Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

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

M.DEVI*[a] AND P.LATHA[b]

[a]Department of Biochemistry, Muthayammal College of Arts and Science, Rasipuram 637408, Namakkal, Tamil Nadu, India, [b]Department of Biochemistry and Molecular Biology, Pondicherry University, Puducherry 605014, India. Email: kmdevi6@gmail.com

Received: 23 Jan 2012, Revised and Accepted: 18 Mar 2012

ABSTARCT

This study was undertaken to investigate the protective effect of methanol extract of *Decalepis 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 resulted 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 *Decalepis 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 *Decalepis hamiltonii*. These results reveal that a methanolic extract of *Decalepis hamiltonii* is able to significantly alleviate the hepatotoxicity induced by acetaminophen in the rat.

Keywords: Acetaminophen; Hepatotoxicity; Decalepis 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 wide spread 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[1]. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [2]. 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[3]. 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 cancer[4]. 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[5]. In order to protect the tissues from damage caused by ROS, organisms possess enzymatic and non-enzymatic antioxidant systems[6].

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[7]. High consumption of fruits and vegetables is associated with low risk for these diseases, which is attributed to the antioxidant vitamins and other phytochemicals[8]. There is a great deal of interest in edible plants that contain antioxidants and health promoting photochemical, 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[9]. Some herbal preparations exist as standardized extracts with major known ingredients or even pure compounds.

Decalepis hamiltonii Wight and Arn (swallow root) (Family: Asclepediaceae) commonly called as maredu kommulu or barre sugandhi or maradu gaddalu or makali beru. *Decalepis hamiltonii* is a monogeneric climbing shrub endemic to the Deccan peninsula, which is used as a culinary spice due to its highly aromatic roots. Earlier studies have shown that *Decalepis hamiltonii* root contain aldehyde, inositols, saponins, amyrins and lupeols[10], as well as volatile compounds such as 2-hydroxy-4-methoxybenzaldehyde, vanillin, 2-phenyl ethyl alcohol, benzaldehyde and others[11]. It has been used as an appetizer, blood purifier, relieves flatulence, preservative and as a source of bioinsecticide for stored food grains, also as a general tonic and as a juice for its alleged health promoting properties[12]. The roots have also been used as a substitute for Hemidesmus indicus in ayurvedic preparations of ancient Indian medicine[13].

Acetaminophen (Paracetamol) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. Acetaminophen is mainly metabolized in liver to excretable glucuronide and sulfate conjugates[14]. However, the hepatotoxicity of acetaminophen has been attributed to the formation of toxic metabolites when a part of acetaminophen is activated by hepatic Cytochrome P-450[15], to a highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI)[16]. NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid[17]. However when the rate of NAPQI formation exceeds the rate of detoxification of GSH, it oxidize tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH.

In recent years, plant derived natural products such as flavanoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological activity[18]. *Decalepis hamiltonii* possesses potent antioxidant properties which could be associated with their health benefits[19], neuroprotective[20] and antiulcer activity[21]. Hence, the present study was designed to evaluate the hepatoprotective activity of an extract of *Decalepis hamiltonii* in an experimental model of acetaminophen -induced hepatotoxicity in Wistar rats. The various biochemical parameters viz., levels of hepatic marker enzymes [serum oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP)] bilirubin and total protein, indicators of oxidative stress [lipid peroxidation (LPO)], antioxidant profile were evaluated and the results were correlated with histopathological observations.

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 coarse powder. 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±2ºC) with a 12-h/12-h day- night cycle, during which time they had free access to food and water ad libitium 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 -80ºC 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 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 asses the membrane damage, the activities of liver marker enzymes like Alkaline phosphatase (ALP) by king's method 1965[22], Alanine transaminase (ALT) and Aspartate transaminase (AST) by Reitman and Frankel method[23] were assayed. Serum Proteins by Lowry method[24] and serum Bilirubin by Malloy and Evelyn method[25] were assayed.

