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RESEARCH ARTICLE

Combined extract evaluation of hydroalcoholic leaf extracts in evaluating the hepatoprotective efficacy of *Sphaeranthus amaranthoides* and *Paerida foetida* against CCl_4-induced hepatotoxicity in wistar rats

Anima Jena[1], Prasanth Damarasingu[1]*, Nityananda Sahoo[2] & Gyanranjan Mahallik[1]

[1]School of Pharmacy and Life Sciences, Centurion University of Technology and Management, Bhubaneswar 752 050, Odisha, India
[2]Nityananda College of Pharmacy, Department of Pharmachemistry, Balasore 756 060, Odisha, India

*Correspondence email - bpharmpharmacy08@gmail.com

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Abstract

Liver disease is becoming the most common cause of death all over the world. Each year 5.9 % of global death is associating with alcohol-related liver disease (ALD), The current study focuses on readily available natural resources. *S.amaranthoides* and *P. foetida* plants hydroethanolic extracts demonstrated a significant hepatoprotective potential on CCl_4-induced hepatotoxic Wistar rats, Alteration of serum SGOT, SGPT and Alkaline Phosphatase concentration caused by CCl_4 administration was recovered by using combined plant extracts. Treatment with 500 mg/kg of both plants' hydroethanolic extracts, *Sphaeranthus amaranthoides and Paerida foetida,* resulted in a significant decrease in the levels of ALT, AST, ALP, total protein, total bilirubin, as well as a significant increase in the levels of MDA and GSH when compared to the disease control group. When compared to the standard group given silymarin at a dose of 10 mg/kg body weight, histopathological studies revealed a good hepatoprotective effect. Combined plant extracts have shown a potential impact, may be due to presence of flavonoids and phenolic content. As both plants are edible, they are safe and effective.

Keywords: *Sphaeranthus amaranthoides*; *Paerida foetida*; hepatoprotective activity

Introduction

The liver is one of the most vital organs, with different functions in the body, like secretion (bile), metabolism, detoxification (waste metabolites) and storage (vitamins). The World Health Organization reports that there are approximately 2.4 million deaths worldwide associated with liver disease, among them cirrhosis accounting for about 800000 of these deaths (1). Damage to the liver's cells, tissues, structure, or function is referred to as "hepatic disease". Biological causes (like bacteria, parasites and viruses) and autoimmune disorders (such as immune hepatitis and primary biliary cirrhosis) may cause this damage. Some other factors are chemicals like some medications in high doses that may cause toxic effects on the liver, for example, paracetamol and anti-tubercular drugs; some poisonous compounds are also responsible for liver disorders like carbon tetrachloride, thioacetamide, dimethylnitrosamine, D-galactosamine or lipopolysaccharide and excessive alcohol consumption. (2). Impairment in the liver is generally linked to oxidative stress, which is an increase in tissue alanine transaminase (ALT), lipid peroxidation, aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), total bilirubin (TB) and cell necrosis (3). Hepatic disorders can be effectively managed using traditional medicine derived from natural sources. Over the past few decades, scientists and researchers have been more motivated to identify potential plant -based hepatoprotective compounds to provide cutting-edge, contemporary treatments for various liver ailments (4, 5). Human beings from the beginning of time have been depending on nature for food as well as for health issues (6). One of the great sources of drugs is medicinal plants (7). *Sphaeranthus amaranthoides* Burm. f. (Asteraceae) is widely dispersed in tropical Asia, Australia and Africa (8). Different therapeutic uses are mentioned in Table 1. *S. amaranthoides* consists of D-carvone, Dodecane, Phytol, Hexadecanoic acid, T-Cadinol, Docosane, tau.-Cadinol, Tricosane, Hexadecanoic acid methyl ester, Tetracosane, Squalene (9).

Paerida foetida L. is a member of Rubiaceae family and it is a widely used medicinal plant in northeastern India. *Paerida foetida* Linn (10). Traditionally well known for its therapeutic uses (Table 1). Leaves were found to contain chemical constituents like sitosterol, alkaloids, carbohydrates, flavonoids, β-sitosterol, ascorbic acid, volatile oil, galacturonic acid, stigmasterol, iridoid glycoside and amino acid (11, 12).

