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Decalepis hamiltonii (Wight & Arn) roots as a hepatoprotectant against ethanol toxicity

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Abstract

The hepatoprotective efficacy of a methanolic extract of *Decalepis hamiltonii* (Wight & Arn) root extract against ethanol-induced hepatotoxcity in rats was investigated. Serum enzymes such as glutamate-pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were used to assess ethanol-induced hepatotoxicity. Glutathione peroxides (GPx), glutathione reductase (GR), glutathione transferase (GTS), catalase (CAT), and glutathione (GSH) were also measured in liver tissue. The results revealed an increase in serum enzymes and lipid peroxidation in the liver of the ethanol-treated group, as well as a decrease in antioxidant enzymes and GSH content in the liver. Pretreatment of extract (50-200m/kg body weight) prior ethanol administration restored the ethanol-induced alterations to normal levels. Histopathological examination further confirmed the protective effect. The findings of this investigation demonstrated that *D. hamiltonii* root extract protects against ethanol-induced hepatocellular damage.

Key words : Hepatoprotection, ethanol, *Decalepis hamiltonii,* antioxidant enzymes.

Hepatocellular carcinoma is a type of chronic liver cancer that has resulted in several deaths around the world.[23, 27]. Alcohol, which is processed by alcohol dehydrogenase in the liver, is the leading cause of liver cancer. The metabolic product of alcohol produces acetaldehyde and free radicals, resulting in oxidative stress[15]. Excessive ethanol consumption can disrupt antioxidant and microsomal cytochrome P450 enzyme functions. Malondialdehyde (MDA) levels in the liver tissues have been shown to be higher, while antioxidants such as SOD, Catalase, peroxidase, and GSH levels have been found to be lower[15]. Although several research is going on to understand the concept of liver diseases, there

is no proper therapies for these pathological changes. Phytotherapeutic approach has gained a great advance in curing this disease with valuable drugs isolated from traditional medicinal plants. Ayurvedic drugs and plant products having hepatoprotecive effect have been reported[15,22,23].

This plant, *Decalepis hamiltonii* (Asclepediaceae) is found to grow majorly in southern part of India in the forest and hilly area of Western Ghats. This roots are consumed as folk medicine as a substitute of *Hemidesmus indcus* due its similar medicinal and aromatic properties.[21] This roots is known to constitute some of the biomoleules which is reported in previous studies such as inositol, saponins paramethoxysalicylaldehyde, aldehyde, sterols, amyrins, ketonic substances, and lupeols[18-20]. It is reported, its roots are consumed as pickles and juices due to its strong aroma[28]. From the earlier studies, it is reported that roots are having bioinsecticidal activity on storage grains; also the dried roots can be stored for long duration without microbe or insect infestation[9]. The presence of 2-hydroxy 4-methoxy benzaldehyde and vanillin are the principle components for antimicrobial properties[24]. We have reported in earlier studies that the roots of *D. hamiltonii* have antioxidant properties with several bioactive compounds which have been isolated and characterized[12]. However, scientific studies of root extract of *D. hamiltonii* as hepatoprotectant in ethanol induced damage, was lacking and in this investigation the root extract was tested against ethanol induced liver injuries to validate its use against hepatic cellular damage.

Materials :

Serum albumin (BSA), 2-oxoglutaric acid, sodium pyruvate, lactate, 2,4-Dinitrophenylhydrazine, DL-alanine, L-aspaaartic acid, *p*-nitrophenyl phosphate, nicotinamide adenine dinucleotide (NAD), Thiobarbituric acid (TBA), bovine Trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2) were procured from Sisco Research Laboratories, India. 1-chloro-2,4-dinitrobenzene (CDNB), glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (GR), cumene hydroperoxide (CHP) was procured from Sigma chemical Co, USA. Remaining chemicals were purchased from Ranbaxy and Qualigens, India.

Preparation of root extract :

D. hamiltonii was obtained from Mysore, India, local sources. The University of Mysore's Department of Botany validated the taxonomy identity. The roots' exterior fleshy portions were removed from the interior hard pith, then sliced into little pieces and dried at room temperature. The dried root material was ground and stored in the refrigerator until needed. Using a soxhlet device, coarsely powdered dried root material (500g) was extracted with methanol. To obtain the dried methanolic extract, the methanolic extract was evaporated under decreased pressure.

