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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF SPHAGNETICOLA TRILOBATA FLOWERS ON EXPERIMENTAL ANIMALS

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ABSTRACT:

Objective: To evaluate the hepatoprotective activity of ethanolic extract of Sphagneticola trilobata flowers in experimental animal models. Methods: Two doses (250mg/kg and 500mg/kg body weight p.o.) of the flower extract of Sphagneticola trilobata were subjected for the evaluation of hepatoprotective potential against PCM (2g/kg, p.o.) and carbon tetrachloride (0.7ml/kg, i.p.) induced liver injury. Silymarin (25mg/kg, p.o) was used as a standard drug. The parameters like ALT, AST, ALP, Total Bilirubin were estimated to assess the liver functions. In addition, histopathological study was also carried out. Results: Both the doses (250mg/kg and 500 mg/kg, p.o) of Sphagneticola trilobata flower extract showed dose dependent significant decrease of biochemical parameter such as ALP, AST, ALT, TOTAL BILIRUBIN. Histopathology of liver showed reduced inflammation, centrilobular and bridging necrosis and it shows normal hepatocytes with significant reduction in areas of necrosis compared to toxic control. The result obtained was comparable with that of the standard drug Silymarin. Conclusion: The finding of the present study provides the evidence that, ethanolic extract of may be beneficial against Paracetamol and Carbon tetrachloride induced hepatotoxicity.

Keywords: Carbon tetrachloride, Hepatoprotective, Paracetamol, Silymarin, Sphagneticola trilobata

Introduction:

The liver, one of the largest organs in the body, plays a crucial role in maintaining and regulating the body's homeostasis through its extensive biotransformation and excretion processes. It is responsible for the metabolism of carbohydrates, proteins and fats, as well as detoxification, bile secretion, and vitamin storage, all of which are vital to its function. [1,2] Maintaining liver health is therefore essential for overall well-being. Liver injury, whether caused by chemical or infectious agents, can lead to liver fibrosis, cirrhosis, and ultimately liver failure. Unfortunately, no effective treatment has yet been discovered to halt the progression of such liver diseases. [3,4] Hepatotoxicity refers to liver damage caused by chemicals. Certain medications can damage the liver when taken in excessive amounts, and in some cases, even within the recommended dosage range. Substances that cause liver damage are known as hepatotoxins. [5]Liver damage is a leading cause of drug withdrawals from the market, with over 900 medications associated with the condition. Often, subclinical liver damage due to chemicals is only detected through abnormal liver enzyme tests. Drug-induced liver injury accounts for 50% of acute liver failures and 5% of hospital admissions. [6] Over 75% of cases involving idiosyncratic drug reactions result in liver transplantation or death. Paracetamol, a commonly used drug with a generally favourable safety profile, can cause severe hepatic necrosis and fatal liver failure when taken in excessive doses. Intravenous N-acetylcysteine is an effective treatment for paracetamol overdose if administered promptly. However, if hepatic encephalopathy develops, the risk of complications and mortality rises significantly. [7] Orthotopic liver transplantation (OLT) is a treatment option for liver failure resulting from a paracetamol overdose. Due to its mild acidity and lipid solubility, paracetamol is rapidly absorbed in the intestines following oral ingestion. Around 50-60% of it is converted into pharmacologically inactive conjugates, which are then excreted in urine. A smaller fraction of paracetamol (5-10%) is metabolized in liver microsomes by cytochrome P450 isoforms (CYP2E1, CYP2A6) into N-acetyl-para-benzoquinone imine (NAPQI), a reactive metabolite primarily responsible for paracetamol's hepatotoxic effects. The amount of paracetamol ingested directly influences the extent of cellular damage caused by NAPQI. With non-toxic doses, NAPQI is quickly conjugated by hepatic glutathione through glucuronidation and sulfonation, forming mercaptate and cysteine complexes that are excreted in urine. However, when paracetamol is consumed at hepatotoxic levels, most of the drug is metabolized via the CYP2E1 pathway, leading to glutathione depletion, activation of GST-S-transferases, and the accumulation of NAPQI at toxic concentrations. [8] The primary factors contributing to CCl_4-induced liver damage include lipid peroxidation (LPO), decreased enzyme activity, free radical production, and hepatotoxicity. CCl_4-induced cell injury can occur either through accelerated lipid peroxidation, where free radical intermediates interact with oxygen and target unsaturated fatty acids, or through the covalent binding of reactive intermediates to cellular components. This results in the breakdown of lipids, particularly unsaturated phospholipids, causing damage to the plasma membrane and intracellular membranes. Reactive aldehydes, which are the primary breakdown products, spread throughout the cell and contribute to further damage, including increased membrane permeability, a key indicator of impending cell death. The partial pressure of oxygen in