Table 1. Ethno botanical, traditional, tribal uses and therapeutic uses of *Sphaeranthus amaranthoides and Paerida foetida*

Plant Name	Ethno botanical / Traditional / Tribal uses / Therapeutic uses
Sphaeranthus amaranthoides	To treat acne, eczema, dermatitis, skin wounds , anti-tumor, anti-oxidative, anti-cancer, anti-inflammatory, anti-fungal, anti-microbial properties, eliminates piles, Vata and Kapha (8, 10, 13).
Paerida foetida	To treat anti-diarrheal, gastro-protective properties, rheumatism and gout, used in Ayurvedic preparations ie. Dasamularista, Prasarani taila, Kubja prasarani taila, Sudard, Maha Narayan Oil and Rhue capsule (Ban lab, Mumbai) (10), constipation, hepatic disorders, diabetes, wounds, itches, coughs, stomachaches, cancer, flatulence, body aches, typhoid, pneumonia and bone fractures (14, 15).

Materials and Methods

Chemicals required

Carbon tetrachloride (CCl_4) and silymarin were acquired from Sigma-Aldrich, USA; standard kits for measuring levels of uric acid, creatinine, urea and bilirubin were purchased from Biosystems, Spain; aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total Protein (TP), total Bilirubin.

Plant Materials

The fresh PF plant and SA plants were collected from the local area of Odisha and Tamil Nadu, India respectively. PF plant was authenticated by Prof. G Mahalik, Department of Botany, Centurion University, Odisha and SA plant was authenticated by Dr. S Mutheeswaran from St. Xavier's college, Xavier research foundation, Palayamkottai, Tamil Nadu, India. The specimen was kept in the Department of Pharmacognosy, Centurion University of Technology and Management, Bhubaneswar, Odisha with the herbarium number CUTM/BOT/2024/02 and XCH-40431 respectively.

Plant Extract preparation

The leaves of PF and SA plants were separately dried completely in shade for 20 days and then were powdered through a mixer grinder. The 500 g of coarsely powdered plant materials were then extracted with the hydroethanolic solvent (water: ethanol; 30:70) separately. The extraction was performed using a Soxhlet apparatus for 6 hrs. by using 1050 mL of ethanol and 450 mL of distilled water. Approximately 81 % and 79 % of ethanol *for S. amaranthoides* and *P. foetida* was recovered respectively. The extract was evaporated by using rotary flash evaporator at 40 °C. The dried extracts were kept in the desiccator and finally stored in screw cap glass bottle for further experiment.

Acute toxicity

It was carried out to find the toxicity doses for the selected plants extract of PF and SA as per OECD guideline (No. 423) with the dose of 2000 mg/kg b.w. An oral dose was given to each animal and observed for 72 hrs and observed for any abnormal activities in animals such as normal food and water intake, poisoning signs, symptoms (diarrhoea, weight loss, tremor, paralysis) and mortality.

Hepatoprotective activity

Animals used in experiment

36 adult wistar rats of good health that weighed between 125 g to 135 g were utilized in the present investigation. To avoid bios male and female rats are selected in 50:50 ratios. The animals were collected from the licensed animal breeders that is SAHA Enterprises, 386/2, Nilachal, Birati, Kolkata- 700051, CPCSEA, Regd. No-1828/PO/Bt/S/15/CPCSEA. Animals are kept in the animal house of the pharmacology department at Centurion University of Technology and Management, Bhubaneswar, Odisha. The rats have resided under environmentally controlled conditions in polypropylene cages (23 ± 2 °C, 12 h dark/12 hrs light) with comfortable bedding, good ventilation and easy accessibility to water and food. All experimental protocols complied as per the institutional animal ethical committee norms (Ref no: CUTM/1AEC-14).

Grouping of animals

Separately, 6 + 6 animals were kept for the acute toxicity study as per OECD guideline (no. 423). Based on the acute toxicity study, the doses were selected for both the plants PF and SA extracts (9, 16). Further, groupings of 6 animals were done for the experiment as follows: Gr-I: Control group where animals were treated with corn oil (10 mL/kg b.w./day, o.p. three days for once); Gr-II (negative control): CCl_4-treated rats received distilled water (10 mL/kg); Gr-III (positive control): CCl_4-treated rats received silymarin (10 mg/kg); Gr-IV: PF and SA extracts (each 125 mg/kg) + CCl_4; Gr-V: PF and SA extracts (each 250 mg/kg) + CCl_4. Except for Gr-I remaining, all four groups of animals were infected by the carbon tetrachloride and corn oil (1:1 v/v) mixture of 2 mL/kg on the 6th day. The PF and SA extracts of two different doses were administered orally once daily for 21 days from the 7th day onwards (9).

