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Combined hepatoprotective potentials of medicinal plants on CCl_4-induced hepatotoxic Wistar rats

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Asparagus racemosus Willd., *Cajanus cajan* (L.) Millsp., *Cassia fistula* L., and *Carissa spinarum* L. (ACCC) are used in ethnomedicine for treating a variety of diseases, including liver disease. The present study investigated the combined antioxidative and hepatoprotective roles of an aqueous methanol extract of ACCC (AMACCC) on CCl_4-induced hepatic damage in the rat model. Thirty male Wistar Albino rats were divided uniformly into five groups. Group I (control) received only the vehicle DMSO (0.5 mL/kg b.w.) and was fed a normal diet for 28 days. Carbon tetrachloride (0.5 mL/kg b.w., 20% CCl_4/olive oil) was used to intoxicate groups II-V. Rats in Group II remained untreated, while groups III, IV, and V were given 50 mg/kg b.w. of sylimarin, 100 mg/kg b.w., and 200 mg/kg b.w. of AMACCC, respectively, for 28 days. Investigations were conducted to evaluate the combined action of AMACCC on liver marker enzymes along with histopathological changes of hepatic tissue before and after treatment. *In vitro* antioxidative activity of AMACCC was also observed. There were significant increases ($p<0.001$) in serum hepatic enzyme markers (ALT, AST, and ALP) activities in rats intoxicated with CCl_4 when compared to the normal group, but administration of AMACCC extract at doses of 100 mg/kg and 200 mg/kg body weight and standard sylimarin drug attenuated ($p<0.01$) the toxic effects of CCl_4. The treated liver was shown to be in repair histopathologically. The combination of the four plant extracts has a strong hepatoprotective effect, indicating that more research is needed.

Keywords: Antioxidant, CCl_4, Hepatoprotection, Liver damage

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The liver is a crucial organ in the human body that is responsible for metabolizing and regulating several physiological activities while also being exposed to xenobiotics through toxin absorption[1]. The multitude of liver physiological processes contributes to the high prevalence of hepatic disorders[2]. Despite tremendous scientific progress in the field of hepatology in recent years, liver disease is on the rise[3]. Unfortunately, there are only a few medications available for the treatment of liver problems that have substantial adverse effects[4]. Because of the side effects of synthetic drugs, more attention is being paid to the therapeutic evaluation of medicinal plants.

Plant-derived medicines have long been investigated for their antioxidant capabilities since they can scavenge both oxygen and nitrogen-derived free radical species (ROS) that cause liver damage[5]. The food system produces highly reactive oxygen free radicals that can oxidize biomolecules, leading to tissue destruction and apoptosis in most cases[6]. In this regard, antioxidants are referred to as free radical scavengers, as they help to prevent and repair damage caused by free radicals[7,8].

Secondary metabolites, which are complex organic molecules synthesized by medicinal plants (alkaloids, flavonoids, phenols, tannins, etc.), have attracted the interest of researchers and consumers as possible source antioxidants[9,10]. Many diseases now have diverse origins, and they can be treated more effectively with a medication combination strategy than with a single administration. Bioactive compounds

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Abbreviations:
CCl_4: Carbon Tetrachloride, IC_{50}: Inhibitory Concentration 50, LC_{50}: Lethal Concentration 50, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline Phosphatase.

generated by plants regulate and buffer one another, working synergistically to boost the systemic effect. In Western nations, effective multidrug therapy is routinely used to treat multifactorial or complicated diseases[11]. Phytotherapy and ethnopharmacology play a major role in this regard, as they rely on herbs or plants for their efficacy of treatment.

A variety of medicinal plants have been known to offer hepatoprotective effects through increasing antioxidant levels. However, the hepatoprotective properties of such plants have not been rigorously studied and hence cannot be confirmed by scientific data. *A. racemosus*, *C. cajan*, *C. fistula*, and *C. spinarum* are some of the medicinal plants available in Bangladesh. These plants have been known to offer a wide range of therapeutic properties in the past. Improved liver damage[12], regeneration[13], and fertility enhancement[14] have all been associated with A. *racemosus. C. cajan* leaf juice is used to treat toothache, jaundice, dysentery, bronchitis, heart disease, and anthehelmintic infection[15]. *C. spinarum* is used for hepatoprotective activity[16], asthma[17], cardiac dysfunction[18], antimicrobial activity[19], wounds and injuries[20]. Another common plant is *C. fistula*, which is used to cure skin problems, inflammatory ailments, rheumatism, anorexia, and jaundice[21]. Flower and pulp showed low level of antioxidant property because of strong presence of flavonoids and reducing sugar[22].