Animals: Male adult rats of the Albino strain (180–200g) raised at the Institute's animal house were kept in consistent hygienic conditions and fed a standard pellet diet and free access to water(CFT/MLP/2004-37).

Single dose pretreatment :

The rats were placed into six groups,

each with four animals. The vehicle water (6.5ml/kg body weight) was given orally to group one (control). The methanolic extract of *D. hamiltonii* was given to group two (200 mg/kg body weight). A single dosage of 6.5ml/kg body weight ethanol was given to group three. Pretreatment with methanolic extracts of *D. hamiltonii* (50mg/kg, 100mg/kg, and 200mg/kg body weight) was given to groups four, five, and six, followed by the administration of ethanol 6.5ml/kg body weight. The rats were slaughtered 24 hours following the treatment with ether anaesthesia.

Multiple doses pretreatment :

The rats were split into five groups, each with four rodents. The vehicle water (6.5ml/kg body weight) was given orally to group one (control). For seven days, group two received only the methanolic extract of *D. hamiltonii* (100mg/kg body weight). Group three was used as a toxin control (ethanol treatment) and was given vehicle for six days before receiving a single dose of ethanol at 6.5ml/kg body weight on the seventh day. For six days, groups four and five were administered methanolic extracts of *D. hamiltonii* (50mg/kg, 100mg/kg body weight), followed by extract + ethanol on the seventh day. Orally administered dosages were used in all case. Rats were sacrificed after the treatment with required period.

Serum enzyme assay :

To obtain the serum, blood was obtained through cardiac puncture, allowed to clot, then centrifuged at 1000 x g. Bergmeyer [5] described the enzymes glutamate-puruvate transaminase (GPT), glutamate oxaloacetate tranaminase (GOT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). The enzyme/protein activity was calculated directly from the absorbance measurements and represented as units/litre[16].

Antioxidant enzymes :

The enzyme activities were measured using liver homogenate (10% w/v) prepared in 0.1 M phosphate buffer (pH 7.2). The activity of catalase (CAT) was evaluated using the Aebi *et al*.[1] technique for reducing H_2O_2 in 0.1M phosphate buffer calculated at 240nm. The molar absorption coefficient was used to compute the activity. The amount of enzyme that converts 1 mol substrate to product in 1 second was defined as one unit.

The indirect test method employing glutathione reductase was used to measure the activity of glutathione peroxidase (GPx). The oxidation of NADPH by glutathione reductase (0.25 U) in tris buffer was measured at 340nm using cumene hydroperoxide (1m) and glutathione (0.25mM) as substrates[17].

In a potassium phosphate buffer, oxidised glutathione (2mM) and NADPH (2mM) were used to test gluathione reductase (GR)[7].

The activity of glutathione transferase (GST) was measured using the method reported by Warholm *et al*.[29] in phosphate buffer with glutathione (2mM) and CDNB (3mM) as substrates, and the change in absorbance at 344nm was measured.

Glutathione :

The content of GSH in a 10% liver

homogenate was determined using Ellman's technique[8]. The glutathione content was calculated using standard GSH and expressed in mol/g liver.

Lipid peroxidation :

The liver was removed, washed with 0.9% saline and 10 % w/v homogenate was prepared in cold 0.1 M phosphate buffer (pH 7.2). The TBA method Buege & Aust[6] was used to determine the total lipid peroxide content in the homogenate. To 1 ml of homogenate, 1 ml each of 20 percent TCA and 0.67 percent TBA solution were added, mixed completely, and heated in a boiling water bath for 15 minutes. After cooling, the supernatant was centrifuged at 40°C for 10 minutes at 2000 rpm, and the absorbance of the supernatant was measured in a spectrophotometer at 535 nm.

Liver histopathological studies :

Section of the liver was removed and fixed in 10% buffered formalin, then dehydrated in ethanol (50-100%), cleaned in xylene, and embedded in paraffin. For photomicroscopic inspection, sections (4-5m thick) were produced and stained with hematotoxylin dye.

Statistical analysis: Duncan's multiple range test was used to discover intergroup differences, with P-values less than 0.05 considered statistically significant.

Effect of D. hamiltonii root extract on serum enzymes :

In the ethanol-treated groups, serum enzymes such as GOT, GPT, ALP, and LDH were higher than in the control group (Fig. 1, 2). When compared to ethanol-treated animals, pretreatment with the extract at doses of 50, 100, and 200mg/kg body weight at single and multiple doses significantly reduced serum enzyme levels.