Collection of blood and organ

Collection of blood was done by retro-orbital route on the 7th, 14th and 21st days by a capillary tube. Blood samples were centrifuged at 3000 rpm to separate the plasma for 10 min at a cold temperature of 40 °C and then stored at 20 °C for further assessments After 21 days. On the 22nd day, all experimental animals were anesthetized by inhaling light ethyl ether and then sacrificed for histological study. The liver was preserved at 20 °C for biochemical analysis after being weighed and preserved. Then, the liver tissues were thinned to about 2 mm afterwards the tissues were embedded in paraffin and preserved in a 10 % buffered formaldehyde solution. They were cut into 2 µm pieces and hematoxylin and eosin-stained liver specimens were finally observed under a light microscope.

Biochemical parameters determination

An autoanalyzer and a commercial kit were used to estimate the biochemical parameter, such as serum enzyme: AST and ALT, ALP, total bilirubin and TP, were evaluated (17). For each sample, the analyses were carried out in triplicate.

Determination of oxidative damage

MDA level: It is calculated using the thiobarbituric acid reaction substance (TBARS) method. Malondialdehyde (MDA) reacted with thiobarbituric acid to form a red TBARS complex under an acidic condition at a high temperature. The absorbance of TBARS was estimated at 532 nm (17).

GSH level: After a 1 hr incubation period at 4 °C, the liver supernatant liquid (1.0 mL) and 4 % sulfosalicylic acid (1.0 mL)

were kept for precipitation. After precipitation, the mixture of 1200 g was centrifuged for 20 min. One mL of the homogenate was mixed to the combined solution of 100 mM dithiobis nitro benzoic acid (DTNB) (2 mL) and 0.1 M potassium phosphate buffer with pH 7.4 (2.8mL). At 412 nm, the yellow product's absorbance was measured. GSH content was calculated as mMol GSH/g of the liver sample (18).

SOD Level: A volume of 880 µl of 0.05 M carbonate buffer (pH 10.2) containing 20 µl of 30 mmol epinephrine and 0.1 mmol EDTA in 0.05 % acetic acid was combined with 100 µl of tissue extract. Changes in activity were monitored at 480 nm for 4 min. The activity was measured in units/mg protein and was defined as the quantity of enzyme that suppresses the oxidation of adrenaline by 50 %, or one unit (18).

Histopathological studies

A small fraction of the liver was collected, appropriately cleaned with normal saline and handled individually for histological examination. The liver tissues were first preserved for at least 48 hrs in 10 % buffered neutral formalin, then dried out in 50 % to 100 % ethanol, cleaned in xylene and embedded in paraffin. A microtome was utilized to prepare the 4 µm slices. After that, the sections of liver were defeated in xylene, rehydrated in various alcohol grades and then given a five-minute rinse with distilled water. Hematoxylin, a primary stain, was applied to the liver sections for 40 sec, followed by eosin, an acidic stain, for 20 sec (H -E) dye (19). The slices were inspected under an Olympus microscope at 100X magnification to look for histopathological alterations.

Statistical analysis

The mean ± SEM presents all values. ANOVA, or one-way analysis of variance, was applied to determine the significant difference. Turkey's post hoc test was used to analyze inter-group significance. A difference was considered highly substantial if $p < 0.001$, marginally significant if $p < 0.01$ and essential if $p < 0.05$.

Results

Acute toxicity study

After 72 hrs, no mortality or toxicity was observed, no alteration of body weight, no skin itching, no morphological alterations, or toxicity. Therefore 250 mg/kg and 500 mg/kg doses, respectively were selected for further hepatoprotective study. Even no changes in body weight after 21 days.

Combined effect PF and SA on ALT, AST and ALP

Elevated levels of ALT, AST and ALP are sensitive indicators of liver injury. Table 2-4 were illustrated that the combined extract of plants showed a comparable and appreciable changes in their AST, ALT, or ALP levels as compared to the normal control and it confirmed the safety doses of PF and SA at 125 mg/kg +125 mg/kg and 250 mg/kg +250 mg/kg. In contrast to the normal control group, the rat's liver damage from the CCl_4 injection showed elevated AST, ALT and ALP serum levels, compared to the CCl_4-treated group-II. The plants extract was (125 mg/kg & 250 mg/kg) significantly decreased the risk of blood enzymes AST, ALT and ALP caused by CCl_4. The impact of silymarin therapy and extracts of PF and SA were similar. These outcomes proved that PF and SA combined extract have a protective effect on rats with liver injuries induced by CCl_4. After 21 days, Gr-V showed the reduction of AST was 94.23 IU/L where the standard showed the reduction of 90.12 IU/L (Table 2) (Fig. 1). Similar results were obtained for ALT and ALP where 500 mg/kg each dose of PF + SA extract revealed a significant decline after 21 days. ALT (IU/L) and ALP (IU/L) reduction was properly shown in Fig. 2-4 respectively.