Hepatoprotective effects of these four plants, *viz., A. racemosus*[12], *C. cajan*[23,24], *C. spinarum*[16], and *C. fistula*[25], have previously been reported. But the combined liver protective effect of these mentioned plants has not been analyzed yet. Regarding this situations our research focuses on the combined potency of *A. racemosus*, *C. cajan*, *C. fistula*, and *C. spinarum* against liver damage, how they synergistically contribute to protecting the CCl_4-induced hepatic damage in the experimental rat.

Methodology

Plant materials

The plant materials, *A. racemosus* (local name: shatamuli, component: root), *C. cajan* (local name: arhar, component: leaf), and *C. fistula* (local name: shonalu, component: bark) were collected from the local market in Dhaka, and *C. spinarum* (local name: koromcha, component: leaf) was from the cultivation area of the Bangladesh Council of Scientific and Industrial Research, Dhaka. Collected plants were confirmed by the researchers of Biomedical and Toxicological Research Institute, Bangladesh Council of Scientific and Industrial Research, Dhanmondi, Dhaka, Bangladesh.

Chemicals

All chemicals and reagents were obtained from Sigma Aldrich Company, USA, and were of analytical quality.

Plant extract preparation

The collected plant materials were washed and air dried for 4 weeks at room temperature (25±2°C) to achieve a consistent weight, after which they were fine powdered using a locally constructed Willy mill. The powdered plant materials (500 g) were defatted with n-hexane before being extracted three times with 3L of 80% aqueous methanol (maceration) at room temperature (25±2°C). A rotary evaporator (RE 200, Bibby Sterling Ltd., England) was used to condense combined filtered (whatman filter paper 1) extracts at 40°C under decreased pressure. Using a water bath (below 40°C) and a petri plate, concentrated extract was collected and allowed to dry for complete evaporation of the solvent, yielding 161.57 g of crude extract. Thus, the AMACCC extract (aqueous methanolic extract of *A. racemosus*, *C. cajan*, *C. fistula*, and *C. spinarum*) was made and stored at 4°C until used.

Methods and Materials

DPPH free radical scavenging assay

The DPPH test was performed using Shimada *et al.'s* (1992) standard technique, with some changes[26]. Here, ascorbic acid was working as a control antioxidant to compare to the AMACCC extract. To make a stock solution of 1 mg/mL, a certain quantity of ascorbic acid was dissolved in methanol. The stock solution was diluted to concentrations of 30, 60, 120, 240, and 480 g/mL. Similarly, an AMACCC stock solution was made by dissolving 10 mg of dried AMACCC extract in 10 mL of a specified solvent. It was diluted to quantities of 30, 60, 120, 240, and 480 g/mL. In methanol, specific quantities of the AMACCC extract/standard were combined with the needed quantity of 0.04 Mm DPPH solutions. After 60 min of reaction at room temperature in the dark, the absorbance values of the AMACCC extract/standard were measured using a UV visible spectrophotometer (Thermo Fisher Scientific-USA) at 517 nm, and the

percentage inhibitory activity was calculated from the following equation:

$$\% I = \{(A_0 - A_1)/A_0\} \times 100$$

Where, A_0 indicates the absorbance of the control (freshly prepared DPPH solution) and A_1 denotes the absorbance of the standard/extract, after that, inhibition was created, and the IC_{50} value was determined using linear regression analysis.