Determination of Lipid peroxidation in hepatic tissue

The extent of lipid peroxidation was assayed by analysis the levels of thiobarbituric reactive substance by Ohkawa et al method[26]. 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 nmoles 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 Sinha[27]. In this test, dichromatic acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2), 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 H_2O_2 consumed/min/mg protein).

Superoxide dismutase (SOD).The activity of SOD in tissue was assayed by the method of Kakkar et al[28]. The assay mixture contained 1.2 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 30ºC 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.

Glutatione peroxidase (GPx). GPx activity was measured by the method described by Rotruck et al[29]. 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 Ellman[30]. 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 (TBA) 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'-dithiois-2-nitrobenzoic acid) (0.1mM) 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. method[31]. 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%thiourea 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 3-5mM sections, and stained with routine haematoxylin-eosin (H-E) dye and finally observed under 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.

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

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 $P \leq 0.05$.

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.

Groups	Hepatic protein (mg/dl)	Serum protein (mg/dl)	Serum bilirubin (mg/dl)
Normal	10.04 ± 0.76^{a}	4.5 ± 0.34^{a}	0.91 ± 0.52^{a}
Acetaminophen	7.5 ± 0.57^{b}	2.5 ± 0.19^{b}	2.41 ± 0.18^{b}
Acetaminophen+100mg MEDH	$8.5 \pm 0.68^{d,c}$	3.25 ± 0.24^{c}	2.02 ± 0.32^{c}
Acetaminophen+200mg MEDH	9.0 ± 0.68^{c}	3.0 ± 0.22^{c}	1.8 ± 0.01^{d}
Acetaminophen+400mg MEDH	$9.6 \pm 0.73^{a,d}$	4.0 ± 0.30^{d}	0.98 ± 0.16^{a}

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 $P \leq 0.05$.

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

Groups	Liver weight (wt/100 g tissue)	Lipid peroxidation (nmol of MDA/mg protein)
Normal	3.2 ± 0.12^{a}	1.43 ± 0.45^{a}
Acetaminophen	6.5 ± 0.19^{b}	3.89 ± 0.89^{b}
Acetaminophen+100mg MEDH	5.9 ± 0.16^{c}	2.25 ± 0.26^{c}
Acetaminophen+200mg MEDH	5.8 ± 0.12^{d}	2.02 ± 0.31^{c}
Acetaminophen+400mg MEDH	3.3 ± 0.19^{a}	1.77 ± 0.15^{a}

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 $P \leq 0.05$.

Table 4: Effect of methanolic extract of *Decalepis hamiltonii* (MEDH) on activities of enzymatic antioxidants in acetaminophen -induced hepatic damage in rats

Groups	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)
Normal	34.88 ± 0.90[a]	3.51 ± 0.592[a]	20.28 ± 0.31[a]
Acetaminophen	14.21 ± 0.27[b]	1.16 ±0 .17[b]	10.08 ± 0.10[b]
Acetaminophen+100mg MEDH	23.23 ±0.31[c]	2.50± 0.569[c]	12.34 ± 0.35[c]
Acetaminophen+200mg MEDH	29.82 ± 0.83[d]	2.48± 0.49[c]	14.35 ± 0.36[d]
Acetaminophen+400mg MEDH	34.74 ±0.84[a]	3.17 ± 0.09[a]	16.48 ± 0.50[e]

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 $P \leq 0.05$.

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

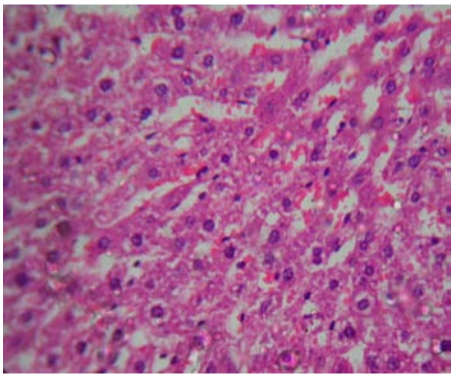
Groups	GSH (µg/mg protein)	Vitamin C (mg/100ml)
Normal	5.5 ± 0.3[a]	8.0 ± 0.60[a]
Acetaminophen	1.26 ± 0.26[b]	2.5 ± 0.19[b]
Acetaminophen+100mg MEDH	3.67 ± 0.29[c]	5.0 ± 0.38[c]
Acetaminophen+200mg MEDH	4.97 ± 0.25[d]	6.21 ± 3.07[d]
Acetaminophen+400mg MEDH	5.6 ± 0.4[a]	7.78 ± 1.34[a]

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 $P \leq 0.05$.

