

Catalase (CAT). CAT activity was determined by the method of Sinha26. In this test, dichromatic acetic acid is reduced to chromic acid when kept in the presence of hydrogen peroxide (H₂O₂), with the formation of perchloric acid as an unstable intermediate. In the test, the green color developed was read at 590nm against blank on a spectrophotometer. The activity of CAT was expressed as units/mg protein (μmol of H₂O₂ consumed/min/mg protein).

Superoxide dismutase (SOD). The activity of SOD in tissue was assayed by the method of Kakkar et al27. Briefly, reaction mixture contained 0.2 ml sodium pyrophosphate buffer (pH 8.3, 0.025 mol/L), 0.1ml phenazine methosulphate (186 mM), 0.3 ml NBT (300 mM), 0.2 ml NADH (780 mM) and approximately diluted enzyme preparation and water in a total volume of 3 ml. After incubation at 30°C for 90 sec, the reaction was terminated by the addition of 1.0 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml n-butanol. The color intensity of the chromogen in the butanol layer was measured at 560 nm against n-butanol and concentration of SOD was expressed as units/mg protein. Absorbance values were compared with a standard curve generated from known SOD.

Glutathione peroxidase (GPx). GPx activity was measured by the method described by Rotruck et al28. Briefly, reaction mixture contained 0.2ml of 0.4 M Tris-HCl buffer pH 7.0, 0.1 ml of 10 mM sodium azide, 0.2 ml of tissue homogenate (homogenized in 0.4 M, Tris-HCl buffer, pH 7.0), 0.2 ml glutathione, and 0.1 ml of 0.2 mM hydrogen peroxide. The contents were incubated at 37°C for 10 min. The reaction was arrested by 0.4 ml of 10% TCA, and centrifuged. Supernatant was assayed for glutathione content by using Ellman’s reagent (19.8 mg of 5,5’-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate).

Determination of levels of non-enzymatic antioxidants

Non-enzymatic antioxidant components of the liver tissue samples of the experimental animals were assayed.

Reduced glutathione (GSH). GSH content was estimated by the method of Ellman29. To measure the reduced glutathione (GSH) level, the tissue homogenate (in 0.1 M phosphate buffer pH 7.4) was taken. The homogenate was added an equal volume of 20% tetrachloroacetic acid (TCA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 200 rpm. The supernatant (200μl) was then transferred to a new set of test tubes and added 1.8 ml of the Ellman’s reagent (5, 5’-dithiobis-2-nitrobenzoic acid (0.1 mM) which was prepared (0.3M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes were made up to the volume of 2 ml. After completion of the total reaction, solutions were measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from standard curve from known GSH.

Ascorbate (Vitamin C). Ascorbic acid concentration was measured by Omaye et al. method30. To 0.5 ml of plasma/0.5 ml liver homogenate, 1.5 ml of supernatant, 0.5 ml of DNPH reagent (2% DNPH) and 4%thiouria in 9 N sulphuric acids) was added and incubated for 3 h at room temperature. After incubation 2.5 ml of 8.5% sulphuric acid was added and color developed was read at 530 nm after 30 min.

Histopathological investigation

After sacrificing the rats by cervical decapitation, hepatic tissues were collected, washed in normal saline and fixed in 10% formalin for 24h and dehydrated with alcohol. Hepatic tissues were cleaned and embedded in paraffin, cut in 3-5μM sections, and stained with routine haematoxylin-eosin (HE) dye and finally observed under light microscope.

light microscope and morphological changes such as cell necrosis, fatty changes or inflammation of lymphocytes were observed.

**Statistical Analysis**

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The values are mean ± SD for six rats in each group. The students' t-test was used to compare the means of specific groups, with p< 0.05 considered as significant.

**RESULTS**

The effect of methanolic extract of *Decalepis hamiltonii* on activities of hepatic marker enzymes on serum transaminases and alkaline phosphatase levels in acetaminophen intoxicated rats are summarized in table 1. A significant (p<0.05) increase in the activities of the serum enzymes AST, ALT and ALP were observed in rats receiving acetaminophen (group II) when compared to normal (group I) rats administered vehicle alone. However, the activities of these serum enzymes were significantly (p<0.05) lower in rats treated with the *Decalepis hamiltonii* extract (groups III, IV & V) than in group II rats. Interestingly, the mean activities of hepatic marker enzymes were significantly lower in group V rats than those in groups III & IV rats.

Table 2 shows the changes in the mean levels of protein and bilirubin in hepatic and serum samples of the experimental rats. Acetaminophen administration in group II rats resulted in significant (p<0.05) decrease in the levels of protein in hepatic and serum samples and a significant increase in level of serum bilirubin when compared to normal rats (group I). Treatment with *Decalepis hamiltonii* extract in groups III, IV & V rats resulted in significantly higher levels of protein and a significantly lower level of bilirubin (p < 0.05) than that in group II rats.