Total protein content

Compared to the normal control group, the serum total protein level was considerably lower in rats that had been intoxicated with CCl_4 (Table 5). When rats were given hydroethanolic extracts of PF + SA and silymarin orally, their total protein levels gradually increased compared to rats given CCl_4 treatment. It has been found that plant extracts and silymarin have shown an approximately same total protein level.

Total bilirubin content

As seen in Table 6, the rats's bilirubin levels increased significantly after receiving CCl_4, suggesting a compromised liver excretory function. Contrarily, treatment with silymarin (10 mg/kg) and PF + SA doses of 125 mg/kg and 250 mg/kg caused a significant drop in bilirubin levels (1.93 mg/dL and 1.84 mg/dL respectively) compared to the rats treated with CCl_4 (3.89 mg/dl). So, the

Table 2. Biochemical parameter (Liver function tests) estimation by auto analyzer: Serum marker enzymes estimation: AST(IU/L)

	AST (IU/L)		
	7 days	**14 days**	**21 Days**
Gr-I	82.11± 1.01	83.02 ± 0.20	85.28 ± 1.31
Gr-II	224.13 ± 0.54^{a***}	227.11 ± 0.15^{a***}	235.32 ± 1.22^{a***}
Gr-III	97.23 ± 0.21^{b***}	94.87 ± 1.04^{b***}	90.20 ± 2.03^{b***}
Gr-IV	128.30± 0.11^{a***b***}	119.03 ± 2.04^{a***b***}	112.48 ± 1.72^{a***b***c*}
Gr-V	114.21 ± 2.10^{a*b***c*}	109.36 ± 1.31^{b***}	94.23 ± 1.06^{b***}

Mean ± SEM; (n=6); ^{a}Against Gr-I; ^{b} Against Gr-II; ^{c} Against Gr-III; *p < 0.05, **p < 0.01, ***p < 0.001

Table 3. Biochemical parameter (Liver function tests) estimation by auto analyzer: Serum marker enzymes estimation: ALT(IU/L)

	ALT(IU/L)		
	7 days	**14 days**	**21 Days**
Gr-I	94.87 ± 1.10	96.24 ± 3.42	97.08 ± 3.98
Gr-II	218.43 ± 1.23^{a***}	221.78 ± 2.31^{a***}	233.20 ± 1.87^{a***}
Gr-III	97.21 ± 1.36^{b***}	94.06± 2.33^{b***}	91.78 ± 2.21^{b***}
Gr-IV	146.31 ± 0.41^{a***b***c***}	138.52 ± 2.33^{a***b***c***}	135.50 ± 2.12^{a***b***c***}
Gr-V	122.41 ± 2.15^{a***b***c**}	117.96 ± 3.02^{a***b***c**}	104.37± 2.48^{b***}

Mean ± SEM; (n=6); ^{a}Against Gr-I; ^{b} Against Gr-II; ^{c} Against Gr-III; *p < 0.05, **p < 0.01, ***p < 0.001

Table 4. Biochemical parameter (Liver function tests) estimation by auto analyzer: Serum marker enzymes estimation: ALP(IU/L)

	ALP(IU/L)		
	7 days	**14 days**	**21 Days**
Gr-I	152.21 ± 1.23	154.33 ± 1.38	162.48 ± 0.56
Gr-II	289.43 ± 0.21^{a**}	299.43± 2.00^{a***}	326.11 ± 1.21^{a***}
Gr-III	154.38 ± 1.14^{b***}	152.20 ± 2.33^{b***}	147.27 ± 1.47^{b***}
Gr-IV	285.31 ± 2.11^{a***c***}	273.22 ± 2.00^{a***b**}	264.38 ± 4.23^{a***b***c***}
Gr-V	268.45 ± 3.20^{a***b**c***}	252.44 ± 2.16^{a***b***}	236.10 ± 4.11^{a**b***c***}

Mean ± SEM; (n=6); ^{a}Against Gr-I; ^{b} Against Gr-II; ^{c} Against Gr-III; *p < 0.05, **p < 0.01, ***p < 0.001

Table 5. Biochemical parameter (Liver function tests) estimation by auto analyzer: Serum marker enzymes estimation: Total protein (g/dL)

	Total Protein (g/dL)		
	7 days	14 days	21 Days
Gr-I	5.08 ± 2.06	5.12 ± 0.32	5.27 ± 2.11
Gr-II	3.78 ± 1.12^{a*}	4.24 ± 0.05	4.31 ± 4.32^{a*}
Gr-III	4.43 ± 1.40	5.06 ± 2.26	5.23 ± 3.27
Gr-IV	4.67 ± 1.22	4.82 ± 0.31	5.18 ± 2.48
Gr-V	4.72 ± 0.21	4.86 ± 0.26	5.21 ±0. 36