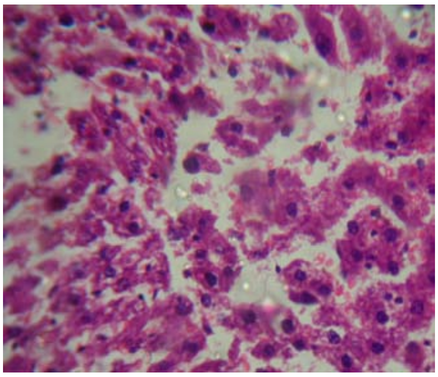
Table 5 lists the levels of non-enzymatic antioxidants in hepatic tissue samples of the experimental animals. A significant ($p<0.05$) decrease in the mean levels of GSH and vitamin C was observed in the hepatic tissue of acetaminophen -induced (group II) rats when compared to normal (group I) rats. 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

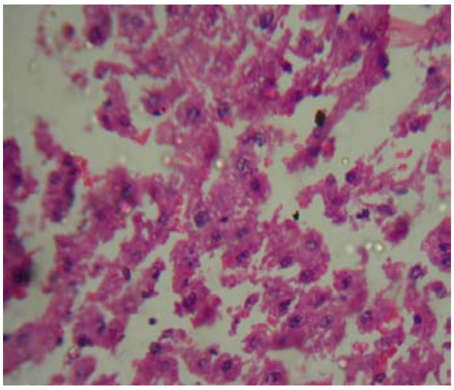
When compared to the histoarchitecture of the hepatic tissue of group I (normal) animals (Fig.1) with hepatic cells of group II rats (acetaminophen -induced) revealed extensive damage, characterized by the disruption of the lattice nature of the hepatocyte, damaged cell membranes, degenerated nuclei, 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.



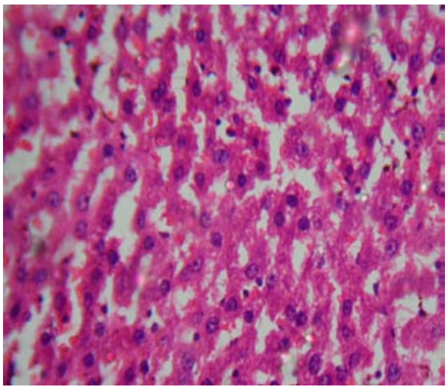
Group1: Normal



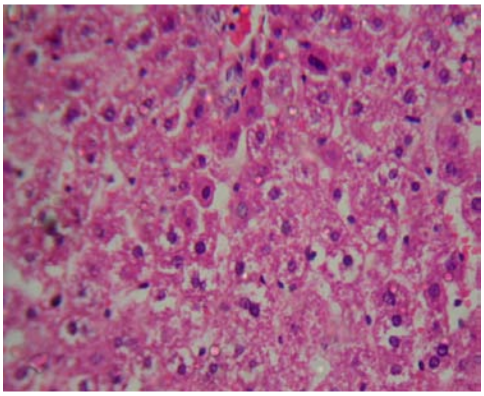
Group 2: Acetaminophen



Group3: Acetaminophen+100mg MEDH



Group4: Acetaminophen+200mg MEDH



Group5: Acetaminophen+400mg MEDH

DISCUSSION

The present studies were performed to asses the hepatoprotective activity of methanolic extract of *Decalepis hamiltonii* in rats against acetaminophen as hepatotoxin to prove its claims in folklore practice against liver disorders.