tissues also influences the progression of CCl_4-induced hepatotoxicity. Low oxygen levels lead to the formation of $CHCl_2$ and CCl_3 radicals and the covalent binding of metabolites. This process results in steatosis, or fatty liver, primarily affecting lipid metabolism by increasing lipid production and reducing their transport out of the hepatocyte. [9] The synthetic medications currently used to treat liver disorders may further worsen liver damage, as they must be metabolized by an already compromised liver, potentially reducing their efficacy and increasing the liver's burden. Steroids, vaccines, and antiviral drugs used for liver diseases can also cause adverse effects, particularly when used frequently or over long periods. As a result, there is a growing demand for the development of pharmacologically effective drugs derived from natural products, which generally have lower toxicity and fewer side effects. Active ingredients for treating various illnesses are compounds extracted from different plant species that exhibit similar therapeutic effects despite having diverse structures. Numerous plant-derived phytomolecules, such as flavonoids, alkaloids, glycosides, and saponins, have demonstrated efficacy as hepatoprotective agents. Plant tissues contain a wide range of substances with antioxidant properties. Phenolic compounds nitrogen compounds, carotenoids, lignans, and terpenes have been shown to suppress the initiation or propagation of chain reactions. Flavonoids and phenolic compounds are the primary antioxidants found in herbal medications. The encyclopedia of Indian ethnobotany and traditional medicine lists 2,532 plants, while India is home to over 45,000 plant species, many of which have been studied for their potential medical benefits. Herbal remedies have been employed to treat liver diseases since ancient times and now show significant promise for addressing a wide range of pathological liver conditions. In India, 160 phytoconstituents from 101 plant families are used alongside more than 40 commercial polyherbal preparations that are claimed to possess hepatoprotective properties. [10] *S. trilobata* (L.), is a medicinal plant that has been used for centuries for treating a variety of conditions, including colds, indigestion, hepatitis, ulcers, sore throats, varicose veins, fever, epilepsy, amenorrhoea, wounds, and snakebite. Several studies have documented the plant's bioactivities, including its anticancer, hepatoprotective, antibacterial, anti-inflammatory, antimalarial, and antifungal properties. [11] The *S. trilobata* aqueous extracts demonstrated significant hypoglycemic action as well as potential antidiabetic effects. It was discovered that the methanol extract of *S. trilobata*'s root, stem, leaves, and flowers had a noteworthy antibacterial effect on *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi*. The antioxidant activity of *W. trilobata* leaf, stem, and flower ethanol extract was evaluated. The leaf and stem ethanol extract possessed strong scavenging activity. Due to its known antidiabetic qualities, this plant's aqueous infusion has been used locally and empirically in the Southern region of Brazil to treat diabetes. It is also sometimes referred to as insulin. *S. trilobata* is regarded as a generally safe herb that has no negative effects on renal or hepatic function when taken as a supplemental diet or traditional medicine. Since there is an insufficiency of scientific data available for the hepatoprotective activity of the plant flowers of *Sphagneticola trilobata* on experimental animal models, this study has been chosen. [12,13]

Methodology:

Experimental animals:

The Albino rats (150 -200 g) of both male and female was used for this study. They were kept under standard conditions (temperature 22 ± 2°C, relative humidity 60±5% and 12 h light/dark cycle) and have free access to standard pellet diet and water *ad libitum*. The animals were caged in sanitized polypropylene cages containing sterile paddy husk as bedding. The Institutional Animal Ethics Committee reviewed and approved the experimental protocol (**Approval no. SCP/IAEC/F150/P219/2023**). All the procedures were conducted in accordance with IAEC constituted as per the direction of the Committee for Control and Supervision of Experiments on Animals (CCSEA). [14]

Collection of plant material and preparation of extract:

Sphagneticola trilobata flowers were collected from Mangalore and authenticated by taxonomist Dr. Siddaraju M.N., PhD. Department of Botany, University College Mangalore. The flowers were cleaned and rinsed with tap water to remove any associated debris. Then it was extracted by mixing 100 g of sample with 400 ml 80% ethanol. The extraction was carried out at room temperature by soaking for 7 days with intermittent stirring during the first day. The extract was filtered with clean muslin cloth and then with Whatman No.1 filter paper. The extraction process was repeated for a second time by adding 400 ml of 80% ethanol to the sample residue. The filtrates were combined and evaporated. [15]

EVALUATION OF HEPATOPROTECTIVE ACTIVITY

Paracetamol Induced Liver Toxicity in Rats:

Group I - Normal group (vehicle 1ml/kg; p.o)

Group II - Toxic control (Vehicle 1ml/kg; p.o. +Paracetamol 2g/kg, p.o. on 9th day)

Group III - Standard group (Silymarin 25 mg/kg,p.o + Paracetamol 2g/kg, p.o. on 9th day)

Group IV - Test group (*Sphagneticola trilobata* flowers extract 250mg/kg, p.o. + Paracetamol 2g/kg, p.o. on 9th day)

Group V - Test group (*Sphagneticola trilobata* flowers extract 500mg/kg, p.o.+ Paracetamol 2g/kg, p.o. on 9th day)

PROCEDURE:

All the drug preparations and *Sphagneticola trilobata* extract will be suspended in suitable solvent for oral administration. All the treatment will be given orally once daily throughout the treatment. All the groups except group I will be made hepatotoxic by oral administration of paracetamol (2 g/kg body weight) on 9th day.

EVALUATION:

On 11th day, blood will be collected through cardiac puncture under light anaesthesia and serum will be separated by centrifugation (2500 rpm for 20 min) and for analyzing various biochemical parameters.

Animals will be sacrificed by overdose of Ketamine anesthesia and the liver will be dissected out and the part of which will be placed in 10% formalin solution for histopathological studies.[16,17]

BIOCHEMICAL ESTIMATION:

The separated blood serum samples were analyzed for estimation of AST, ALT, ALP and Bilirubin content using commercially available diagnostic kits.

HISTOPATHOLOGICAL STUDIES:

The isolated liver was preserved in 10 % formalin. The sections were taken using microtome. The different histopathological indices were evaluated by subsequent staining with haematoxylin and eosin.

Carbon tetrachloride (CCl_4) liver toxicity:

The Wistar albino rats (150-200g) of either sex will be randomly divided into five groups of six animals each. The different groups will be assigned as follows,

Group I - Normal group (vehicle 1ml/kg; p.o)

Group II - Toxic control (Vehicle 1ml/kg; p.o. +CCl_4/olive oil (1:1 v/v 0.7ml/kg,i.p.)

Group III - Standard group (Silymarin 25 mg/kg,p.o + CCl_4/olive oil (1:1 v/v0.7ml/kg, i.p.)

Group IV- Test group (*Sphagneticola trilobata* flowers extract 250mg/kg, p.o. + CCl_4/olive oil (1:1 v/v 0.7ml/kg,i.p.)

Group V - Test group (*Sphagneticola trilobata* flowers extract 500mg/kg, p.o.+ CCl_4/olive oil (1:1v/v 0.7ml/kg,i.p.).

PROCEDURE:

All the animals except 1st group was made hepatotoxic by administered of CCl_4 (1:1 of CCl_4 in olive oil 0.7ml/kg, i.p.) on 2nd, 4th & 6th day of treatment. All the treatment was given orally once daily for seven days.[18]

EVALUATION:

On 7th day, 2 h after the treatment, blood will be collected through cardiac puncture under light anaesthesia and serum will be separated by centrifugation (2500 rpm for 20 min). The serum was separated by centrifuge (2500rpm for 20 minutes) and used for estimation of various biochemical parameters. The animals were sacrificed by euthanasia (overdose of ketamine) and liver will be dissected out for histopathological studies.