Mean ± SEM; (n=6); ^{a}Against Gr-I; ^{b} Against Gr-II; ^{c} Against Gr-III; *p < 0.05, **p < 0.01, ***p < 0.001

Table 6. Biochemical parameter (Liver function tests) estimation by auto analyzer: Serum marker enzymes estimation: Total bilirubin (mg/dL)

	Total Bilirubin (mg/dL)		
	7 days	14 days	21 Days
Gr-I	1.28 ± 0.40	1.31 ± 0.21	1.32 ± 0.22
Gr-II	2.76 ± 0.24	3.45 ± 0.25^{a***}	3.89 ± 0.33^{a***}
Gr-III	2.47 ± 1.11	2.01 ± 0.23	1.78 ± 0.15
Gr-IV	2.09 ± 0.22^{c*}	1.98 ± 0.64 ^{b***}	1.93 ± 0.34 ^{b***}
Gr-V	2.03 ± 1.00^{c*}	1.96 ± 1.08 ^{b***c*}	1.84 ± 0.39^{b***}

Mean ± SEM; (n=6); ^{a}Against Gr-I; ^{b} Against Gr-II; ^{c} Against Gr-III; *p < 0.05, **p < 0.01, ***p < 0.001

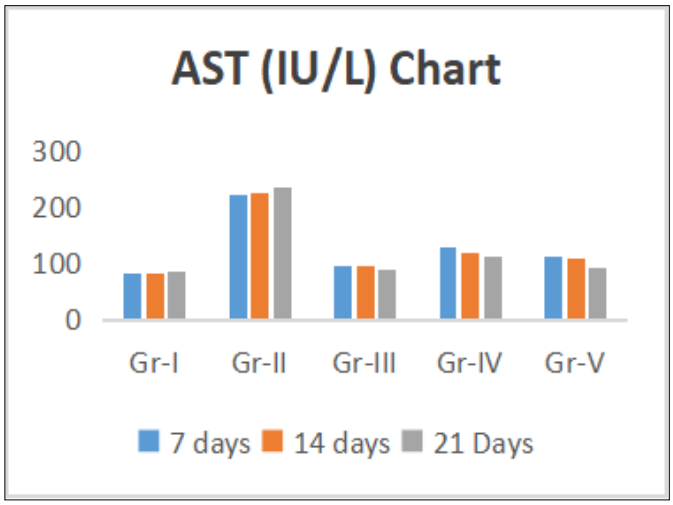

Fig. 1. Serum marker enzymes estimation: AST(IU/L).

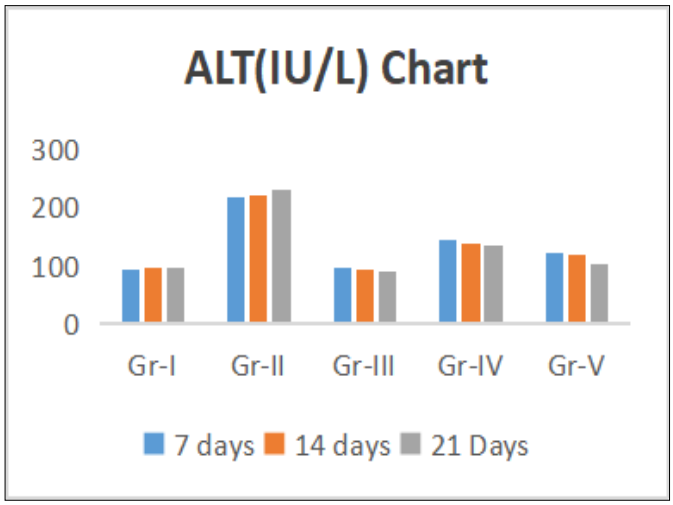

Fig. 2. Serum marker enzymes estimation: ALT(IU/L).