Effect of D. hamiltonii root extract on lipid peroxidation :

Ethanol treatment resulted in a rise in malondialdehyde (MDA) levels (Fig. 1, 3). In ethanol-treated rats, pretreatment with *D. hamiltonii* root extract decreased the production of MDA in a dose-dependent manner.

Effect of D. hamiltonii root extract on antioxidant enzymes :

In rats treated with ethanol, antioxidant enzymes such as CAT, GPx, and GSR were lowered, whereas GST was enhanced. Pretreatment of the extract (50-200mg/kg body weight) at single and multiple doses increased CAT, GPx, and GSR activity while decreasing GST activity (Fig 4, 5).

Effect of D. hamiltonii root extract on GSH:

In comparison to the control group, the ethanol treatment reduced the level of liver GSH. The liver GSH content was significantaly raised in the animals pretreated with *D. hamiltonii* root extract (50-200mg/kg body weight) at single and multiple doses (Fig. 6).

Histopathological studies :

Histopathological changes of liver are

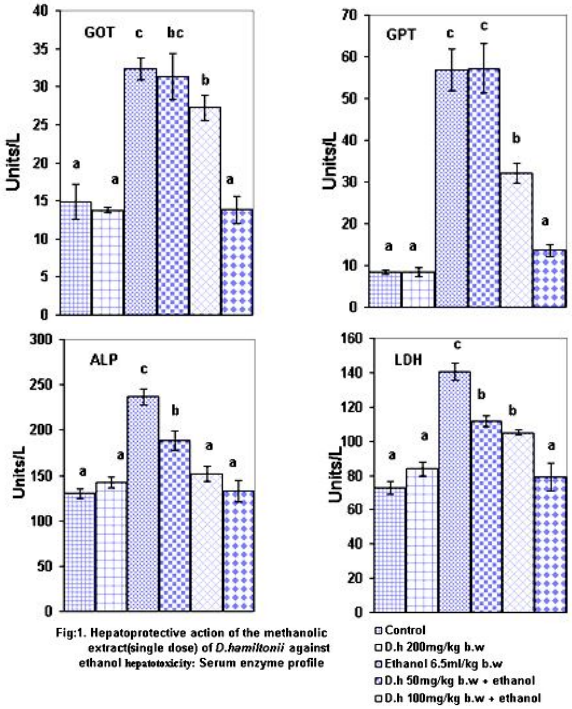

Fig:1. Hepatoprotective action of the methanolic extract(single dose) of *D.hamiltonii* against ethanol hepatotoxicity: Serum enzyme profile

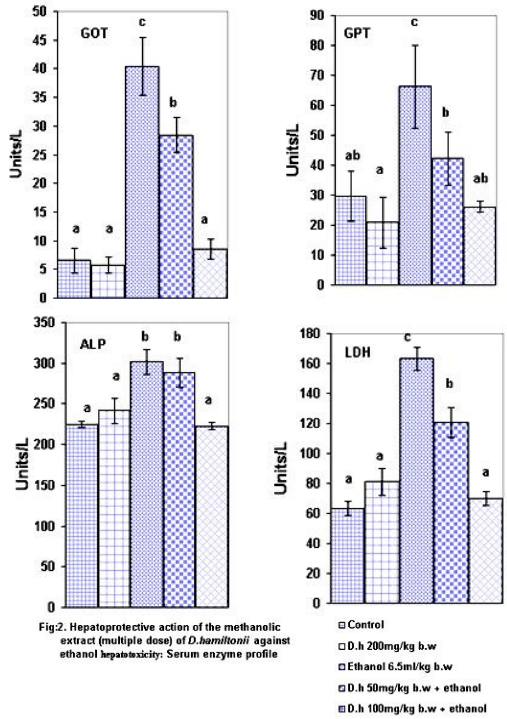

Fig:2. Hepatoprotective action of the methanolic extract (multiple dose) of *D.hamiltonii* against ethanol hepatotoxicity: Serum enzyme profile