BIOCHEMICAL ESTIMATION:

The separated blood serum samples were analyzed for estimation of AST, ALT, ALP and Bilirubin content using commercially available diagnostic kits.

HISTOPATHOLOGICAL STUDIES:

The isolated liver was preserved in 10 % formalin. The sections were taken using microtome. The different histopathological indices were evaluated by subsequent staining with haematoxylin and eosin.[19,20]

Statistical analysis

All data were expressed as mean ± SEM. The statistical significance between groups was compared using one way ANOVA, followed by Dunnett's (multiple comparisons) test.

Results :

Evaluation of hepatoprotective activity of ethanolic extract of *Sphagneticola trilobata* on paracetamol induced hepatic damage in rats

Administration of paracetamol to rats caused significant liver damage, as evidenced by the altered serum biochemical parameters. Pre-treatment of rats with *Sphagneticola trilobata extract* exhibited marked protection against paracetamol induced hepatotoxicity, (shown in Table 1). The effects produced by *Sphagneticola trilobata extract* were comparable with that produced by the standard; Silymarin.

I. Biochemical parameters

The effect of *Sphagneticola trilobata* extract on various biochemical parameters are shown in Table No.1 and Fig. 1 and 2

Group I (Control)

The ALP, AST, ALT and Total Bilirubin levels were 107.70±6.26, 31.98±1.50, 35.83±2.22 and 0.65±0.03 respectively. Histopathological study showed normal portal triad, sinusoids, and cord arrangement of hepatocytes. (Figure no: 3A)

Group II

The paracetamol induced group showed elevation in ALP, AST, ALT and Total Bilirubin levels up to 221.31±6.19, 220.50±5.44, 185.08±4.52 and 1.80±0.17 respectively when compared to the control group. Histopathological study showed portal tract inflammation. (Figure no: 3B)

Group III

There was a significant ($p<0.001$) reduction in the ALP, AST, ALT and Total Bilirubin levels (118.21±2.68, 32.65±1.23, 42.96±2.60 and 0.75±0.03 respectively) after the treatment with Silymarin (25mg/kg). Histopathological study showed almost normal appearing hepatocytes. (Figure no: 3C).

Group IV

There was a reduction ($p<0.05$) in the ALP, AST, ALT and Total bilirubin levels (175.83±3.29, 65.63±4.57, 60.03±4.25 and 1.27±0.1 respectively) after the treatment with 250 mg/kg *Sphagneticola trilobata extract*. Histopathological study showed mild portal tract inflammation. (Figure no:3D).

Group V

There was a significant ($p<0.01$) reduction in the ALP, AST, ALT and Total bilirubin levels (155.50±4.3, 52.53±4.94, 49.73±4.43 and 0.86±0.09 respectively) after the treatment with 500mg/kg *Sphagneticola trilobata extract*. Histopathological study showed minimal portal tract inflammation. (Figure no: 3E).

Table No 1: Effect of *Sphagneticola trilobata* extract on AST, ALT, ALP and Total Bilirubin in PCM induced liver toxicity

Groups	Treatment	ALP(U/I)	AST(U/I)	ALT(U/I)	TB(mg/dl)
Normal Control	Vehicle (1ml/kg)	107.70±6.26	31.98±1.50	35.83±2.22	0.65±0.03
Toxic Control	PCM (2g/kg)	221.31±6.19[a]	220.50±5.44[a]	185.08±4.52[a]	1.80±0.17[a]
Standard	Silymarin (25mg/kg)	118.21±2.68[***]	32.65±1.23[***]	42.96±2.60[***]	0.75±0.03[***]
Dose 1	*Sphagneticola trilobata* extract (250 mg/kg)	175.83±3.29[*]	65.63±4.57[*]	60.03±4.25[*]	1.27±0.1[*]
Dose 2	*Sphagneticola trilobata* extract (500mg/kg)	155.50±4.3[**]	52.53±4.94[**]	49.73±4.43[**]	0.86±0.09[**]

All the values are Mean± SEM, n=6. One way ANOVA followed by Dunnett"s t test. [a] $p< 0.001$ when compared with vehicle treated control group.*$p<0.05$, **$p<0.01$,

***$p<0.001$ when compared with toxic control

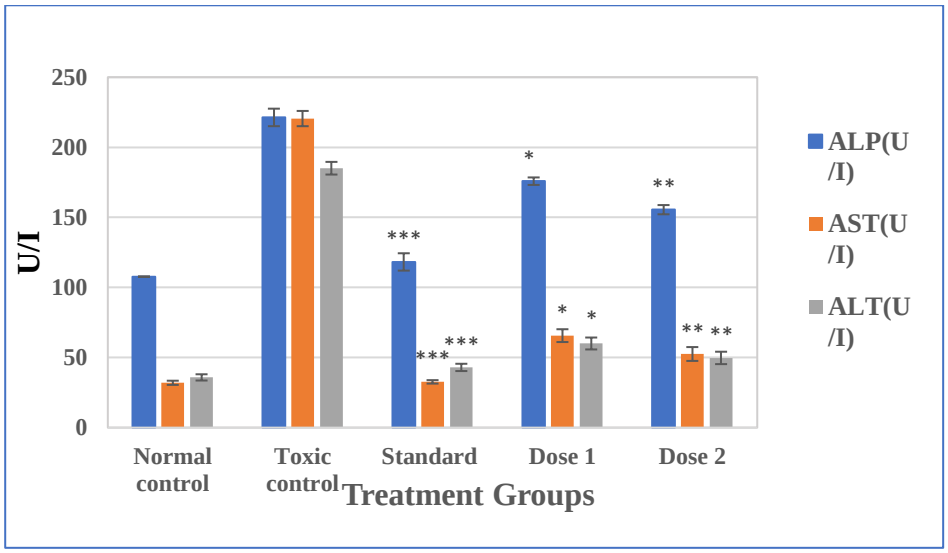
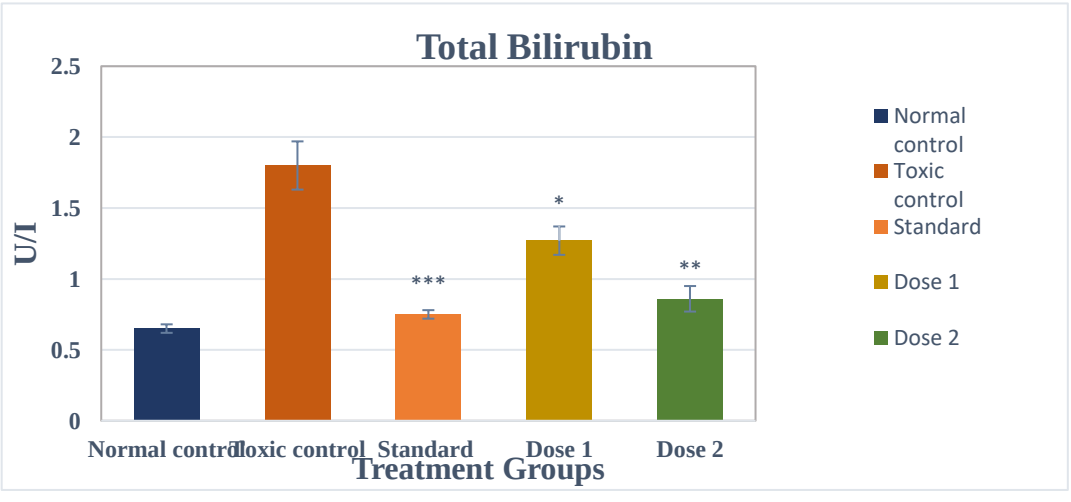


