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Evaluation of phytonutrients, mineral composition, antimicrobial and hepatoprotective activities of leaves of *Actinodaphne madraspatana* Bedd (Lauraceae)

Devarajan Saravanan[1], Dhakshanamurthy Thirumalai[2] and Indira Viswambaran Asharani[3*]

[1]*Ratnam Institute of Pharmacy, Pidathapolur, Nellore, Andhra Pradesh, India*
[2]*Department of Chemistry, Thiruvalluvar University, Vellore, Tamil Nadu, India*
[3]*School of Advanced Sciences, VIT University, Vellore, Tamil Nadu, India*

ABSTRACT

The objective of the present study was to evaluate the phytonutrients, mineral composition, antimicrobial and hepatoprotective activities of leaf of Actinodaphne madraspatanaBedd (A.madraspatana). The content of β-carotene and lycopene in ethanol extract was determined by UV-Visible spectroscopy. The mineral composition of the leaf powder was determined by atomic absorption spectroscopy. The antimicrobial activity of the ethanol extract (200µg/mL) was tested by disc diffusion method against six bacterial and four fungal strains, which was compared with Ciprofloxacin (5µg/disc) for bacteria and Nystatin (100 µg/disc) for fungi. The hepatoprotective activity of extract (200 mg/kg and 400 mg/kg) was investigated in carbon tetrachloride induced hepatotoxicity, which was compared with silymarin at a dose level of 50 mg/kg. The average contents of β-carotene and lycopene in ethanol extract was found to be 1.20±0.002 mg/gm and 0.21±0 .002 mg/gm dry weight of extract respectively. The composition of minerals present in leaf powder was within in the permissible limit. The results of antimicrobial activity showed that ethanol extract proved the antimicrobial activity against the tested microorganisms. Results of hepatoprotective activity revealed that the ethanol extract showed the significant ($p<0.001$) hepatoprotective activity. The ethanol extract of leaves of A.madraspatana has potent antimicrobial and hepatoprotective activities.

Key words: Phytonutrients, minerals composition, antimicrobial study, leaves, ethanol extract

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INTRODUCTION

Medicinal plants are the sources of phytoconstituents such as flavonoids, tannins, glycosides, alkaloids, and terpenoids that have different pharmacological activities including those useful in the treatment of human and animal diseases. Medicinal plants are used medicinally in different countries and are rich sources of potent drugs. Medicinal plant parts such as leaves, stem, root, flower, twigs, and fruit are used in the extract as a raw drug and theirposse's different medicinal values[1].

Actinodaphne madraspatana Bedd (*A.madraspatana*) is one the most used herbal remedy in the natural medicine. It belongs to the family Lauraceae in the major group of angiosperms (flowering plants). It is commonly known as 'Putta Thali' in Tamil, 'Ray Laurel' in English, 'Irolimarom', 'Mungali' in Malayalam, 'Kovangutti' in Telugu[2]. It is a medium-sized evergreen tree and Shrub, widely distributed common on the Rock Hill slopes at higher elevations, Aruku Valley, Vishakhapatnam District, Talakona, Dharmagiri, Microwave station, on the way to Thumburu Theertham[3].Leaves, flowers and fruits constitute the drug. It is a precursor of vitamin A[4]. The

benzene extract of the Heartwood was reported to contain 5, 7, 8-Trimethoxyflavone[5]. The Leaves of the plant are used traditionally to cure wounds, cure mania, fickle minded behavior and diabetic[6].From the literature survey, *Actinodaphne* plants have been reported to produce isoquinoline alkaloids such as aporphines, oxoaporphines and lactones[7-9]. Oxoaporphine was reported to have antimicrobial and antitumor activities[10].Therefore, the objective of the present study was to analysis the phytonutrients, minerals content and antimicrobial study of leaves of *A.madraspatana*.