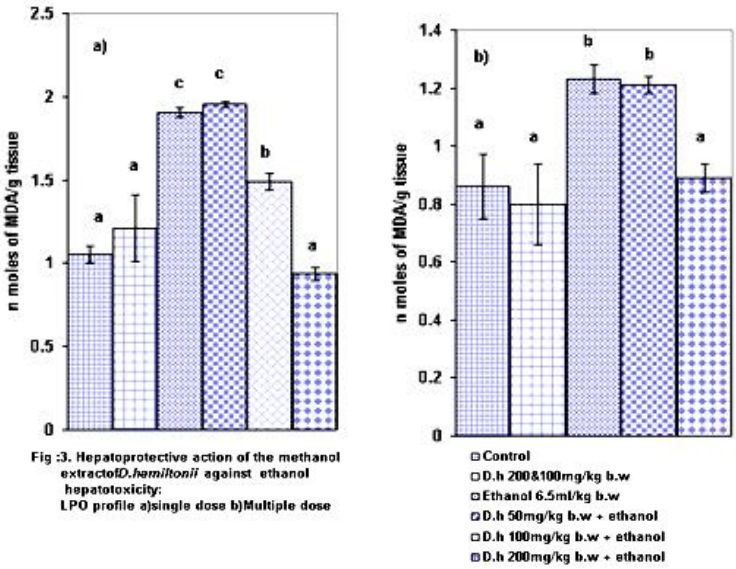

Fig :3. Hepatoprotective action of the methanol extractof*D.hemiltonii* against ethanol hepatotoxicity:
LPO profile a)single dose b)Multiple dose

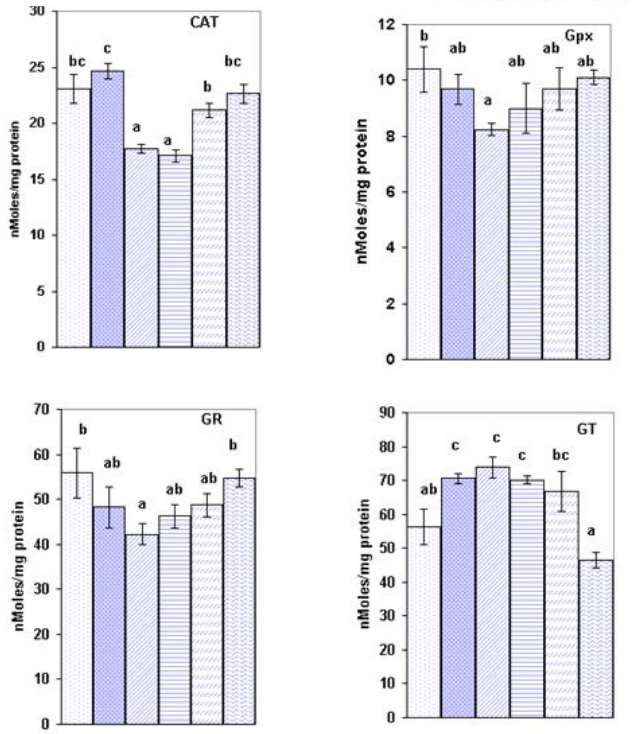

Fig:4. Hepatoprotective action of methanolic extract of the roots of D.hamiltonii (single dose) against ethanol hepatotoxicty : Hepatic antioxidant enzyme profile.

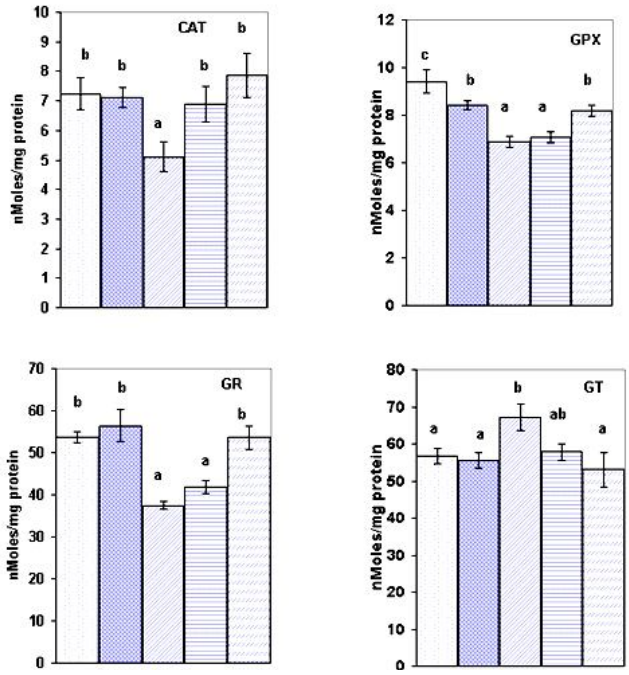

Fig:5. Hepatoprotective action of methanolic extract of the roots of D.hamiltonii (multiple dose) against ethanol hepatotoxicty : Hepatic antioxidant enzyme profile.