### Table 1: Effect of methanolic extract of *Decalepis hamiltonii* (MEDH) on activities of hepatic marker enzymes in serum samples of rats in acetaminophen -induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>61.32 ± 4.25a</td>
<td>53.85 ± 2.67b</td>
<td>66.23 ± 1.48a</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>175.41 ± 7.82a</td>
<td>126.64 ± 1.72b</td>
<td>120.23 ± 2.16a</td>
</tr>
<tr>
<td>Acetaminophen+100mg MEDH</td>
<td>145.79 ± 8.53c</td>
<td>102.29 ± 1.77c</td>
<td>85.37 ± 2.52c</td>
</tr>
<tr>
<td>Acetaminophen+200mg MEDH</td>
<td>106.32 ± 6.11d</td>
<td>96.93 ± 2.38d</td>
<td>77.75 ± 1.75d</td>
</tr>
<tr>
<td>Acetaminophen+400mg MEDH</td>
<td>66.21 ± 5.54e</td>
<td>68.95 ± 1.37e</td>
<td>68.95 ± 1.37e</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats in each group. ANOVA followed by Duncan’s multiple range tests. Values not sharing a common superscript differ significantly at P<0.05.

### Table 2: Effect of methanolic extract of *Decalepis hamiltonii* (MEDH) on levels of protein and bilirubin in hepatic and serum samples of rats in acetaminophen -induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hepatic protein (mg/dl)</th>
<th>Serum protein (mg/dl)</th>
<th>Serum bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10.04 ± 0.76a</td>
<td>4.5 ± 0.34a</td>
<td>0.91±0.52a</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>7.5 ± 0.57b</td>
<td>2.5 ± 0.19b</td>
<td>2.41±0.18b</td>
</tr>
<tr>
<td>Acetaminophen+100mg MEDH</td>
<td>8.5 ± 0.68c</td>
<td>3.25 ± 0.24c</td>
<td>2.02±0.32c</td>
</tr>
<tr>
<td>Acetaminophen+200mg MEDH</td>
<td>9.0 ± 0.68c</td>
<td>3.0 ± 0.22c</td>
<td>1.8±0.01d</td>
</tr>
<tr>
<td>Acetaminophen+400mg MEDH</td>
<td>9.6 ± 0.73d</td>
<td>4.0 ± 0.30d</td>
<td>0.98±0.16a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats in each group. ANOVA followed by Duncan’s multiple range tests. Values not sharing a common superscript differ significantly at P<0.05.

The mean levels of LPO and weight in the hepatic tissue of group II (acetaminophen -induced) rats were significantly higher than that in group I (normal) rats (table 3). Treatment with *Decalepis hamiltonii* extract in groups III, IV and V rats were found to result in a significant (p < 0.05) lowering of the mean levels of LPO and liver weight, presumably by limiting lipid peroxidation in the hepatic tissue. Interestingly, the mean levels of LPO and liver weight were significantly lower in group V rats than those in groups III & IV rats.

A significant decrease in CAT, SOD and GPx activity was observed in the hepatic tissue of acetaminophen -induced (group II) rats when compared to normal (group I) rats that had received vehicle alone (table 4). Treatment with the extract of *Decalepis hamiltonii* appeared to exert a beneficial effect since the activities of CAT, SOD and GPx were significantly (p < 0.05) higher in hepatic tissue of groups III, IV and V than those in group II rats. The mean activities of antioxidant enzymes were significantly higher in group V rats than those in groups III & IV rats.

### Table 3: Effect of methanolic extract of *Decalepis hamiltonii* (MEDH) on levels of lipid peroxidation and liver weight in Acetaminophen -induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver weight (wt/100 g tissue)</th>
<th>Lipid peroxidation (nmol of MDA/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.2 ± 0.12a</td>
<td>1.43 ± 0.45a</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>6.5 ± 0.19b</td>
<td>3.89 ± 0.89b</td>
</tr>
<tr>
<td>Acetaminophen+100mg MEDH</td>
<td>5.9 ± 0.16c</td>
<td>2.25 ± 0.26c</td>
</tr>
<tr>
<td>Acetaminophen+200mg MEDH</td>
<td>5.8 ± 0.12d</td>
<td>2.02 ± 0.31c</td>
</tr>
<tr>
<td>Acetaminophen+400mg MEDH</td>
<td>3.3 ± 0.19e</td>
<td>1.77 ± 0.15c</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats in each group. ANOVA followed by Duncan’s multiple range tests. Values not sharing a common superscript differ significantly at P<0.05.
Table 4: Effect of methanolic extract of *Decalepis hamiltonii* (MEDH) on activities of enzymatic antioxidants in acetaminophen-induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>CAT (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>34.88 ± 0.90</td>
<td>3.51 ± 0.592</td>
<td>20.28 ± 0.31</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>14.21 ± 0.27</td>
<td>1.16 ± 0.17</td>
<td>10.08 ± 0.10</td>
</tr>
<tr>
<td>Acetaminophen+100mg MEDH</td>
<td>23.23 ±0.31</td>
<td>2.50 ± 0.569</td>
<td>12.34 ± 0.35</td>
</tr>
<tr>
<td>Acetaminophen+200mg MEDH</td>
<td>29.82 ± 0.31</td>
<td>2.48 ± 0.49</td>
<td>14.35 ± 0.36</td>
</tr>
<tr>
<td>Acetaminophen+400mg MEDH</td>
<td>34.74 ±0.84</td>
<td>3.17 ± 0.09</td>
<td>16.48 ± 0.50</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats in each group. ANOVA followed by Duncan’s multiple range tests. Values not sharing a common superscript differ significantly at P ≤ 0.05.