EXPERIMENTAL SECTION

Plant collection and authentication

The leaves of *A.madraspatana* were collected from Talakona forest near to Tirupathi and were authenticated by Dr. K. Madavachetty, S. V. University, Tirupati, Andhra Pradesh, India. A voucher specimen (ACD) has been kept in the Herbarium.

Drugs and chemicals

Carbon tetrachloride (CCl_4) was purchased from Loba Chemie Pvt Ltd, Mumbai, India. Silymarin and carboxy methyl cellulose (CMC) were purchased from M/S Hi media Ltd, Bombay. Aspartate aminotransferase (AST), and alanine aminotransferase (ALT) kits were purchased from Span Diagnostic Pvt Ltd, Mumbai, India. Alkaline phosphatase (ALP), total protein (TP), and total bilirubin (TB) kits was purchased from Excel Diagnostic Pvt Ltd, Hyderabad, Andhra Pradesh, India. All other chemicals and reagents used in the study were of analytical grade.

Preparation of the extracts

The leaves of *A.madraspatana* were shade dried and powdered. About 500 gms of dry powder was successively extracted by soxhlation using the solvent ethanol. The extract was filtered in hot condition and concentrated in vacuum under reduced pressure and dried in desiccators.

Determination of β-carotene and lycopene content by UV spectroscopy

β-Carotene and lycopene content in the ethanol extract were determined according to the method of Tevfik Ozen (2010). The dried ethanol extract (100 mg) was vigorously shaken with 10 mL of acetone-hexane mixture (4:6) for 1 minute. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations:

lycopene (mg/100 mL) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$
β-carotene (mg/100 mL) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$

The results were mean values ± SEM. The values were expressed as mg of β-carotene/gm and mg of lycopene/gm of dry extract[11].

Analysis of mineral composition

Instrument

Atomic absorption spectrophotometer (Shimadzu-AA 7000) equipped with a deuterium lamp for background correction. The hollow cathode lamp was employed as a radiation source. The flames used where air/acetylene and N_2O/acetylene. Nitrogen can be used as a carrier gas.

Sample preparation

Powdered leaves of *A.madraspatana* (2 gms) were accurately weighed and transferred into a silica crucible and kept in a muffle furnace for ashing at 450 °C for three hours and the 5 mL of the 6M hydrochloric acid solution was to silica crucible. Then, the crucible kept on a hot plate and digested. The final residue was dissolved in 0.1M nitric acid solution and made up to 50 mL. The working standard solutions were prepared by diluting the stock solution with 0.1M nitric acid for checking the linearity[12].

Antimicrobial activity

Micro-organisms tested

The test microorganisms of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, Aspergillus niger, Candida albicans, Mucor racemosus, and Aspergillus fumigatus* were selected based on their pharmacological and clinical importance. The bacterial and

fungal stock cultures were obtained from National Chemical Laboratory (NCL), Pune and maintained by periodical sub culturing on Nutrient agar and Sabouraud dextrose agar medium for bacteria and Fungi respectively.The bacterial strains were grown at 37 °C in Muller-Hinton agar medium, whereas the fungal strains were grown at 28°C in Sabouraud's dextrose agar medium. The stock cultures were maintained at 4°C.

Determination of zone of inhibition

The antimicrobial activity of the ethanol extract of leaves of *A.madraspatana* was carried out by Disc Diffusion Technique against two gram positive- *Staphylococcus aureus, Bacillus subtilis*; four gram negative-*Escherichia coli, Klebsiella aerogenes, Pseudomonas aeruginosa, and Proteus vulgaris* human pathogenic bacteria, and four fungal strains-*Aspergillus niger, Candida albicans, Mucor racemosus, and Aspergillus fumigatus*. The purified ethanol extract and standard drug Ciprofloxacin and Nystatin were dissolved in dimethyl sulfoxide, sterilized by filtration and stored at 4 °C.