Fig No 1: Effect of *Sphagneticola trilobata* extract on Serum ALT, AST& ALP in PCM induced liver toxicity

Fig no 2: Effect of Sphagneticola trilobata extract on TB in PCM induced liver toxicity



Histopathological studies of the liver: The histopathological evaluation of PCM toxicity in all the groups was examined and shown in Fig no. 3. Liver section of normal group shows liver parenchyma with intact architecture. Most hepatocytes appear normal. In toxic control group shows inflammation, centrilobular degeneration and necrosis. Treatment with extract of *Sphagneticola trilobata* (250mg/kg & 500mg/kg) found to reduce inflammation, centrilobular and bridging necrosis. Liver section of this group shows normal hepatocytes with significant reduction in areas of necrosis when compared to toxic group. These changes show protective effect of the drug against hepatic damage induced by PCM.

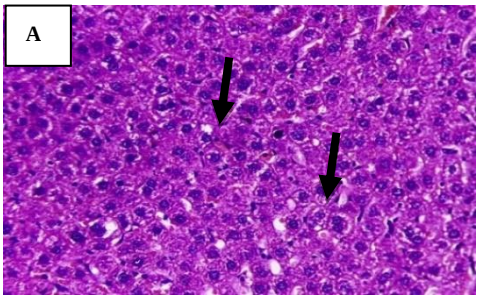


Fig no.3A: Section of the liver tissue of control showing normal central vein and radiating hepatocytes.

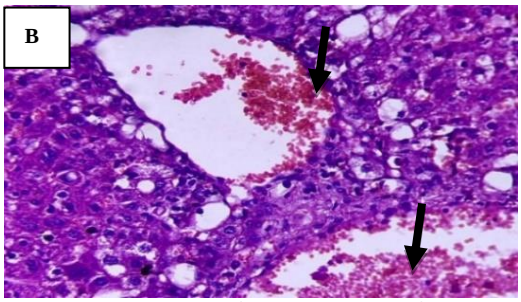


Fig no. 3B: Section of the liver tissue of animal treated with PCM showing fatty degeneration, necrosis and fibrosis.

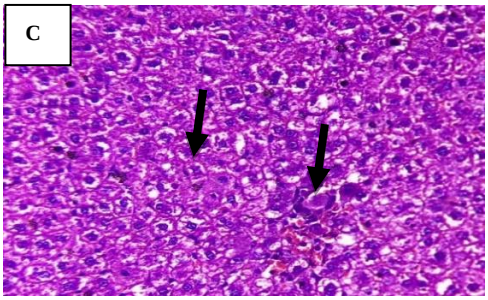


Fig no. 3C: Section of the liver tissue of silymarin treated animals showing normal hepatocytes with central hepatic vein.

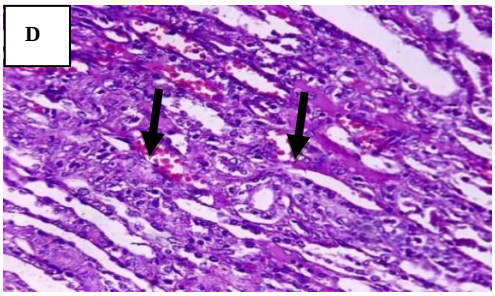


Fig no. 3D: Section of the liver tissue of animal treated with ethanolic extract of *Sphagneticola trilobata* showing absence of necrosis and minimal fatty change.

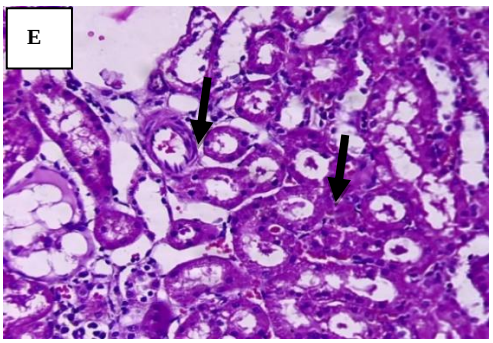


Fig no. 3E: Section of the liver tissue of animal treated with ethanolic extract of *Sphagneticola trilobata* showing absence of necrosis and moderate fatty change.

Fig No 3: Effect of *Sphagneticola trilobata* extract on liver histology in PCM-induced liver toxicity.

A: Normal rat; B: PCM-induced rat; C: Silymarin-treated rat; D: *Sphagneticola trilobata* extract (250 mg/kg) treated rat; E: *Sphagneticola trilobata* extract (500mg/kg) treated rat.

Evaluation of hepatoprotective activity of *Sphagneticola trilobata extract* on carbon tetrachloride(ccl_4) induced hepatic damage in rats.

In the present study, the hepatotoxicity was successfully produced by administration of carbon tetrachloride 0.7ml/kg body weight for once daily and the hepatoprotective activity of *Sphagneticola trilobata extract* was determined from the serum parameters AST, ALT, ALP and TB.