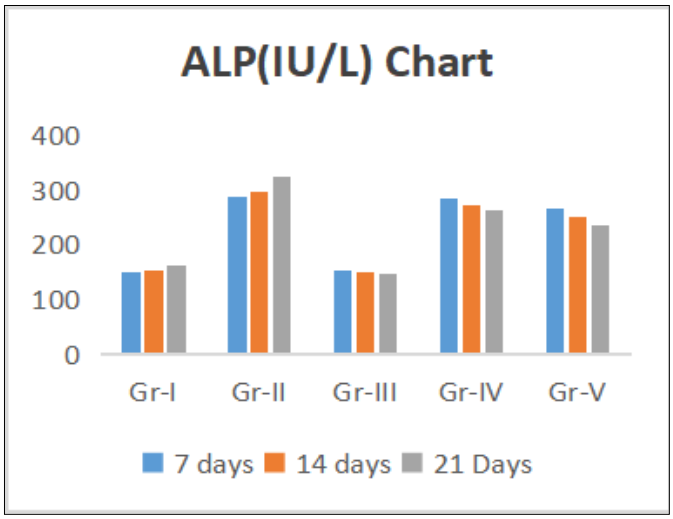

Fig. 3. Serum marker enzymes estimation: ALP(IU/L).

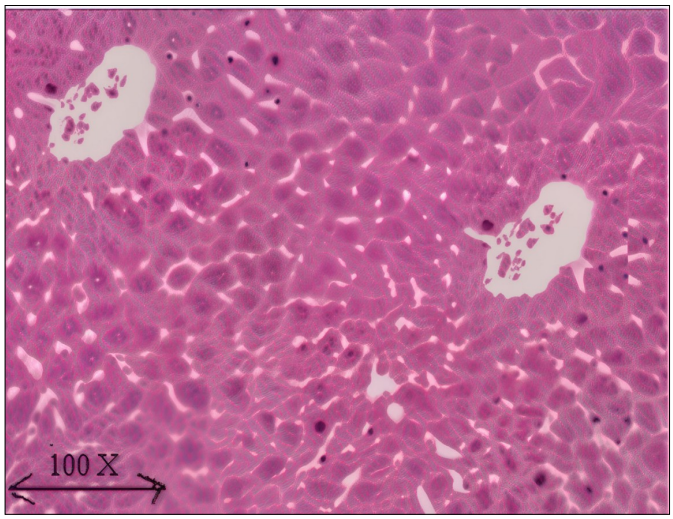

Fig. 4. Gr-I: Control liver.

bilirubin levels of plants PF & SA have shown approximately same effect as compared to silymarin (1.78 mg/dl).

Determination of oxidative damage

MDA level

Lipid peroxidation yields malondialdehyde (MDA) as a by-product. MDA is produced during the production of arachidonic acid or free oxygen radicals. MDA is a well-known oxidative stress biomarker. It was observed that, MDA level was decreased (1.29 µmol/g) with each 250 mg/kg doses of PF + SA where standard drug showed 1.19 µmol/g. The result was better than CCl_4 induced rats (2.37 µmol/g of tissue) (Table-7).

GSH level

GSH is the main antioxidant which is protective type enzyme that increases their activity under oxidative stress conditions. It was observed that GSH level was increased. PF + SA extract at dose level of 125 mg/kg showed good result of GSH (2.97 µmol/g tissue) than CCl_4 induced animal (2.10 µmol/g tissue) (Table 7).

SOD level

One extremely potent antioxidant enzyme is SOD. SOD makes sure that the body gets rid of a lot of dangerous and poisonous substances. Additionally, it cleanses the cell of free radical by products. The result revealed that 500 mg/kg dose of PF + SA showed increased SOD level (86.67 U/mg proteins) than CCl_4 induced rats (26.73 U/mg proteins) (Table 7).

Combined effect of PF and SA on liver histopathological alteration

The rat's livers were examined histologically. CCl_4- induced rats

Table 7. Determination of oxidative damage by MDA level, GSH level & SOD level

	After 21 days		
	MDA level (µmol/g tissue)	**GSH level (µmol/g tissue)**	**SOD (U/mg protein)**
Gr-I	1.07 ±0.05	3.42 ± 0.22	120.22 ± 0.03
Gr-II	2.37 ± 0.10	2.10 ± 0.01	27.82 ± 0.42
Gr-III	1.19 ± 0.31	3.38 ± 0.21***	102.10 ± 0.26***
Gr-IV	2.11± 0.16	2.97 ± 0.03	61.04 ± 0.36***
Gr-V	1.29 ± 0.15***	3.03 ± 0.12***	86.67 ± 0.21***

Mean ± SEM (n =6); (***)= when compared with Gr-II;

showd significant hepatocellular damages, bleeding and inflammation, whereas the control group's healthy rats's hepatocytes showed a normal architecture. During the treatment with PF + SA extract, the degree of liver poisoning caused by CCl_4 was diminished in a dose-dependent manner, while silymarin administration showed recovery of hepatic cells (Fig. 4-8). It has been found that 250 mg/kg doses of both plant extracts have shown a good effect on healing the liver tissue (Fig. 8).