The discs of 6mm diameter were prepared from Whatmann filter paper and were sterilized in hot air oven at 160 °C for one hour. The discs were impregnated with ethanol extract, standard Ciprofloxacin, standard Nystatin and the solvent dimethyl sulfoxide. The seeding was done on Muller-Hinton agar mediumfor bacteria and Sabouraud's dextrose agar medium for fungi with a sterile swab. The seeded plates were allowed to dry and then the Ciprofloxacin, Nystatin, extract, and dimethyl sulfoxide discs were placed on the seeded medium. Then the plates were kept at 4 °C for 30 minutes to allow the prediffusion of Ciprofloxacin, Nystatin, extract and dimethyl sulfoxide. The zones of growth inhibition around the disks were measured after 18 to 24 hours of incubation of plates at 37 °C for bacteria and two to four days for fungi at 28 °C. The effect produced by the ethanol was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 µg/disc for fungi). The sensitivities of the microorganism to the ethanol extract were determined by measuring the sizes of inhibitory zones around the surface of the disks. The zone of inhibition less than 8mm was considered not as active against pathogenic microorganisms[13].

Experimental Animals

Swiss Albino rats (190-250 gms) of either sex used for this study. The animals were housed in polypropylene cages in a controlled room temperature 22±10 °C and relative humidity of 60-70%. They were kept under standard conditions of 12/12 hours light and dark cycle. The animals were maintained with standard pellet diet (Kamadenu Enterprises, Bangalore) and water *ad libitum*. The animals were acclimatized to laboratory condition for seven days before commencement of the experiment. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee (Reg. No. 1558/PO/a/11/CPCSEA) and was cleared by the same before starting the experiment.

Acute toxicity studies

Acute toxicity study of ethanol extract of *A.madraspatana* was carried out in Swiss Albino rats of either sex (190-250g) according to OECD (Organization for Economic Cooperation and Development) guidelines No. 423. Extract at different doses up to 2000 mg/kg p.o. was administered and the animals were observed for behavioral changes, toxicity, and mortality up to 48 hours.

Evaluation of hepatoprotective activity

Rats of either sex were randomly divided into five groups (I-V) with six animals in each group (n = 6) and treated as follows. Group I served as normal control and it was a received 1mL of 0.5% CMC for 5 days; Group II, received a 1mL of 0.5% CMC for 5 days along with the i.p dose of CCl_4 in liquid paraffin (1:1v/v; 1 mL/kg) on day 3 of the experiment after 30 minutes of administration of CMC; Group III, received a ethanol extract (200 mg/kg) daily for 5 days along with the i.p dose of CCl_4 in liquid paraffin (1:1v/v; 1 mL/kg) on day 3 of the experiment after 30 minutes of administration of ethanol extract; Group IV, received a ethanol extract (400 mg/kg) daily for 5 days along with the i.p dose of CCl_4 in liquid paraffin (1:1v/v; 1 mL/kg) on day 3 of the experiment after 30 minutes of administration of ethanol extract; Group V, received a standard drug silymarin (50 mg/kg) daily for 5 days along with the i.p dose of CCl_4 in liquid paraffin (1:1v/v; 1 mL/kg) on day 3 after 30 minutes of administration of silymarin. At the end of the experiment blood was collected from all animals by puncturing the retro orbital plexus. The blood sample was allowed to clot for 15 minutes and serum was separated by centrifugation at 3000 rpm for 15 minutes. The separated serum was subjected for estimation of various biochemical parameters, namely AST, ALT, ALP, TB, and TP[14].

Histopathological studies
After the study, rats were sacrificed under anesthesia. Their livers were excised immediately, washed in ice cold normal saline (0.9 %w/v) and fixed overnight in 10% formalin solution. Liver section were made by microtome, dehydrated in graduated ethanol (50-100%), cleared in xylene and embedded in paraffin. The hepatic sections (4-5 µm) were stained with haematoxylin and eosin dye and examined with a photomicroscope.

Statistical analysis
The data are expressed as the mean ±SEM. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnett's test for the multiple comparisons using prism Graphpad version 5.0. The p values less than 0.001 were considered statistically significant.