I. Biochemical parameters

The effect of *extract* on various biochemical parameters are shown in Table No.2 and Fig no.4 and 5.

Group I (Control)

The ALP, AST, ALT and Total Bilirubin levels were 93.31± 2.91, 37.51± 3.29, 27.43± 2.93and 0.45± 0.12 respectively. Histopathological study showed normal portal triad, sinusoids, and cord arrangement of hepatocytes. (Figure no: 6A)

Group II

The carbon tetrachloride induced group showed elevation in ALP, AST, ALT and Total Bilirubin levels 209.15± 4.19, 107.68± 3.62, 104.56± 3.28 and 1.23± 0.16 respectively when compared to the control group. Histopathological study showed portal tract inflammation. (Figure no: 6B)

Group III

There was a significant ($p<0.001$) reduction in the ALP, AST, ALT and Total Bilirubin levels 119.56±3.38, 53.88±3.33, 33.46±3.2 and 0.48±0.03 respectively after the treatment with Silymarin (25mg/kg). Histopathological study showed almost normal appearing hepatocytes. (Figure no: 6C)

Group IV

There was a reduction ($p<0.05$) in the ALP, AST, ALT and Total bilirubin levels (195.6± 3.26 , 79.73± 2.27, 72.66± 3.26 and 0.76±0.21 respectively) after the treatment with 250 mg/kg *Sphagneticola trilobata extract*. Histopathological study showed mild portal tract inflammation. (Figure no:6D).

Group V

There was a significant ($p<0.01$) reduction in the ALP, AST, ALT and Total bilirubin levels (162.75± 3.41, 67.91± 2.57 , 59.83± 3.37and 0.59±0.03 respectively) after the treatment with 500mg/kg *Sphagneticola trilobata extract*. Histopathological study showed minimal portal tract inflammation. (Figure no: 6E)

Table 2: Effect of *Sphagneticola trilobata extract* on AST, ALT, ALP & TB in carbon tetrachloride(ccl_4) induced liver toxicity.

Groups	Treatment	ALP(U/I)	AST(U/I)	ALT(U/I)	TB(mg/dl)
Normal Control	Vehicle (1ml/kg)	93.31± 2.91	37.51± 3.29	27.43± 2.93	0.45± 0.12
Toxic Control	Carbon tetrachloride	209.15± 4.19[a]	107.68± 3.62[a]	104.56± 3.28[a]	1.23± 0.16[a]
Standard	Silymarin (25mg/kg)	119.56±3.38***	53.88±3.33***	33.46± 3.2***	0.48±0.03***
Dose 1	*Sphagneticola trilobata extract* (250 mg/kg)	195.6± 3.26*	79.73± 2.27*	72.66± 3.26*	0.76± 0.21*
Dose 2	*Sphagneticola trilobata extract* (500 mg/kg)	162.75± 3.41**	67.91± 2.57**	59.83± 3.37**	0.59±0.03**

All the values are Mean± SEM, n=6. One way ANOVA followed by a Dunnett's t test. [a]p< 0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

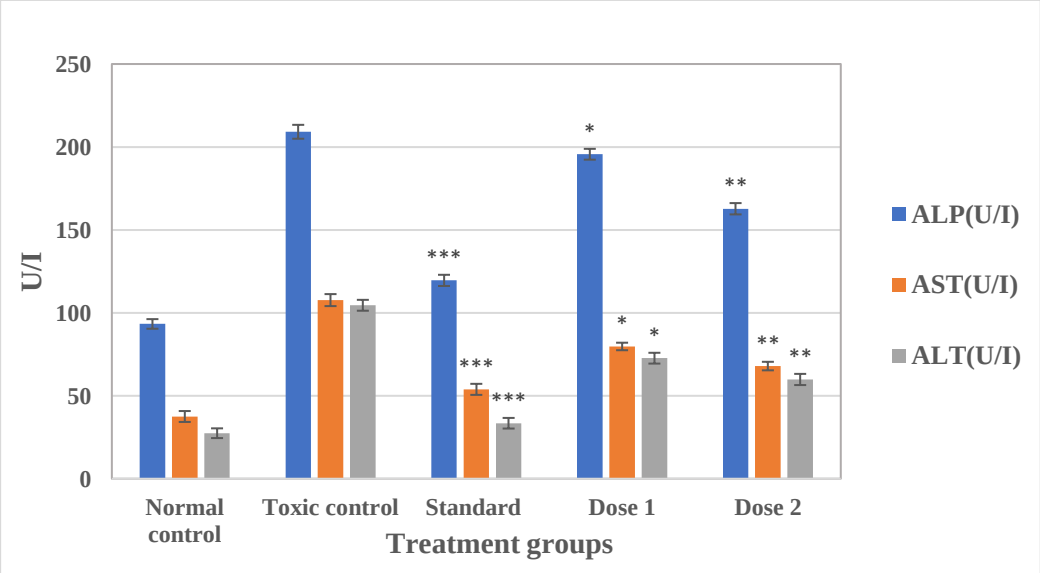


Fig No 4: Effect of *Sphagneticola trilobata* extract on AST, ALT& ALP in CCL_4 induced liver toxicity

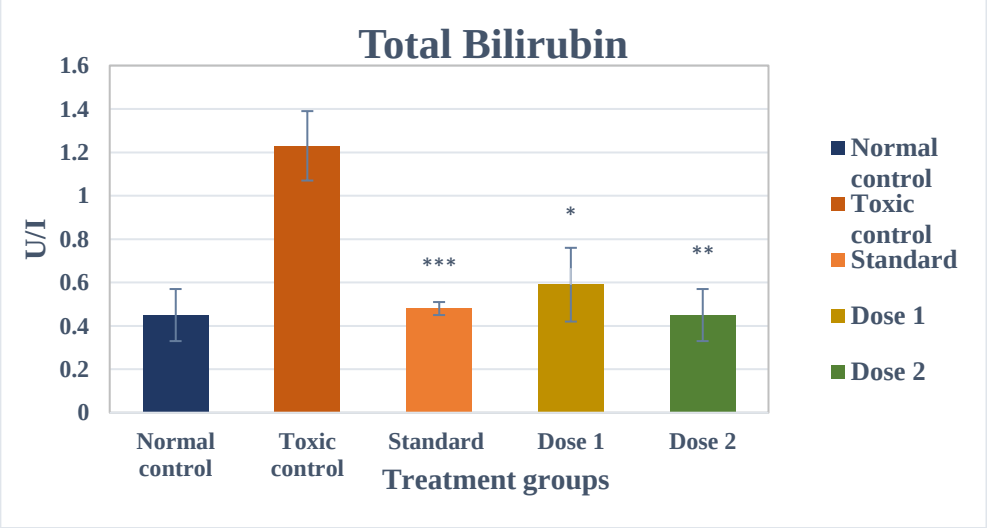


Fig No 5: Effect of *Sphagneticola trilobata* extract on serum TB in CCL_4 induced liver toxicity