$FeCl_3$ reducing power assay

The reducing power of AMACCC extract was evaluated using the method reported by Tundis *et al.'s* (2013)[27]. Both the standard (ascorbic acid) and the sample were prepared in phosphate buffered saline (pH 6.6) at the concentrations of 30, 60, 120, 240, and 480 μg/mL. Then 2.5 mL of 1% potassium ferric cyanide were added to each concentration of sample and standard. For 20 min at 50°C, the reaction mixtures were digested. After incubation, 2.5 mL of 10% trichloroacetic acid was added, thoroughly mixed, and centrifuged at 3000 rpm for 10 min. 2.5 mL of distilled water and in 2.5 mL of supernatant, 1 mL of 0.1% ferric chloride were added. Finally, absorbance was measured at 700 nm by a UV visible spectrophotometer (Thermo Fisher Scientific-USA) after 10 min of incubation of the reaction mixtures. The following formula was used to compute the reduction powers of the two parallel experiments:

$$\% \text{ increase of reducing power} = [A_{(test)}/A_{(control)} - 1] \times 100$$

Where, $A_{(test)}$ is the absorbance of test solution and $A_{(control)}$ is the absorbance of control.

An increase in reducing power is shown by a rise in the absorbance of the reaction mixture.

Animals used in research

30 male Wistar albino rats (6 weeks, 150-200 g) were selected for hepatoprotective activities from Bangladesh Council of Scientific and Industrial Research, Dhaka. They were healthy and were acclimatized to laboratory conditions for 7 days before conducting experiment. Throughout the experimental period, the animals were housed in cages under room temperature (23±2)°C with relative humidity of 60-70% and were exposed to 12:12 h light: dark cycle with food and water provided *ad libitum.* All rats were from the Biomedical and Toxicological Research Institute (BTRI) of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. All procedures performed in studies involving animals were in accordance with the ethical standards of the 'Ethical Committee of BCSIR for Animal Research (ECAR)'. Approval no: 39.309.006.00.00.163.2014/ 403 (2019-3).

Acute toxicity study

The test was conducted according to OECD guideline 425[28]. Briefly, 40 animals (8 weeks) were divided into eight equal groups of five rats each received a single oral dose of 375, 550, 820, 1200, 1750, 2600, 3750, and 5000 mg/kg body weight of AMACCC extract. Each animal was kept in close observation during the first 30 min after dosing, and occasionally the first 24 h and afterward for 14 days to identify any delayed toxicity.

Experimental procedure

Animals were divided into five groups of six animals each. Group I was maintained as the normal control received only the vehicle DMSO (0.5 mL/kg b.w.) and was fed with a normal diet for four weeks. Groups II–V were each injected with carbon tetrachloride (0.5 mL/kg b.w., 20% CCl_4/olive oil) intraperitoneally (ip) twice a week for four weeks. Rats in Group II remained untreated and served as the hepatic control. Group III worked as reference control and received the standard drug silymarin at a dose of 50 mg/kg b.w. orally for four weeks. For the treatment group, 1/50 (100 mg/kg b.w.) and 1/25 (200 mg/kg b.w.) of the maximum dose selected in the acute toxicity test were considered as two treatment doses for the experimental model.

Groups IV and V served as the treatment groups and received 100 mg/kg b.w. and 200 mg/kg b.w. oral dosages of AMACCC extract, respectively. At the end of 4 weeks, 24 h after the last treatment, the weights of the animals were finally measured. After that, the animals were anesthetized and sacrificed. Blood was collected through the inferior vena cava, then serum was separated by centrifugation and stored at -80°C for further enzymatic analysis.

Biochemical investigation

All biochemical measurements (AST, ALT, and ALP) were determined using the kit manufacturers' instructions using a biochemistry analyzer (LCMS-8050, Randox Laboratories Ltd., UK).

Results

Acute toxicity

In the acute toxicity study, oral administration of all doses of AMACCC extract did not produce any

morbidity or mortality in animals. The rats did not show tremors, convulsions, salivation, diarrhea, lethargy, sleep, or coma up to the dose of 5 g/kg body weight. Furthermore, treatment with AMACCC extracts of 100 mg/kg and 200 mg/kg body weight was well tolerated by the animals and did not produce any behavioral changes during long-term treatment (Table 1).

DPPH free radical scavenging activity

The radical scavenging activity of AMACCC extract was determined by measuring the absorbance of increasing concentrations of extract and ascorbic acid at 517 nm. DPPH was successfully scavenged by AMACCC extract, and scavenging capacity was found to be concentration-dependent. The extract's inhibitory concentration (IC_{50}) was obtained by plotting scavenging activity against log concentration (Fig. 1). The IC_{50} value was found to be 85.11 µg/mL for the AMACCC extract, and ascorbic acid (standard antioxidant) showed 50% inhibition at a concentration of 13.80 µg/mL.