Acetaminophen a widely used antipyretic and analgesic drug produces acute liver damage if accidental overdoses are consumed. The covalent binding of N-acetyl p- benzoquinoamine, an oxidation product of acetaminophen, to sulphydryl groups of proteins resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier[32]. Hepatic cells appear to participate in a variety of enzymatic metabolic activities and acetaminophen produced marked liver damage at the given doses as expected[33].

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 al[34] 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 membrane[35]. 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 injury[36].

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 pressure[37]. The rise in the level of serum bilirubin is most sensitive and confirms the intensity of jaundice[34]. 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 hepatocyte[38]. 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 secretary 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, which 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 administration[39]. Lipid peroxide levels were significantly increased in acetaminophen intoxicated rats were revealed in our study. Guillen- Sans et al[40] 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 radicals[41]. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical induced cellular damage. Gupta et al[42] 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 al[43] 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 liver[44]. Its functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals and maintanence of membrane protein thiols and as a substrate for GPx and GST[45]. 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 activity[46]. 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 radicals[47]. Excessive production of free radicals resulted in the oxidative stress, which leads to damage to biomolecules e.g., lipids and can induce lipid peroxidation[48]. 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 scavenge the free radicals thereby increase the antioxidant defense in the body. Vitamin C is an excellent hydrophilic antioxidant; it readily scavenges ROS and peroxy radical[49]. Also acts as a co- antioxidant by regenerating the vitamin A, E and GSH from radicals[50]. In our study we have observed a decreased level of vitamin C in serum of acetaminophen induced hepatic damaged rats. Chatterjee[50] 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 some what 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[52].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[53]. 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.

ACKNOWLEDGEMENT

Authors are thankful to the department of Biochemistry of Muthayammal College of Arts and Science, Rasipuram, Tamil Nadu and Dr.B.Duraiswamy, Department of pharmacognosy, ooty, Tamil Nadu for their encouragement and technical support in testing the extracts for activity.