Histopathological studies of the liver:

The histopathological evaluation of Carbon tetrachloride toxicity in all the groups was examined and shown in Fig no.6. Liver section of normal group shows liver parenchyma with intact architecture. Most hepatocytes appear normal.

In toxic control group shows inflammation, centrilobular degeneration and necrosis. Treatment with *Sphagneticola trilobata* (250 mg/kg & 500mg/kg) found to reduce inflammation, centrilobular and bridging necrosis. Liver section of this group shows normal hepatocytes with significant reduction in areas of necrosis when compared to toxic group. These changes show protective effect of the drug against hepatic damage induced by Carbon tetrachloride.

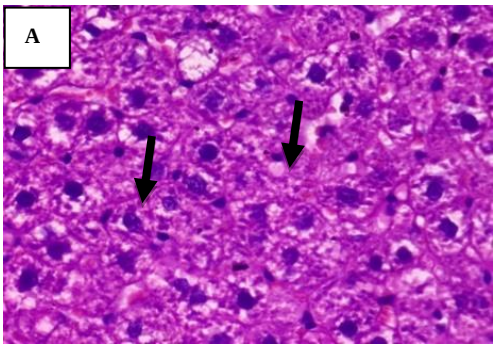


Fig no. 6A: Section of the liver tissue of control showing normal central vein andradiating hepatocytes.

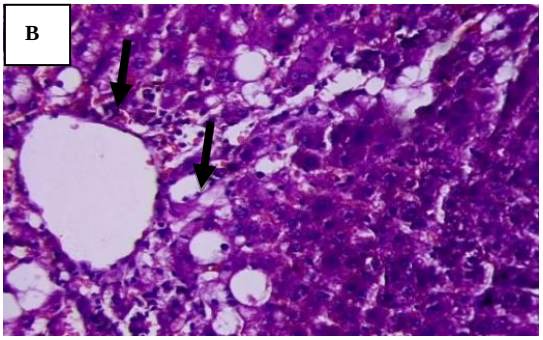


Fig no. 6B: Section of the liver tissue of animal treated with CCL_4 showing fatty degeneration, necrosis and fibrosis.

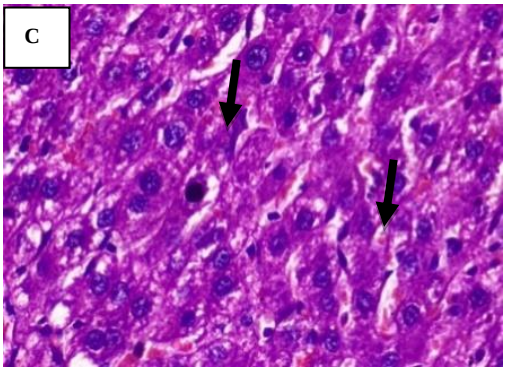


Fig no. 6C: Section of the liver tissue of silymarin treated animals showing normal hepatocytes with central hepatic vein.

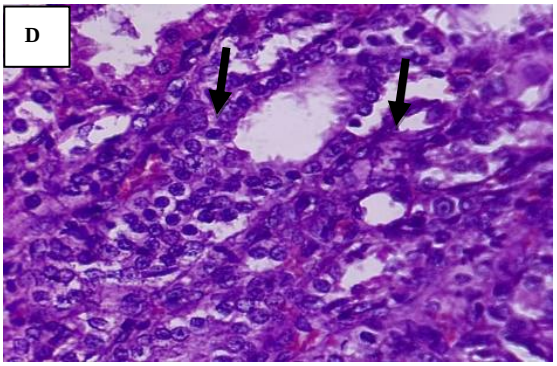


Fig no 6D : Section of the liver tissue of animal treated with ethanolic extract of *Sphagneticola trilobata* showing absence of necrosis and minimal fatty change.

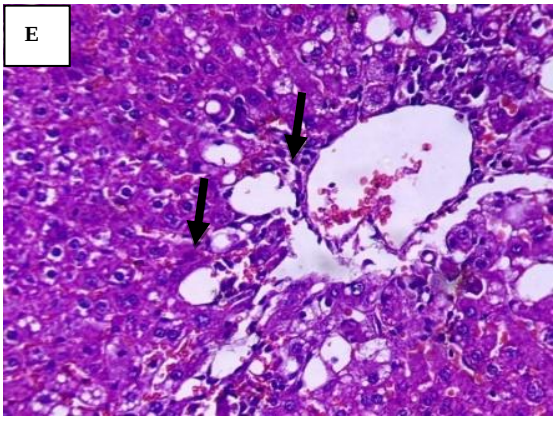


Fig no. 6E: Section of the liver tissue of animal treated with ethanolic extract of *Sphagneticola trilobata showing* absence of necrosis and moderate fatty change.

Fig No 6: Effect of *Sphagneticola trilobata* extract on liver histology in carbon tetrachloride induced liver toxicity.

A: Normal rat; B: CCL_4 induced rat; C: Silymarin treated rat; D: *Sphagneticola trilobata* extract (250 mg/kg) treated rat; E: *Sphagneticola trilobata* extract (500mg/kg) treated rat.