Discussion

Hepatocellular damage is directly correlated to the serum level of different enzymes like AST, ALT. Assessing serum aminotransferase (AST and ALT) activity levels, which are indicators of hepatocellular injury, was analysed in the present study, which showed similar results as per earlier research (19). In this work, we demonstrate that hydroethanolic extracts of PF + SA can prevent CCl_4-induced acute liver damage. Liver damage resulting from CCl_4 is comparable to that caused by viral hepatitis. Due to the simple fact that Liver injuries cause the release of cytoplasmic AST, ALT and ALP into the circulation, the weakened structural integrity of the liver has been connected to their elevated serum enzyme levels (21). According to our research, rats receiving CCl_4therapy alone had higher AST, ALT and ALP activities than rats in the normal control group. Combined extracts PF + SA improved the liver's excretory function, as seen by its ability to prevent the rise in serum bilirubin levels. Protein synthesis was facilitated by the stability of the endoplasmic reticulum, which decreased the quantity of total protein (22). The study demonstrated that other research findings regarding the elevated CCl_4-treated group's liver MDA concentration were concordant. The level of MDA in the liver of the CCl_4-treated group was significantly decreased by the plant extract. This can be explained by inhibiting the liver's lipid peroxidation from spreading. One of the main antioxidant methods to prevent CCl_4's hepatotoxicity and lessen liver damage is the scavenging of free radicals (23). In contrast, the groups that received two different doses of PF +SA showed a notable increase in GSH levels and a reduction in MDA content

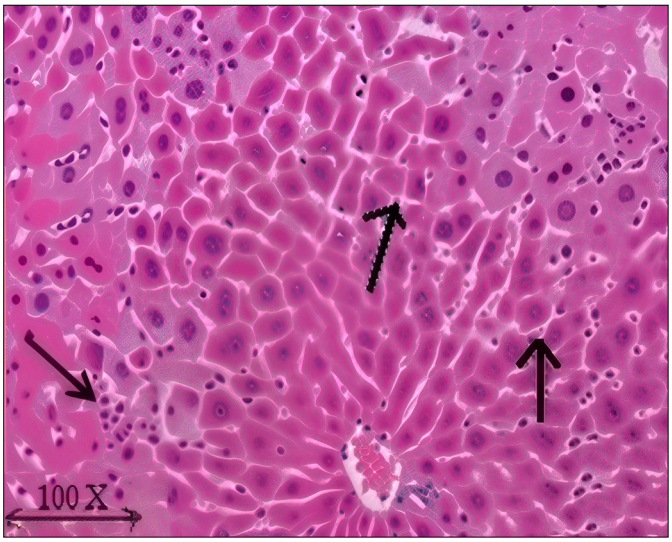

Fig. 5. Gr-II: CCl4 induced liver.

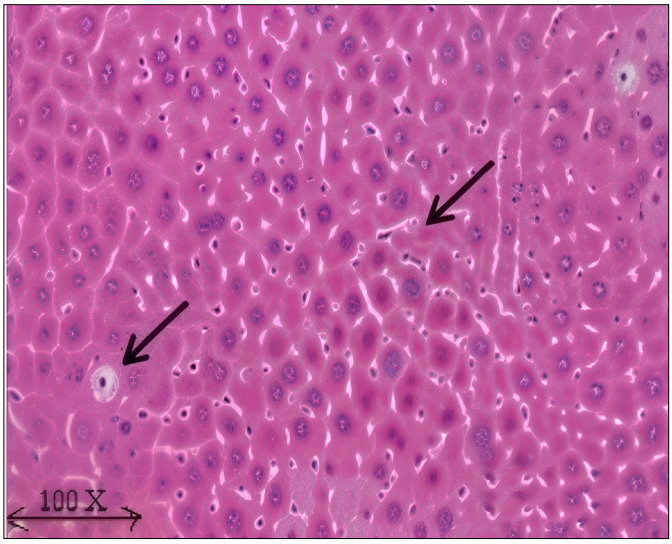

Fig. 6. Gr-III: Silymarin + CCl4.