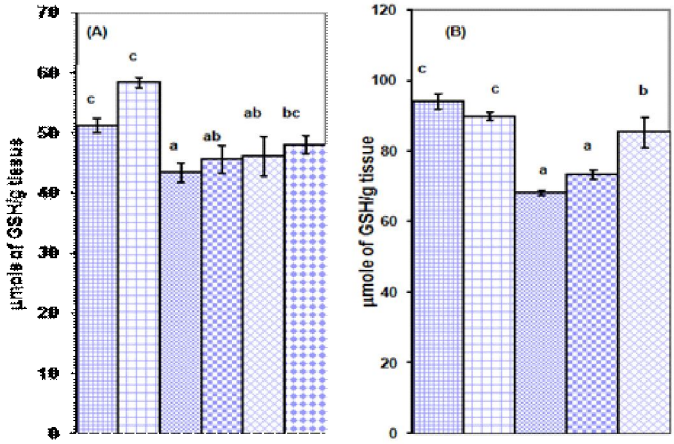

Fig. 6 . Hepatoprotective action of the methanol extract of *D.hamiltonii* against ethanol hepatotoxicity: GSHprofile
A) Single dose B) Multiple dose.
The values are mean±SEM (n=3), bars with different letter differ significantly at P<0.05 by DMRT

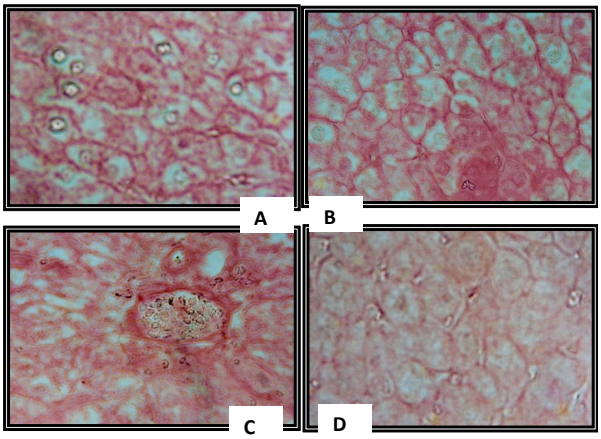

Fig. 7. Hepatoprotective action of *D. hamiltonii* root extract on ethanol hepatotoxicity :

Liver Histopathology

A. Liver of control rats showing normal histoarchitecture.
B. Liver of rats pretreated with single dose of methanolic extract of *D. hamiltonii* (200mg/kg body weight) alone.
C. Liver of Ethanol treated rats showing extensive necrosis
D. Liver of rats pretreated with single dose of methanolic extract of *D. hamiltonii* (200mg/kg.body weight) followed by single dose of Ethanol

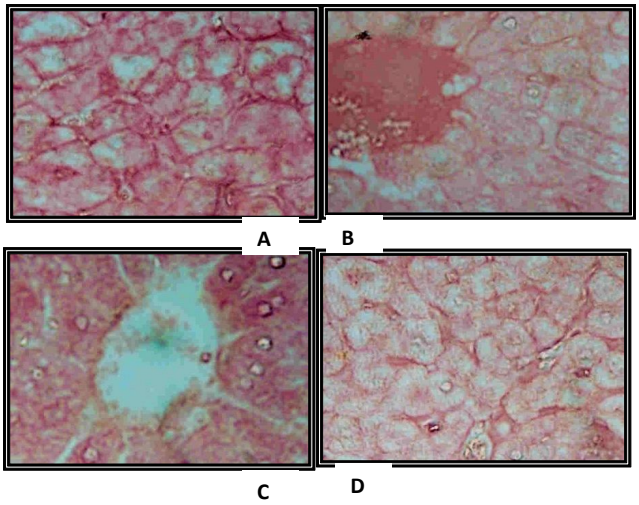

Fig. 8. Hepatoprotective action of *D.hamiltonii* root extract on ethanol hepatotoxicity :

Liver histopathology

A. Liver of control rats showing normal histoarchitecture.
B. Liver of rats pretreated with multiple dose of methanolic extract of *D.hamiltonii* (100mg/kg body weight) alone.
C. Liver of ethanol treated rats showing extensive necrosis
D. Liver of rats pretreated with multiple dose of methanolic extract of *D.hamiltonii* (100mg/kg.body weight) followed by single dose of ethanol.

shown in Fig 7, 8. The liver section of alcohol administered groups showed feathery degeneration, micro and macro cellular fatty changes, periportal fibrosis and vascular congestion. On treatment with *D. hamiltonii* root extract the liver showed almost normal histology. Control rats treated with extract showed normal histology.