Table 1 — Determination of median lethal dose (MLD) (LD_{50}) for orally administrated AMACCC extract

AMACCC extract (mg/Kg)	No. of animals used	No. of survivors	No. of deaths	MLD (LD_{50})
375	5	5	0	
550	5	5	0	
820	5	5	0	>5000
1200	5	5	0	mg/kg
1750	5	5	0	
2600	5	5	0	
3750	5	5	0	
5000	5	5	0	

$FeCl_3$ reducing power assay

As seen in Figure 2, the AMACCC extract's reducing power seems to have a dose-response curve. The reducing power of the AMACCC extract was observed to rise when the concentrations were raised. The reducing power of the AMACCC extract was equivalent to that of ascorbic acid. The AMACCC extract showed antioxidant activity with a correlation coefficient (r) of 0.999, and an IC_{50} value of 24.69 µg/mL. The standard ascorbic acid showed antioxidant activity with a correlation coefficient (r) of 0.994, and an IC_{50} value of 4.43 µg/mL.

Effect of AMACCC extract on liver ALT, AST, ALP

Administration of CCl_4 (0.5 mL/kg b.w.; 20% CCl_4/olive oil) induced a marked increase in the serum hepatic enzyme levels (AST, ALT, and ALP) as compared with normal control (*p<0.001) (Table 2), indicating, that liver damage was successfully induced. Treatment of the rats with AMACCC extract (100 mg/kg b.w. and 200 mg/kg

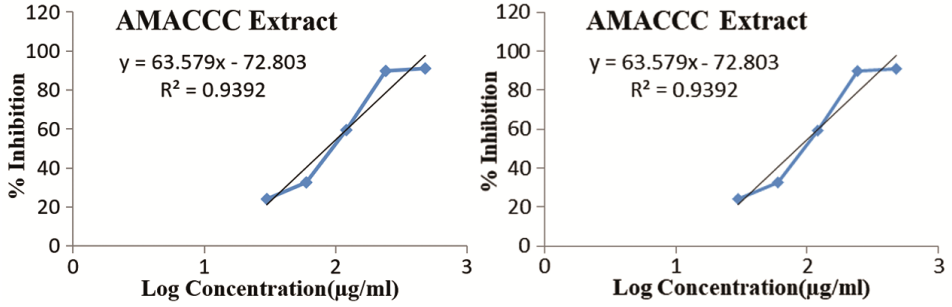

Fig. 1 — Regression analysis to calculate the IC_{50} values of AMACCC extract

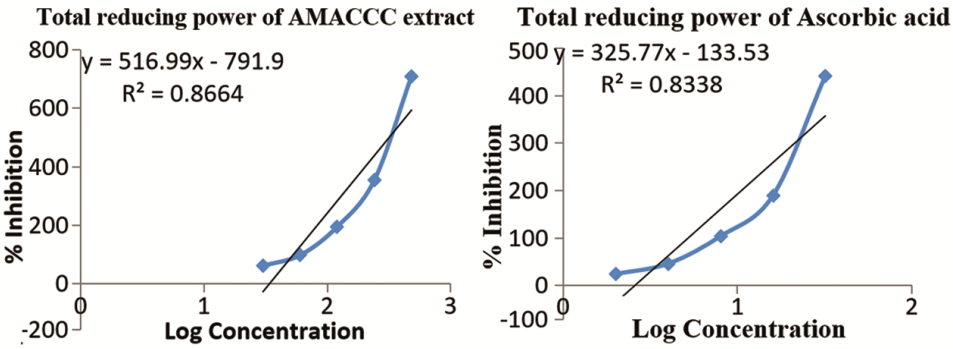

Fig. 2 — Regression analysis to calculate the IC_{50} values of AMACCC extract

b.w.) showed a significant reduction in the level of serum AST, ALT, and ALP (***p≤0.001), which were almost comparable with the silymarin-treated group (Group III). The serum hepatic indicators were recovered in a dose-dependent manner by both treatment groups in this study (Table 2).