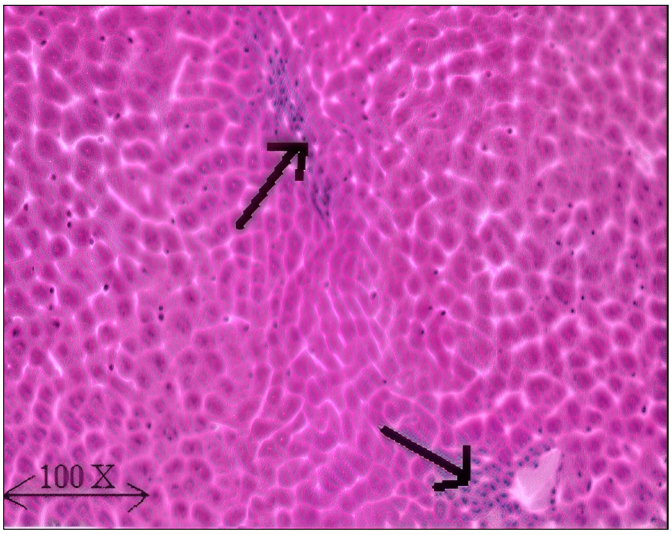

Fig. 7. Gr-IV: *Paerida* (125 mg/kg) + *Sphaeranthus* (125 mg/kg) + CCl4.

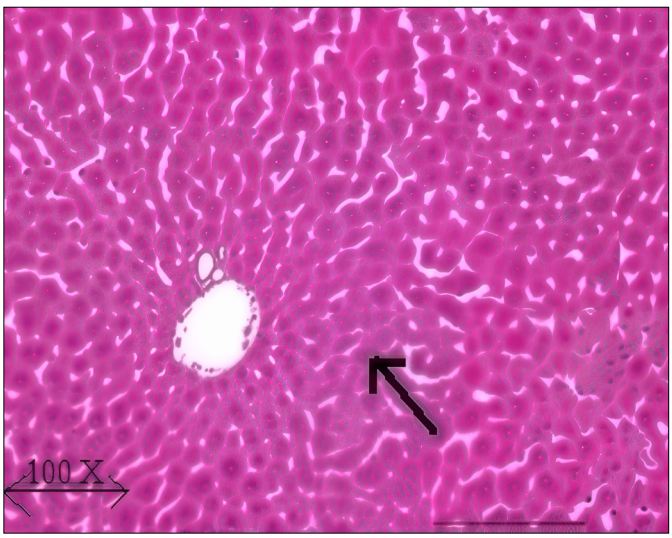

Fig. 8. Gr-V: Paerida (250 mg/kg) + Sphaeranthus (250 mg/kg) + CCl4.

(also known as the lipid peroxidation level) in the liver and an increase in SOD and GSH activities. The hepatic generation of ROS may have decreased due to the synthesis of counter-enzymes like SOD and GSH in the groups that received supplementation with PF + SA leaves extract. Thus, one of the primary reasons for the disease-preventing effect of PF + SA may be the initiation of cellular antioxidant defence.

Furthermore, the liver's histological examination supported the biochemical study by demonstrating the protective effects of the components under investigation against acute CCl_4 induced liver injury. According to the histology, CCl_4 treatment resulted in severe oxidative liver damage, which was consistent with other research on liver injury. Severe necrosis, inflammation, cytoplasmic vacuolation, hepatocellular degeneration and loss of cellular boundaries were the characteristics of this injury. In a dose-dependent way, PF treatment was notable because it lessened the severity of oxidative damage. Thus, it denotes the safeguard by the administration of combined extracts PF + SA. The high dose of PF (500 mg/kg) +SA (500 mg/kg) appeared to have similar effects to those of normal emergence, suggesting that the lower dose (250 mg/kg) was also effective but somewhat less compared to the high dose. At 500 mg/kg, PF + SA had a preventative effect comparable to silymarin therapy. This hepatoprotective study may be due to the presence of phytochemicals from both plants. In a previous study, it was found that the combined plants' effect was showing good protection against CCl_4-induced hepatotoxic rats, may be combined plant extract is having a good protection against hepatotoxicity (24). As a result, the current study offers scientific support for the pharmacological application of PF+SA as a successful substitute for treating liver diseases.

Conclusion

In general, the study's outcomes revealed that the PF + SA plant extract has hepatoprotective action in rats that are affected by exposure to CCl_4. The hydroethanolic extract of both the plant PF & SA increased serum enzyme levels and decreased liver antioxidant markers in a dose-dependent manner. The lower lipid peroxidation and improved hepatocyte defence may have contributed to the protective activity due to the availability of several bioactive chemicals and may be due to combined effect.

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Authors' contributions

AJ, PD and NS participated in the design of the study and performed the statistical analysis; AJ helped in funding acquisition. AJ and PD conceived of the study and participated in its design and coordination. GM participated in the sequence alignment. AJ contributed to writing original draft and AJ and PD contributed to writing reviews and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors have not any conflict of interest to declare.

Ethical issues: Protocol for animal handling was approved by the Animal Ethics Committee, Pharmacology department, Centurion University of Technology and Management, Bhubaneswar, Odisha (Ref no: CUTM/1AEC-14) in 2024.

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