Table 5: Effect of methanolic extract of *Decalepis hamiltonii* (MEDH) on levels of non-enzymatic antioxidants in acetaminophen induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (μg/mg protein)</th>
<th>Vitamin C (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5.5 ± 0.3</td>
<td>8.0 ± 0.60</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>1.26 ± 0.26</td>
<td>2.5 ± 0.19</td>
</tr>
<tr>
<td>Acetaminophen+100mg MEDH</td>
<td>3.67 ± 0.29</td>
<td>5.0 ± 0.38</td>
</tr>
<tr>
<td>Acetaminophen+200mg MEDH</td>
<td>4.97 ± 0.25</td>
<td>6.21 ± 3.07</td>
</tr>
<tr>
<td>Acetaminophen+400mg MEDH</td>
<td>5.6 ± 0.24</td>
<td>7.78 ± 1.34</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats in each group. ANOVA followed by Duncan’s multiple range tests. Values not sharing a common superscript differ significantly at P ≤ 0.05.

Table 5 lists the levels of non-enzymatic antioxidants in hepatic tissue samples of the experimental animals. A significant (p<0.05) decrease in the mean levels of GSH and vitamin C was observed in the hepatic tissue of acetaminophen-induced (group II) rats when compared to normal (group I) rats. Treatment with *Decalepis hamiltonii* extract in groups III, IV & V rats resulted in a significantly higher concentration of GSH and vitamin C than that in group II rats. The mean levels of GSH and vitamin C were significantly higher in group V rats than those in groups III & IV rats.

**Histopathological examinations**

When compared to the histoarchitecture of the hepatic tissue of group I (normal) animals (Fig.1) with hepatic cells of group II rats (acetaminophen-induced) revealed extensive damage, characterized by the disruption of the lattice nature of the hepatocyte, damaged cell membranes, degenerated nuclei, disintegrated central vein and damaged hepatic sinusoids (Fig. 2). Treatment with methanolic extract at the dose 100, 200 and 400mg/kg body weight is shown in Fig.3, 4 and 5 respectively.
in glutathione in the liver as the cause of hepatotoxicity have been resulting in cell necrosis and lipid peroxidation induced by decrease reported earlier32. Hepatic cells appear to participate in a variety of enzymatic metabolic activities and acetaminophen produced marked liver damage at the given doses as expected32.

In the assessment of liver damage by acetaminophen, the determination of enzyme levels such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) is largely used. Drotman et al14 reported that the elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane35. This results in decreased levels of SGOT, SGPT and ALP in the hepatic cells and a raised level in serum. High levels of SGOT indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manner. Therefore, SGPT is more specific to the liver and is thus a better parameter for detecting liver injury36.

Serum ALP and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure37. The rise in the level of serum bilirubin is most sensitive and confirms the intensity of jaundice38. Bilirubin is one of the most useful clinical clue for the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte39. In our study treatment with methanolic extract of Decalepis hamiltonii significantly reduced the levels of these enzymes which are an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Effective control of ALP and bilirubin levels points towards an early improvement in the secretory mechanism of the hepatic cell.

Administration of methanolic extract of Decalepis hamiltonii significantly increased the decreased level of protein and also preserves the structural integrity of the hepatocellular membrane and liver cell architecture damaged by acetaminophen, was confirmed by histopathological studies. Previous studies have proved that lipid peroxidation has been postulated as being the destructive process in liver injury due to acetaminophen administration35. Lipid peroxide levels were significantly increased in acetaminophen intoxicated rats were revealed in our study. Guillen-Sans et al38 reported an increase in MDA levels (in terms of TBARS) suggested an enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. The treatment with methanolic extract of Decalepis hamiltonii significantly reversed these changes. Hence it may be possible that the mechanisms of hepatoprotection of methanolic extract of Decalepis hamiltonii is due to its antioxidant effect.