RESULTS AND DISCUSSION

Determination of β-carotene and lycopene content
The content of β-carotene and lycopene in the ethanol extract *A.madraspatana* was found to be 1.20±0.002 mg carotenoid/gm and 0.21±0.002 mg lycopene/gm dry weight of ethanol extract respectively. Carotenoids, such as β-carotene and lycopene, are natural phytoconstituents of many medicinal plants and protect our body against disease. Carotenoids are vitamin A precursor and are a group of pigments, which cause the color yellow orange and red on the medicinal plants. Carotenoids are natural antioxidants, which can scavenge the free radicals, increase the immunity and metabolic alteration of cancer [15].

β-Carotene is commonly known as a physical and radical scavenger of singlet oxygen and is to play an important role in the inhibition of the initial stages of lipid peroxidation. Lycopenes are lipid soluble power full antioxidant that is synthesized by many plants and microorganisms, but not by human and animals that are closely related to β-carotene. It is a highly unsaturated open straight chain hydrocarbon consisting of 11 conjugated and 2 unconjugated double bonds.

Analysis of mineral composition
A total of six minerals like copper, zinc, chromium, manganese, lead, and iron were determined in the powdered leaves of *A.madraspatana* using an atomic absorption spectrometer (AAS). The study of mineral composition revealed that, a composition elements was founded within in the permissible limit and has the level of minerals in the range of Fe>Zn>Mn>Cu>Cr>Pb. The results were presented in Table 1.Living organisms require varying amounts of minerals such as iron, lead, cobalt, molybdenum, zinc, copper, chromium, and manganese. All the minerals are toxic to our body at higher concentrations. Excessive levels of minerals can be damaging to the organism. These minerals take part in electron transfer, redox reactions, structural functions in nucleic acid metabolism, water distribution, cell permeability, generation of electrical potential and cofactors in enzymatic reactions [16].

Copper is one of the essential mineral which required for iron metabolism, cofactor for metallo protein and play major role in the metabolic pathway. Copper is very important for oxidative phosphorylation (ATP production) and it is also constituents of cytochrome oxidase [17]. Zinc is an essential mineral and it is widely distributed in the body. Zinc associated with certain metallo enzymes biochemically such as lactic dehydrogenase, alcohol dehydrogenase, aldolase, carbonic anhydrase, alkaline phosphatase and glutamic dehydrogenase. It also binds to RNA, thus stabilizing secondary and tertiary structures [18]. Manganese is associated with RNA and play vital role in oxidative phosphorylation, protein synthesis, cholesterol biosynthesis, and fatty acid metabolism. Iron is considered to be one of the essential components of the body. It is essential to the elementary metabolic process in the cell and it is responsible for transport of molecular oxygen in higher microorganism. Chromium is an essential mineral and it is necessary for optimal growth of animals. Chromium is an essential cofactor for insulin. Insulin is a very important hormone that regulates the metabolism of carbohydrates, protein and fats and can be helpful for people with diabetes mellitus or hypoglycemia. Chromium is playing the vital role in the synthesis of cholesterol, proteins and fats [19-20].

From the study, the leaves of *A.madraspatana* contain variable concentration of minerals. The variation in concentration of these minerals may be mainly due to environmental condition where the constituent of plant grown, the use of pesticide, and fertilizers. But generally it is concluded that the studied leaves of *A.madraspatana* are a rich source of essential minerals Cu, Cr, Mn, Pb, Fe and Zn and hence play an important role in the in electron transfer in

respiratory chain (ATP Production), carbohydrate metabolism, redox reactions, structural functions in nucleic acid metabolism, water distribution, cell permeability, generation of electrical potential and co factors in enzymatic reactions [21-22].