Discussion :

Drug-induced liver injury (DILI) is a significant issue in hepatic pathology due to its complexity and the potential impact of a patient's comorbidities on interpretation. Although liver biopsies can be performed for a number of reasons, they are most frequently performed to aid in the diagnosis of suspected DILI when there is clinical uncertainty. Biopsies may also be used to assess the degree of liver damage or to rule out serious conditions including severe fibrosis, necrosis, or duct loss. Differential diagnosis is often problematic as well. Due to the widespread perception that herbal remedies are safe and do not cause severe side effects, their usage in treating liver disorders has grown globally.[21,22] Using two models the carbon tetrachloride-induced liver damage model and the paracetamol-induced liver damage model, the current study was conducted to assess the hepatoprotective activity of *Sphagneticola trilobata* extract. Serum enzyme measurements such as ALT, AST, ALP, and total bilirubin, as well as histological investigations were the criteria utilised to evaluate the hepatoprotective effectiveness. The administration of paracetamol considerably raises the serum levels of total bilirubin, ALT, AST, and ALP. Its bioactivation to the hazardous electrophile N-acetyl-p-benzoquinone-imine is the cause of this. Normally, paracetamol is mostly excreted as glucuronide and sulphate. The amount of paracetamol that is transformed into N-acetyl-p-benzoquinone-imine is just 5%. The sulfation and glucuronidation pathways, however, become saturated when lethal quantities of paracetamol are administered; as a result, a greater proportion of paracetamol molecules are oxidised by cytochrome-450 enzymes to the extremely reactive N-acetyl-p-benzoquinone-imine (NAPQI). One electron reduction of NAPQI produces a semi-Quinone radical, which can covalently attach to cellular membrane. Histopathological investigations that revealed portal tract inflammation further supported the hepatotoxic impact of paracetamol. A nine-day pretreatment with *Sphagneticola trilobata* extract prevented paracetamol from raising ALT, AST, ALP, and total bilirubin. These metabolic effects could be the result of cytochrome P450 inhibition and/or glucuronidation promotion. Histopathological investigations that revealed nearly normal hepatocytes further supported this. [23,24]

Perfused livers, isolated or cultured hepatocytes, and in vivo models of acute and chronic CCl_4 poisoning have all been used in this investigation. It is now widely accepted that CCl_4 toxicity is multifaceted. This process results in the creation of inflammatory cytokines, lipid peroxidation, covalent attachment to macromolecules, loss of calcium homeostasis, hypomethylation of nucleic acids, and free radicals created from CCl_4. Most studies on the initial phases of CCl_4 toxicity, which occur within minutes to many hours, have been conducted in vitro with isolated hepatocytes. According to these studies, early warning indicators include hepatocyte enlargement, endoplasmic reticulum disorder and mitochondrial morphological damage. Therefore, little is known about this organic solvent's first effects in vivo, particularly in relation to mitochondrial function. Long-term CCl_4 injection for six weeks causes modifications to mitochondrial DNA (mtDNA), reduced glutathione (GSH) depletion, and lowered aconitase activity, according to recent studies using a mouse model of liver fibrosis. In this investigation, overexpression of Bcl-2 initially reduced liver fibrosis by protecting hepatocytes from mitochondrial damage during the first three weeks of treatment. However, this protective effect did not prevent fibrosis with repeated exposure. Other CYPs (CYP2B and CYP3A) only partially activate CCl_4, which is primarily activated by cytochrome P450 (CYP)2E1 to generate the trichloromethyl ($CCl_3\cdot$) free radical. This radical can produce the trichloromethyl peroxy radical ($CCl_3OO\cdot$) when it reacts with oxygen. Both radicals are extremely reactive and have the ability to generate lipid, protein, and nucleic acid adducts by covalently attaching to macromolecules. However, there isn't much evidence that these interactions with liver DNA take place in vivo. [25,26] Several studies utilising radiolabelled CCl_4 have indicated moderate binding to hepatocyte DNA; however, mass spectrometry detection of adducts has not confirmed this. Lipid peroxidation is initiated by the $CCl_3O\cdot$ radical when a hydrogen atom near a double bond in polyunsaturated fatty acids is abstracted. As the peroxidation process goes on, lipids are finally broken down into small molecules like malondialdehyde (MDA) and 4-hydroxynonenal (HNE). [27,28]

These highly reactive aldehydes can form adducts with proteins and DNA. Lipid peroxidation and mitochondrial changes were directly linked when the CYP2E1 inhibitor diethyldithiocarbamate (DDTC) inhibited CCl_4 activation and antioxidants inhibited CCl_4-induced lipid peroxidation. [29,30] The study indicates the presence of saponins, flavonoids, and polyphenols in *Sphagneticola trilobata* extract and suggests that these flavonoids may play a

significant role in enhancing antioxidant and free radical scavenging capabilities across various assays. Plant extracts rich in natural antioxidants can produce radical scavengers, which help mitigate oxidative damage to the liver. Overall, the findings highlight that the diverse bioactive compounds found in extract work together to bolster antioxidant defenses, thereby supporting liver health and potentially preventing oxidative stress-related damage.

Conclusion :

This study aimed to evaluate the hepatoprotective properties of *Sphagneticola trilobata* extract. The extract showed notable hepatoprotective activity in oxidative stress response, which reduces oxidative stress and safeguards liver cells from harm. These findings suggest that *Sphagneticola trilobata* extract holds promise for use as a hepatoprotective agent. To optimize the medicinal benefits of *Sphagneticola trilobata*, it is essential to extract and refine the bioactive constituents that are responsible for its protective properties. Identifying compounds such as alkaloids, phenols and flavanoids could enable more precise therapeutic applications and help ensure consistent efficacy across extracts. Future research should emphasize investigating how these compound combinations work together to enhance hepatoprotection, which could support the development of more efficacious and robust formulations. The exploration of these interactions could also uncover novel therapeutic pathways that individual compounds might not achieve alone. Additionally, understanding the underlying mechanisms of these combined effects will be crucial for optimizing dosage and maximizing the therapeutic potential of the plant's phytochemicals. Such insights could lead to the discovery of new applications and benefits beyond hepatoprotection. Furthermore, this knowledge could pave the way for more personalized and targeted therapeutic approaches. Overall, thorough investigation into the plant's phytochemical interactions will be essential for unlocking its full medicinal potential.