Antioxidants and radical scavengers were to study the mechanism of acetaminophen toxicity as well as to protect liver cells from acetaminophen induced damage. In this study, we observed a decrease in catalase activity in liver tissue during chronic administration of acetaminophen. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals40. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical induced cellular damage. Gupta et al41 reported that the excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to accumulation of superoxide radicals and hydrogen peroxide. Administration of methanolic extract of Decalepis hamiltonii increases the activity of catalase in acetaminophen induced liver damage in rats to prevent the accumulation of excessive free radicals and protects the liver from acetaminophen intoxication.

SOD dismutates superoxide radicals O$_2^-$ into hydrogen peroxide plus O$_2$, thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. In this study, SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues. Curtis et al42 reported that the decrease in serum activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury. In the present study, it was observed that methanolic extract of Decalepis hamiltonii caused a significantly increased in the hepatic SOD activity of the acetaminophen induced liver damage in rats. This shows methanolic extract of Decalepis hamiltonii may be associated with decreased oxidative stress and free radical mediated tissue damage.

Glutathione is a non-enzymatic biological antioxidant present in the liver43. Its functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoy radicals and maintenance of membrane protein thikos and as a substrate for GPx and GST44. Deficiency of GSH within living organisms can lead to tissue disorder and injury. Example includes liver injury induced by consuming alcohol or by taking drugs like acetaminophen, lung injury by smoking and muscle injury by intense physical activity45. All are known to be correlated with low levels of GSH.

Acetaminophen intoxication produces significant depletion of GSH and imbalance of GSH/GSSG ratio. The reduced form of GSH becomes readily oxidized to GSSG on interacting with free radicals46. Excessive production of free radicals resulted in the oxidative stress,
which leads to damage to biomolecules e.g., lipids and can induce lipid peroxidation. In our present study, decreased level of GSH has been associated with an enhanced lipid peroxidation in acetaminophen treated rats. Administration of methanolic extract of *Decalepis hamiltonii* significantly increased the level of GSH in a dose dependent manner.

Vitamin C acts as an antioxidant in biological systems and scavenges the free radicals thereby increase the antioxidant defense in the body. Vitamin C is an excellent hydrophilic antioxidant; it readily scavenges ROS and peroxy radicals. Also acts as a co-antioxidant by regenerating the vitamin A, E and GSH from radicals. In our study we have observed a decreased level of vitamin C in serum of acetaminophen induced hepatic damaged rats. Chatterjee proved that the decreased level could be the increased utilization of vitamin C in deactivation of the increased levels of ROS or too decreased in the GSH level. Since, the GSH is required for the recycling of vitamin C. Administration of methanolic extract of *Decalepis hamiltonii* increase the serum level of vitamin C may be expected to enhance the GSH level or stimulation of the system to recycle the dehydroascorbic acid to ascorbic acid.

**Histopathological Study**

Histology of liver sections of normal control animals (group I) showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and central vein (Fig 1). Acetaminophen treated animals (Fig 2) shows that the liver cells of rats intoxicated with cells have high degree of damage, characterized by necrosis along with various gradation of fatty changes of tiny to large sized vacuoles (fatty droplets). The normal architecture of liver was completely damaged. The hepatic cells of rats treated with 100 and 200mg of methanolic extract of *Decalepis hamiltonii* showed mild fatty change with tiny vacuolation, but is somewhat similar to normal (Fig 3 and 4). The hepatic cells of rats treated with 400mg of methanolic extract of *Decalepis hamiltonii* showed almost normal hepatic cells but some damaged cells could also been seen (Fig 5), but as compared to acetaminophen damaged cells, the number of hepatocytes with normal nucleus are much more, and vacuolation in cytoplasm are observed to be low. Methanolic extract of *Decalepis hamiltonii* treatment exhibited protection against liver damage caused by acetaminophen which is confirmed by the results of biochemical studies.

**CONCLUSION**

Our photochemical study showed the presence of flavanoids, steroids, tannins and saponins in methanolic extract of *Decalepis hamiltonii*. It is known that some flavanoids are able to reduce xenobiotic induced hepatotoxicity in animals\(^1\). The inhibitory activity of flavanoids on free radical production could be related their hepatoprotective effects since exogenous antioxidants may counteract the damaging effects of oxidative stress, co-operating with natural systems like glutathione, tocopherol or protective enzymes\(^2\). Our results shows that the hepatoprotective and antioxidant effect of methanolic extract of *Decalepis hamiltonii* may be due to its antioxidant and free radical scavenging properties. In conclusion, the results of this study demonstrate that methanolic extract of *Decalepis hamiltonii* has a potent hepatoprotective action upon acetaminophen induced hepatic damage in rats.

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