Table 1: Minerals content in leaves of *A.madraspatana*

S. No	Name of Minerals	Minerals content (mg/kg)
1	Copper	11.16
2	Zinc	52.68
3	Chromium	0.81
4	Manganese	51.04
5	Lead	0.59
6	Iron	455.11

Antimicrobial study

The antimicrobial activity of the ethanol extract of leaves of *A.madraspatana* was studied (200 µg/mL) against six pathogenic bacterial strains (two gram positive and four gram negative) and four fungal strains. The antimicrobial activity of the extract was assessed in terms of zone of inhibition of microbial growth. The extract of the leaves of *A.madraspatana* showed antibacterial activity against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella aerogenes, Pseudomonas aeruginosa, Proteus vulgaris* and antifungal activity against *Aspergillus niger, Candida albicans, Mucor racemosus, and Aspergillus fumigatus*. The zone of inhibition of the extract of the leaves of *A.madraspatana* was compared with Standard Ciprofloxacin for bacteria and Nystatin for fungi as shown in Table 2,3, and Figure 1. The diameters of inhibition varied for extract and standard drug. The growth of inhibition measured ranged from 25 to 30 mm for all the bacteria as compared with Ciprofloxacin (26-40 mm) and growth of inhibition measured ranged from 20 to 28 mm for all the fungi as compared with Nystatin (30-35 mm).Thus the present study clearly demonstrated the antibacterial property of the plant *A.madraspatana* against both gram positive and gram negative bacteria.

Table 2: Antibacterial activity of ethanol extract of leaves of *A.madraspatana*

S. No	Microorganisms	Zone of inhibition		
		Ethanol extract (200 µg/mL)	DMSO	Ciproflaoxacin (5 µg/disk)
1	*Staphylococcus aureus* (NCIM 2079)	30	-	35
2	*Bacillus subtilis* (NCIM 2063)	26	-	40
3	*Escherichia coli* (NCIM 2065)	25	-	38
4	*Klebsiella aerogenes* (NCIM 2098)	28	-	30
5	*Pseudomonas aeruginosa* (NCIM 2036)	26	-	35
6	*Proteus vulgaris* (NCIM 2027)	25	-	26

Table 3: Antifungal activity of ethanol extract of leaves of *A.madraspatana*

S. No	Microorganisms	Zone of inhibition		
		Ethanol extract (200 µg/mL)	DMSO	Nystatin (100 µg/disk)
1	*Aspergillus niger* (NCIM 105)	25	-	35
2	*Candida albicans* (NCIM 3102)	30	-	32
3	*Mucor racemosus* (NCIM 108)	28	-	30
4	*Aspergillus fumigatus* (NCIM 204305)	20	-	32

The antimicrobial activity of the ethanol extract can be attributed to the presence of various phytoconstituents such as flavonoids, triterpenoids, steroids, and glycosides found in this extract [23]. The variation observed in zone of inhibition of the tested microbes can be attributed either to their mechanism of action on gram positive and gram negative bacteria and fungi or to the difference in the composition of phytoconstituents present in the ethanol extract. The mechanism of action of the alkaloids, glycosides, tannins and flavonoids on gram positive and gram negative bacteria and fungi was demonstrated. Several researches clearly demonstrated that polyphenols and phenolic compounds have antimicrobial activities and other researchers have shown that the sites and the number of hydroxyl groups on the phenol are thought to be related to their relative toxicity to microorganisms [24-25]. The presence of multiple phytoconstituents confers to the antimicrobial activity of ethanol extract of leaves of *A.madraspatana*. A detailed investigation into the potential plant constituents responsible for the antimicrobial

property may provide scope for lead molecules that may be of use in treating humans affected with infections caused by various pathogens.