Ethanol is a well-known model for studying liver injury, and it has been used to assess the efficacy of numerous phytochemicals and medications[11]. The liver is known to be damaged after consuming ethanol. Leakage of cellular enzymes into plasma is a marker of hepatic damage[4]. Enhanced levels of liver serum enzymes like GOT, GPT, and ALP have been seen in alcohol-treated rats, indicating increased hepatocyte permeability, injury, or necrosis[10]. Further prior *D. hamiltonii* extract administration at different concentrations provided considerable protection by reversing the alterations caused by ethanol, implying that *D. hamiltonii* extract protects the liver's structural integrity against the harmful effects of ethanol.

Chronic ethanol administration causes hepatic lipid peroxidation, which is a sign of oxidative stress. Most studies have discovered excessive lipid peroxidation as indicated by the production of TBARS[22]. In line with these findings, ethanol-treated rats had higher amounts of TBARS in their liver tissue than control rats. When *D. hamiltonii* extract was given to alcohol- administered rats, lipid peroxidative indicators were found to be significantly lower than in ethanol-administered rats. This observation demonstrates the antiperoxidative and antioxidant effects of *D. hamiltonii* root extract. In this aspect Srivastava *et al*.[26] has reported that *D. hamiltonii* root extract has inhibitory effect on lipid peroxidation *in vitro* which is convincing to our results obtained. From these evidences hepatoprotective effect could be attributed due to antioxidant activity.

The defense of the cells against oxidative stress involves both non- enzymatic and antioxidants such as glutathione, ascorbic acid and alpha – tocopherol which directly react with free radicals as well as antioxidant enzymes like catalase, peroxidase which scavenge free radicals[22]. The levels of antioxidant enzymes (catalase,Gpx, Gr, and GSH-T), which were altered by ethanol administration, were normalized by pretreatment with the *D. hamiltonii* root extract. While the exact role of *D.hamiltonii* extract could be boosting of scavenging radical enzymes which prevents from the oxidative damage of hepatocytes.

Glutathione is a non-protein thiol found in living creatures that plays a key function in coordinating our body's antioxidant defense system. It has a role in maintaining normal cell structure and function, most likely through its redox and detoxifying reactions. We observed a lower level of GSH in alcoholic treated rats, which could be due to free radical generated during the metabolism of ethanol, which lead to glutathione oxidation. However, *D. hamiltonii* extract co administered rats showed significantly improved GSH level, possible mechanism could be extract boost the antioxidant enzymes and prevent the damage of hepatocytes by radicals, ameliorating the level of oxidative stress. It is reported in our earlier studies that *D.*

hamiltonii extract as shown protection in CCl_4 induced damage in liver of rats[13,14,19].

Alcohol administration produces a spectrum of histological abnormalities in the liver as described earlier[15]. The liver histology of ethanol-treated animals revealed pathomorphologic changes. These changes are predominant in the centrilobular region having reduced oxygen perfusion. Hepatic damage may be caused in part by cytochrome P450-dependent enzyme activity in the liver, which are seen in higher concentrations near the central vein and lower concentrations near the peripharal locations[2,25]. Treatment with *D. hamiltonii* extract (single and multiple dose) reduced the morphological changes produced by ethanol and reverted the microanatomy of the liver to almost normal. Histopathological section of liver pretreated with extract followed by ethanol reveals hepatoprotection activity of *D. hamiltonii* root extract. Hepatocytesobserved are normal, with neligible damage in intralobular vein and endothelium lining. Pyknosis is absent in the nucleus. Histopathological and biochemical assay results clearly shows that *D. hamiltonii* root can be considered as effective hepatoprotectant, which could be consumed in our daily food habits which boost our immune systems and protects from liver disorders.

Oxidative damage induced to the liver by ethanol was significantly protected by the methanoic extract of roots of *D. hamiltonii*. The hepatoprotective activity could be due to the active biomolecules of the methanoic extract that we have been identified and reported in earlier studies.

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