Histopathological explanation

Histopathological assessment at 200X magnification of liver sections from all the groups was performed to visualize and analyze the effect of AMACCC (100 mg/kg b.w. and 200 mg/kg b.w.) and silymarin (50 mg/kg b.w.) on the liver. Histopathological observations of liver sections from the normal control group showed normal cellular architecture with distinct hepatic cells radiating from the central vein. The nucleus and sinusoidal spaces were in a normal arrangement (Fig. 3A). In contrast, the CCl_4 administered group (hepatic control) exhibited severe liver injuries (Fig. 3B). The hepatic control section shows disruption of the hepatic cord, loss of lobular structure, necrosis, depletion of hepatocytes, increased sinusoidal space and fat infiltration. The silymarin-treated rats exhibited areas of normal liver architecture and mild necrosis of hepatic cells (Fig. 3C). The liver sections of the rats treated with AMACCC (Fig. 3D-E) showed a relatively normal lobular pattern with a slight degree of necrosis compared to group 2 with ameliorating functional hepatic cells.

Discussion

The development of many degenerative illnesses has been linked to oxidative stress. Antioxidants are

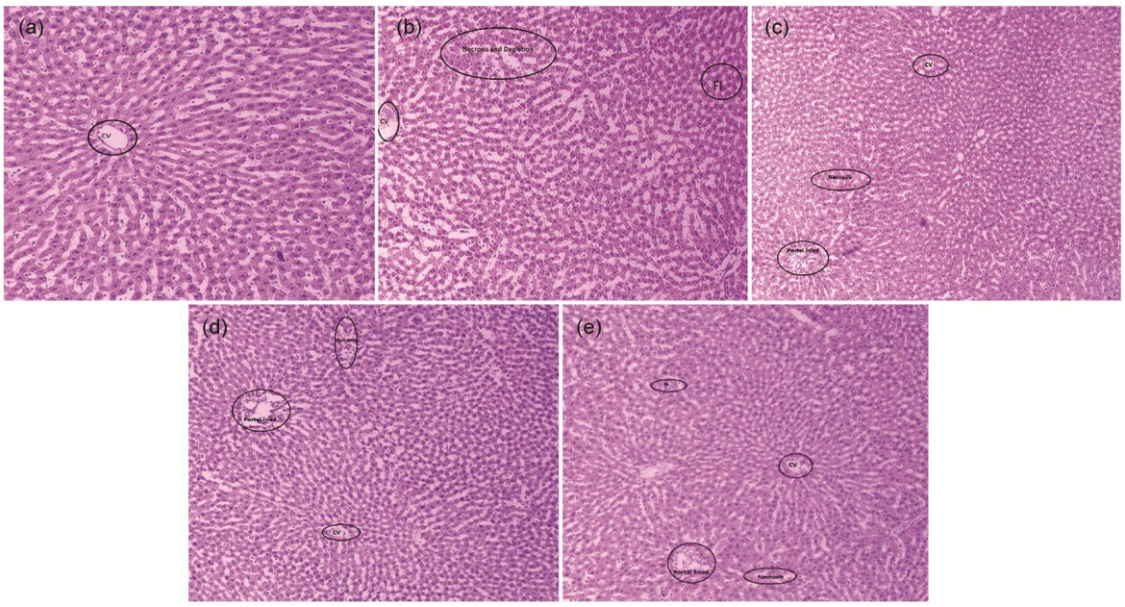

Fig. 3 — Effect of AMACCC and silymarin on liver histopathology of CCl_4 treated male Wistar rats. Magnification: 200 xs, Stain: haematoxylin–eosin. (A) vehicle control group (B) Hepatic control, only CCl_4 treated group section (C) Silymarin (50 mg/kg), CCl_4 treated group section (almost near normal); (D) AMACCC (100 mg/kg bw), CCl_4 treated group section and (E) AMACCC (200 mg/kg bw), CCl_4 treated group section