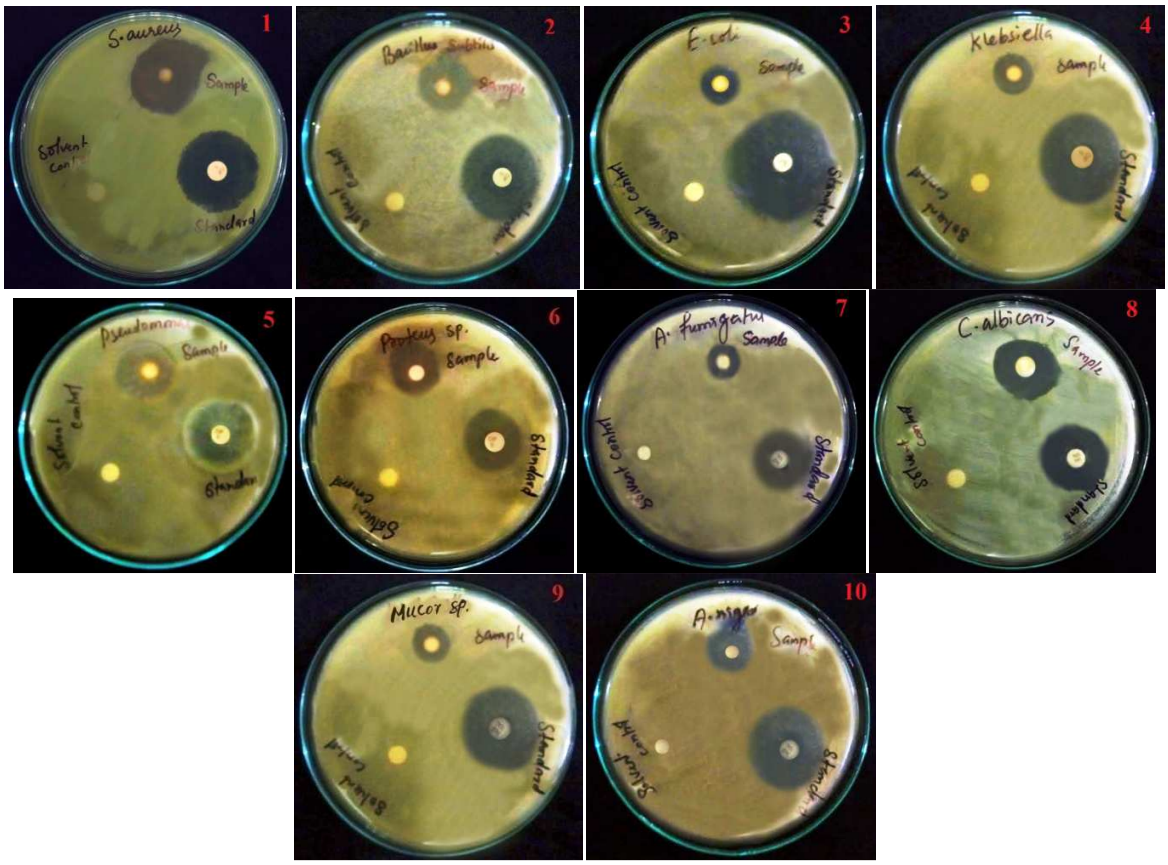

Figure 1: Antimicrobial activity of ethanol extract of leaves of *A.madraspatana*. (1) *Staphylococcus aureus*; (2) *Bacillus subtilis*; (3) *Escherichia coli*; (4) *Klebsiella aerogenes*; (5) *Pseudomonas aeruginosa*; (6) *Proteus vulgaris*; (7) *Aspergillus fumigates*; (8) *Candida albicans*; (9) *Mucor racemosus*; (10) *Aspergillus niger*

Evaluation of hepatoprotective activity

The results of present study revealed that the treatment of rats with carbon tetrachloride in liquid paraffin (1:1; 1ml/kg) increases in serum AST, ALT, ALP, TB levels and decreased levels of TP when compared to normal control rats. The administration of both doses of ethanol extract (200 and 400 mg/kg) and standard drug silymarin (50 mg/kg) for 7 days significantly reduced the elevated level of AST, ALT, ALP, and TB and significantly increased the reduced level of TP when compared to carbon tetrachloride alone treated group. The results were shown **Table 4**. The liver damage is produced by administration of carbon tetrachloride. CCl_4 is widely used as experimental hepatotoxicant which activates liver metabolizing enzyme cytochrome P_{450} to form highly reactive metabolites such as peroxy trichloromethyl radical ($CCl_3OO^{\bullet}$) and trichloromethyl radical ($CCl_3^{\bullet}$). Both free radicals are abstracting hydrogen atom from poly unsaturated fatty acid, covalently bind with proteins or lipids of cell membrane or organelles, and initiating lipid peroxidation thus causing the damage of cell membrane, disturbing Ca^{+} homeostasis, changing the enzyme activities and inducing hepatic injury. This leads to the leakage of a variety of liver enzymes such as AST, ALT and ALP from liver cytosol into the blood, which in turn increases their level in serum [26].