REFERENCES

1. Kodavanti PR, Joshi UM, Young YA, Meydrech EF, Mehendale HM. Protection of hepatotoxin and lethal effects of carbon tetrachloride by partial hepatectomy. Toxicology and Pathology 1989; 17: 494-505.
2. Wendel A, Feurensteins S, Konz KH. Acute paracetamol intoxication of starved mice leads to lipid peroxidation in vivo. Biochem Pharmacol 1987; 28: 2051-2053.
3. Pryor W. Oxy- radicals and related species, their formation, life times and reactions. Ann Rev Physiol 1986; 48: 657-667.
4. Halliwell B, Gutteridge JM.C, Cross CE. Free radicals, antioxidants and human disease: Where are we now? J Lab Clin Med 1992; 119: 598-620.
5. Brattin W J, Glenda E A. Pathological Mechanisms in Carbon Tetrachloride hepatotoxicity. J Free Radical Biol. Med 1985; 1: 27-38.
6. Parthasarathy S, Santanam N, Ramachandran S, Meilhac O. Potential role of oxidized lipids and lipoproteins in antioxidant defense. Free Radic Res 2000; 33: 197-215.
7. Halliwell B, Gutteridge JMC. Free radical in biology and medicine, 3rd ed. Oxford University Press, London chapter 3.1998.
8. Prior RL. Fruits and vegetables in the prevention of cellular oxidative damage. Am J Clin. Nutr 2003; 78 (Suppl), 570S-578S.
9. Girish C, Chandra Koner B, Jayanthi S, Ramachandra Rao K, Rajesh B, Suresh CP. Hepatoprotective activity of six polyherbal formulations in carbon tetrachloride induced liver toxicity in mice. I J Exp Biol 2009; 47: 257-263.
10. Murti PBR, Sheshadiri TR. A study of the chemical compounds of *Decalepis hamiltonii*. Proceedings of Indian Academy of Science 1940; 13: 221-232.
11. Nagarajan S, Rao LJN, Gurudutt KN. Chemical composition of the volatile of *Decalepis hamiltonii* (Wight and Arn). Journal of Flavor and Fragrance 2001; 16: 27-29.
12. George J, Pereira J, Divakar S, Udaysankar K, Ravishankar GA. A method for the preparation of active fraction from the root of *Decalepis hamiltonii*, useful as bioinsectiside. Indian patent No. 1301/Del/98. 1998.
13. Nayar RC, Shetty JKP, Mary Z, Yiganasshimhan. Pharmacognostical studies on the root of *Decalepis hamiltonii* (wt and Arn) and comparison with Hemidesmus indicus (L) R.Br. Proceeding of Indian Academy of Science 1978; 87: 37-48.
14. Jollow DJ, Thorgeireson SS, Potter WZ, Hashimoto M, Mitchell JR. Acetaminophen induced hepatic necrosis VI. Metabolic disposition of toxic and non toxic doses of acetaminophen. Pharmacology 1974; 12: 251-271.
15. Savides MC, Oehme FW. Acetaminophen and its toxicity. J Appl Toxicol 1983; 3: 95-111.
16. Vermesslen N.P.E., Bessems J.G.M., Van de streat R. Molecular aspects of paracetamol induced hepatotoxicity and its mechanisms based prevention. Drug Metab Rev 1992; 24: 367-407.
17. Moore M, Thor H, Moore G, Molders P, Orrenius S. The toxicity of Acetaminophen and N- acetyl p- benzoquinonimine in isolated hepatocytes is associated with thio depletion and increased cytosolic Ca^{2+}. J Biol Chem 1985; 260: 13035-40.
18. Takeoka GR, Dao LT. Antioxidant constituent of almond [Prunus dulcis (mill) DA. Webb] hulls. J Agri Food Chem 2003; 51: 496 – 501.
19. Srivastava A, Shereen Harish R, Shivanandappa T. Antioxidant activity of the roots of *Decalepis hamiltonii* (wt and Arn). LWT-Food Science and Technology 2006; 39: 1059-1065.
20. Srivastava A, Shivanandappa T. Neuroprotective effect of *Decalepis hamiltonii* root against ethanol induced oxidative stress. Food chem 2009; 119(2): 626-629.
21. Yogender N, Jayaram S, Harish Nayaka MA, Lakshman, Dharmesh SM. Gastroprotective effect of *Decalepis hamiltonii* extract: Possible involvement of H^+- K^+- ATPase inhibition and antioxidative mechanism. J Ethanopharmacol 2007; 112: 173-179.
22. King J. The hydrolases – acid and alkaline phosphatase. *Practical Clinical Enzymology*, Van, D (ed.), Nostrand Company Ltd, London 1965: 191-208.
23. Reitman S, Frankel S. Colorimetric method for the determination of serum glutamine Lowry O.H., Rosebrough N.J., Farr A.L., Rendall R.J. Protein measurement with the Folin's phenol reagent. J Biol Chem 1951; 193: 265-275.
24. Malloy HJ, Evelyn KA. The determination of bilirubin with photoelectric colorimeter. J Biol Chem 1937; 119: 481.

25. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
26. Sinha KA. Colorimetric assay of Catalase. Anal Biochem 1972; 47: 389-394.
27. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. I J Biochem Biophys 1984; 21: 130-132.
28. Rotruck JT, Pope A.L, Gauther HE, Swanson AB, Hageman DG. Selenium: Biochemical roles as a component of glutathione peroxidase. Science 1973; 179: 588-590.
29. Ellman GL. Tissue sulphydryl groups. Arch. Biochem. Biophys 1959; 82: 70 – 77.
30. Omaye ST, Tumbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and body fluids. Methods Enzymol 1979; 62: 3-11.
31. Jollow DJ. Acetaminophen induced hepatic necrosis: (II) Role of covalent binding in vivo. J Pharmol Exp Ther 1973; 187: 195.
32. Roderick NMM, Peter PA, Peter JS. Pathology of liver. Livingstone, Great Britain, Churchill; 1989: Vol 534.
33. Drotman RB, Lawhorn GT. Serum enzymes are indicators of chemical induced liver damage. Drug Chem Toxicol 1978; 1: 163-171.
34. Zimmerman HJ, Seeff LB. Enzymes in hepatic disease In. Goodly, E.L. (Ed.), Diagnostic Enzymology. Lea and Febiger, Philadelphia; 1970.
35. Moss DW, Butterworth PJ. Enzymology and Medicine. London: pitman Medical; 1974: p 139.
36. Muriel P, Garciapina T, Perez- Alvarez V, Murelle M. Silymarin protect against paracetamol induced lipid peroxidation and liver damage. J Appl Toxicol 1992 12: 439-442.
37. Manoharan S, Jaswanth A, Sengottuvelu S, Nandhakumar J. Hepatoprotective activity of Aerva lanata Linn. Against Paracetamol induced hepatotoxicity in rats. Res J Pharm Tech 2008; 1(4): 398-400.
38. Muriel P. Peroxidation of lipids and liver damage. In: Baskin S.I., Salem H. (Ed.), Antioxidants, oxidants and free radicals. Taylor and Francis, Washington, DC 1997: p 237.
39. Guillen – Sans R, Guzman – Chozas M. The thiobarbituric acid (TBA) reaction in foods: A Rev. Crit. Rev. Food. Sci. Nutr 1998; 38: 315-330.
40. Chance B, Green Stein DS, Riughton RJW. The mechanism of Catalase action 1- steady state analysis. Arch Biochem Biophys 1952; 37: 301-339.
41. Gupta M, Muzumder UK, Sambathkumar R. Hepatoprotective and antioxidant role of Caesalpinia boundrcella on paracetamol induced hepatic damage in rats. Nat Pdt Sci 2003; 9(3): 186-191.
42. Curtis SJ, Moritz M, Snodgrass PJ. Serum enzymes derived from liver cell fraction and the response to carbon tetrachloride intoxication in rats. Gastroenterology 1972; 62: 84-92.
43. Anderson ME. Glutathione: an overview of biosynthesis and modulation. Chem Biol Interact. 1998; 111 – 112: 1-14.
44. Prakash J, Gupta SK, Kochupillai V, Singh N, Gupta YK, Joshi S. Chemopreventive activity of Withania somnifera in experimentally induced fibro sarcoma tumors in Swiss albino mice. Phytother Res 2001; 15: 240-244.
45. Kuruta M, Suzuki M, Agar NS. Antioxidant systems and erythrocyte life span in mammals. Biochem physiol 1993; 106: 477-487.
46. Ip SP, Poon MKT, Che CT, Ng KH, Kongi YC, Ko KM. Schisandrin B protects against carbon tetrachloride toxicity by enhancing the mitochondrial glutathione redox status in mouse liver. Free Radiac Biol Med. 1996; 21(5): 709-712.
47. Sinclair AJ, Barnett AH, Lunie J. Free radical and antioxidant systems in health and disease. J Appl. Med 1991; 17: 409.
48. Edge R, Truscott TG. Pro- oxidant and antioxidant reaction mechanism of Carotene and radical interaction with Vitamin E and C. Nutrition 1997; 13: 992.
49. Atanasiu RL, Stea D, Mateescu M. Direct evidence of ceruloplasmin antioxidant properties. Mol Cell Biochem 1998; 189: 127-135.
50. Chatterjee IB, Nandi A. Ascorbic acid, a scavenges of oxyradicals. I J Biochem Biophys 1991; 28: 233-236.
51. Paya M, Ferrandiz ML, Sanz MJ, Alcaraz MJ. Effects of phenolic compounds on bromobenzene- mediated hepatotoxicity in mice. Xenobiotica 1993; 23: 327-333.
52. Kodarian C, Browsalis AM, Mino J, Lopex P, Gorzalczany S, Ferraro G, Acevedo C. Hepatoprotective activity of Achyrocline satureioides (Lam) D.C. Pharm Res 2002; 45 (1): 57- 61.