REFERENCES:

1. Natikar, N. A., Mangannavar, C. V., Shalavadi, M. H., & Kolli, S. S. (2020). Hepatoprotective activity of *Curcuma vamana* ethanolic rhizome extract against paracetamol and CCl_4 induced hepatotoxicity in albino rats. RGUHS Journal of Pharmaceutical Sciences, 10(4).
2. Williams, R. (2006). Global challenges in liver disease. Hepatology, 44(3), 521–526.
3. McGill, M. R. (2016). The past and present of serum aminotransferases and the future of liver injury biomarkers. EXCLI Journal, 15, 817.
4. Stickel, F., & Schuppan, D. (2007). Herbal medicine in the treatment of liver diseases. Digestive and Liver Disease, 39(4), 293–304.
5. Bhawna, S., & Kumar, S. U. (2009). Hepatoprotective activity of some indigenous plants. International Journal of PharmTech Research, 4, 1330–1334.
6. Służały, P., Paśko, P., & Galanty, A. (2024). Natural products as hepatoprotective agents—A comprehensive review of clinical trials. Plants, 13(14).
7. Asrani, S. K., Devarbhavi, H., Eaton, J., & Kamath, P. S. (2019). Burden of liver diseases in the world. Journal of Hepatology, 70(1), 151–171.
8. Caparrotta, T. M., Antoine, D. J., & Dear, J. W. (2018). Are some people at increased risk of paracetamol-induced liver injury? A critical review of the literature. European Journal of Clinical Pharmacology, 74, 147–160.
9. Taniguchi, M., Takeuchi, T., Nakatsuka, R., Watanabe, T., & Sato, K. (2004). Molecular process in acute liver injury and regeneration induced by carbon tetrachloride. Life Sciences, 75(13), 1539–1549.
10. Kumar, C. H., Ramesh, A., Kumar, J. S., & Ishaq, B. M. (2011). A review on hepatoprotective activity of medicinal plants. International Journal of Pharmaceutical Sciences and Research, 2(3), 501.
11. Buddhakala, N., & Talubmook, C. (2020). Toxicity and antidiabetic activity of ethanolic extract of Sphagneticola trilobata (L.) Pruski flower in rats. Journal of Ethnopharmacology, 262, 113128.
12. Mardina, V., Ilyas, S., Halimatussakdiah, H., Harmawan, T., Tanjung, M., & Yusof, F. (2021). Anticancer, antioxidant, and antibacterial activities of the methanolic extract from *Sphagneticola trilobata* (L.) J. F Pruski leaves. Journal of Advanced Pharmaceutical Technology & Research, 12(3), 222.
13. Adewusi, E. A., & Afolayan, A. J. (2010). A review of natural products with hepatoprotective activity. Journal of Medicinal Plants Research, 4(13), 1318–1334.
14. Kumar, T., & Jain, V. (2014). Antinociceptive and anti-inflammatory activities of *Bridelia retusa* methanolic fruit extract in experimental animals. Scientific World Journal, 2014, 1–12.
15. Junlatat, J., Peng-ngummuang, K., Thorajake, P., Fangkrathok, N., & Sripanidkulchai, B. (2023). Biological activities supporting development of cosmeceutical skin products from *Wedelia trilobata* (L.) Hitch. flower extract. Agriculture and Natural Resources, 57(1), 77–86.
16. Prasanna, K. S., Reddy, G. J., Kiran, M., & Raju, K. T. (2019). Biological activities and phytochemical constituents of trailing daisy trilobata: A review. Journal of Drug Delivery and Therapeutics, 9(4-s), 888–892.
17. Balekar, N., Nakpheng, T., & Srichana, T. (2014). *Wedelia trilobata* L.: A phytochemical and pharmacological review. Chiang Mai Journal of Science, 41(3), 590–605.
18. Mardina, V., & Sufriadi, E. (2020). Flower of *Sphagneticola trilobata* (L.) J. F Pruski from Aceh, Indonesia: Antioxidant and cytotoxic activity on HeLa cells. IOP Conference Series: Materials Science and Engineering, 1007(1), 012182.
19. Babu, D. V., Kulkarni, R., & Bhatt, R. (2017). Evaluation of hepatoprotective activity of *Jasminum sambac* in rats. International Journal of Research in Pharmacology and Pharmacotherapeutics, 6(2).
20. Jacob, J. M., Olaleye, M. T., & Olugbuyiro, J. A. (2014). Hepatoprotective effect of *Alchornea cordifolia* leaf on liver damage in albino rats. International Journal of Applied Sciences and Biotechnology, 2(2), 217–221.

21. Ramachandran, R., & Kakar, S. (2009). Histological patterns in drug-induced liver disease. Journal of Clinical Pathology, 62(6), 481–492.
22. Gasmi, B., & Kleiner, D. E. (2020). Liver histology: Diagnostic and prognostic features. Clinics in Liver Disease, 24(1), 61–74.
23. Prescott, L. F. (2000). Paracetamol, alcohol, and the liver. British Journal of Clinical Pharmacology, 49(4), 291–301.
24. Caparrotta, T. M., Antoine, D. J., & Dear, J. W. (2018). Are some people at increased risk of paracetamol-induced liver injury? A critical review of the literature. European Journal of Clinical Pharmacology, 74, 147–160.
25. Mistry, S., Dutt, K. R., & Jena, J. (2013). Protective effect of Sida cordata leaf extract against CCl_4-induced acute liver toxicity in rats. Asian Pacific Journal of Tropical Medicine, 6(4), 280–284.
26. Dong, S., Chen, Q. L., Song, Y. N., Sun, Y., & Wei, B. (2016). Mechanisms of CCl_4-induced liver fibrosis with combined transcriptomic and proteomic analysis. Journal of Toxicological Sciences, 41(4), 561–572.
27. Bjornsson, E. S. (2016). Hepatotoxicity by drugs: The most common implicated agents. International Journal of Molecular Sciences, 17(2), 224.
28. Gong, J., Tu, W., Liu, J., & Tian, D. (2023). Hepatocytes: A key role in liver inflammation. Frontiers in Immunology, 13, 1083780.
29. Czaja, A. J. (2014). Hepatic inflammation and progressive liver fibrosis in chronic liver disease. World Journal of Gastroenterology, 20(10), 2515–2532.
30. Wang, K. (2014). Molecular mechanisms of hepatic apoptosis. Cell Death and Disease, 5(1), 1–10.