The treatment of rats with carbon tetrachloride in liquid paraffin (1:1; 1ml/kg) resulted in dramatic increases in serum AST, ALT, ALP, TB levels and decreased levels of TP was observed in carbon tetrachloride induced hepatotoxicity in rats when compared to normal control rats. It observed that liver damage occurred in carbon tetrachloride induced hepatotoxicity in rats. The oral administration of both doses of ethanol extract (200 and 400 mg/kg) and standard drug silymarin (50 mg/kg) for 7 days significantly reduced the elevated level of AST, ALT,

ALP, and TB and significantly increased the reduced level of TP when compared to carbon tetrachloride alone treated group and restored the damaged liver cell morphology. The tendency of these liver enzymes to return to normality in the ethanol extract treated groups due to presence of flavonoid in *A.madraspatana* and it is a clear feature of the hepatoprotective effect of the extract. Flavonoids block the lipid peroxidation in cell membranes due to their high free radical scavenging activity. A new flavone (5,7,8-trimethoxy flavone) was a reported [5] in benzene extract of *A.madraspatana* and has strong antioxidant activity which help to prevent the oxidative harm to cells, and lipids. *A.madraspatana* contain betasetosterol which is a reported hepatoprotective agent [27]. In this view, the reduction in levels of AST, ALT, ALP, and bilirubin and increased in levels of total protein by ethanol extract is an indication of repair of hepatic tissue damage and stabilization of plasma membrane caused by carbon tetrachloride metabolite peroxy trichloromethyl radical ($CCl_3OO^{\bullet}$) and trichloromethyl radical ($CCl_3^{\bullet}$).

Table 4: Effect of ethanol extract on serum parameters in carbon tetrachloride induced toxicity in rats

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TB (mg/dl)	TP (gm/dl)
I	48.6±0.21	42.7±0.13	127.0±0.17	1.25±0.02	6.58±0.02
II	[a]102.0±0.18*	[a]96.0±0.23*	[a]283.9±0.25*	[a]5.26±0.02*	[a]4.12±0.01*
III	[b]78.9±0.15*	[b]60.9±0.16*	[b]196.9±0.18*	[b]2.18±0.01*	[b]6.04±0.02*
IV	[b]66.0±0.15*	[b]55.9±0.20*	[b]162.9±0.27*	[b]1.84±0.01*	[b]6.21±0.01*
V	[b]52.0±0.21*	[b]46.7±0.22*	[b]138.7±0.15*	[b]1.50±0.02*	[b]6.48±0.02*

*All values are expressed as mean ±SEM for six animals, [a]p<0.001 compared to normal control, [b]p<0.001 compared to negative control, *statistically significant.*

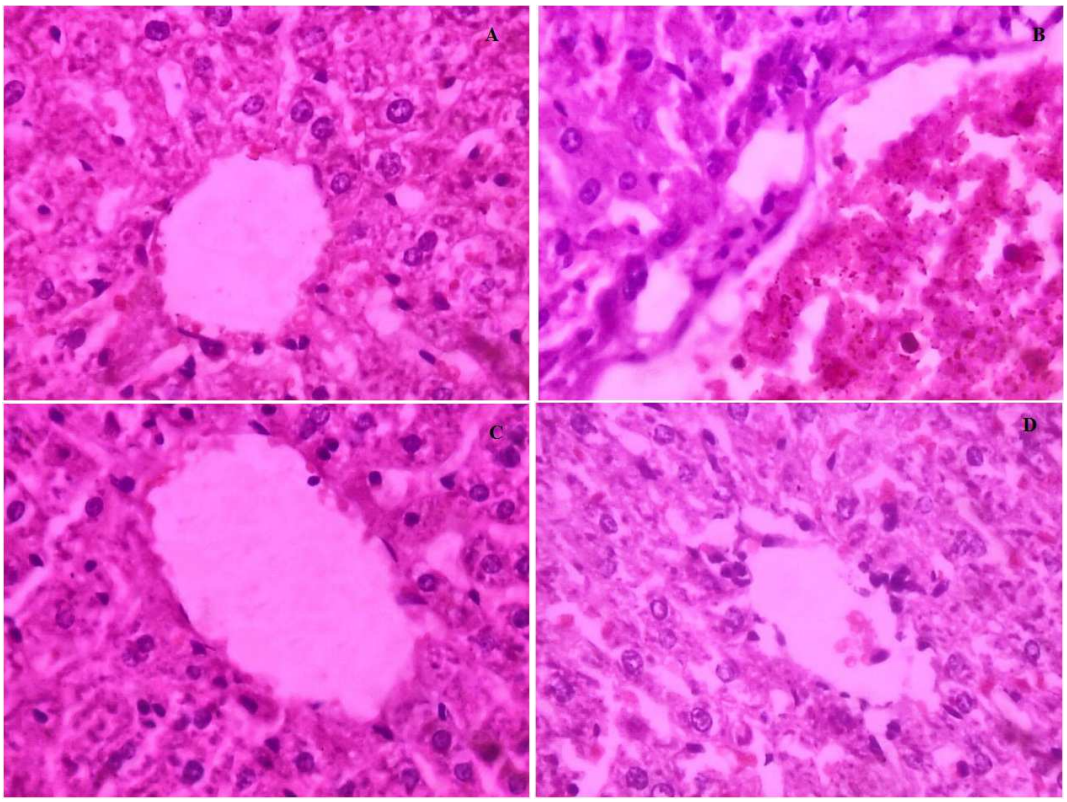

Figure 3: Histopathological slides of livers of different animal groups. Normal control (A); Carbon tetrachloride negative control (B); Test group (C); Standard control (D)

The hepatoprotective activity of ethanol extract was further confirmed by histopatholgical study of livers (**Figure 3A-3D**). Histology of the liver sections of the control rats showed the normal hepatic cells with prominent nucleus and nucleolus, well preserved cytoplasm, and visible central veins. The liver sections of carbon tetrachloride treated rats showed the massive fatty changes, loss of cellular boundaries, necrosis, ballooning degeneration and broad infiltration of the lymphocyte. These events were because of peroxy trichloromethyl radical ($CCl_3OO^{\bullet}$) and

trichloromethyl radical ($CCl_3^{\bullet}$) generated due to carbon tetrachloride metabolism. The liver sections of ethanol extract treated rats showed the mild degree of fatty changes, lymphocytes infiltration, and necrosis.

The histopathological study of liver showed the that administration of carbon tetrachloride to rats causes the massive fatty changes, loss of cellular boundaries, necrosis, ballooning degeneration and broad infiltration of the lymphocyte. These events were because of peroxy trichloromethyl radical ($CCl_3OO^{\bullet}$) and trichloromethyl radical ($CCl_3^{\bullet}$) generated due to carbon tetrachloride metabolism. The ethanol extract of *A.madraspatana* at both doses restored the disturbed hepatic cell morphology to normal, when compared to the silymarin treated and control group's rats.

CONCLUSION

The leaves of *A.madraspatana*have potentials for use in food formulations in view of it β-carotene, lycopene, and mineral contents. The ethanol extract of leaves of *A.madraspatana* possess the antimicrobial and hepatoprotective activities and further studies are required to isolate and characterize the active phytoconstituents, which are responsible for the antimicrobial and hepatoprotective efficacy.

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