Table 2 — Effects of AMACCC on liver function test in rat

Groups	ALT (U/I)	AST(U/I)	ALP(U/I)
I. Normal	52.50±5.95	109.75±4.96	247.00±4.98
II. CCL_4	109.00±5.28*	182.50±5.12*	419.00±14.02*
III.CCL_4+SIlymerin	68.25±5.28***	124.75±3.84***	286.75±10.97***
IV.CCL_4+100mg/kg ex	83.75±3.30**	146.50±3.88***	319.50±11.58***
V.CCL_4+200mg/kg ex	77.25±3.86***	134.75±2.39***	300.25±11.66***

Note: AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase.
n=6; values were expressed Mean±SEM; Group II was compared to Group I. Groups III to V were compared to group II. A one-way ANOVA was used to evaluate the data, followed by a Dunnett's t-test. *p<0.001 as compared with the normal control group I, **p<0.01 as compared with the CCl_4 only treated group II; very significant, ***p≤0.001 as compared with the CCl_4 only treated group; highly significant.

vital chemicals that can protect the body from the effects of oxidative stress generated by free radicals[29]. Radicals and other reactive oxygen species (ROS) are continually produced in the human body and ejected by the antioxidant defense mechanisms, both enzymatic and non-enzymatic[30]. As a result, we investigated the combined therapeutic benefits of these four medicinal herbs in terms of their free radical scavenging capacity both *in vitro* and *in vivo*.

In the case of plant extracts, it is considered that a LD_{50} of more than 5 mg/kg is non-toxic[31]. Again, the Organization for Economic Cooperation and Development (OECD) guidelines classified a dose of $LD_{50} \geq 2000$ mg/kg as unclassified. Therefore, the AMACCC extract can be expected to be orally safe as its LD_{50} value is > 5 g/kg. Similar results were also reported in the case of the individual plant extracts; the LD_{50} of aqueous extract of *A. racemosus* root, the methanol extract of *C. spinarum* leaves, and the ethanol extract of *C. cajan* leaves were more than 1 g/kg[12], 2 g/kg[25], and 5 g/kg[32], respectively.

The DPPH free radical scavenging method is a common spectrophotometric procedure for defining the antioxidant activity of medicinal plants[33,34]. The DPPH test is widely used for screening plant products and may be used to analyze the substrate activity or plant extract activity in quenching free radicals[35].

According to the researchers, the lower the IC_{50} value, the greater the antioxidant activity of the samples[36]. In the present study, the IC_{50} value of AMACC extract was found to be 85.11 µg/mL in comparison to the IC_{50} value of the standard ascorbic acid which was 13.80 µg/mL (Fig. 1). According to Phongpaichit *et al.*[37], samples with an IC_{50}value more than 50 µg/mL were categorized as having moderate antioxidant activity, whilst those with an IC_{50}value less than 50 µg/mL were deemed to have a strong antioxidant capacity. In this case, the present experiment gives an estimation of the antioxidant activity of AMACCC extract, which can be concluded as AMACCC extract possessed intermediate antioxidant activity by the DPPH method.

The principle behind reducing power activity is that substances with reduction potential react with potassium ferric cyanide (Fe^{+3}) to form potassium ferrous cyanide (Fe^{+2}), which then reacts with ferric chloride to form ferrous complex with a 700 nm absorption maximum[38]. The reducing power of the AMACCC extract increased with increasing concentrations in our investigation. This is due to the presence of reductants in the samples, such as antioxidants, which cause the Fe^{3+}/ferricyanide complex to be reduced to the ferrous form[39]. The AMACCC extract showed antioxidant activity with correlation coefficient values (r) of 0.999, which was very close to standard ascorbic acid's coefficient value (0.994), indicating that the AMACCC extract has good reducing power.

Hepatotoxicity in rats induced by carbon tetrachloride is a well-characterized experimental model for assessing the heptoprotective activity of many natural compounds/herbal extracts[40,41]. It is well recognized that the cytochrome P450 system biotransforms CCl_4 into the reactive metabolite trichloromethyl radical (CCl_3^-)[42]. In the presence of oxygen, trichloromethyl radicals present in this system react very quickly to form more toxic trichloromethylperoxyl radicals (CCl_3O_2)[43]. This highly reactive ROS initiates lipid peroxidation by abstracting hydrogen from polyunsaturated fatty acids of the cell membrane and forming various kinds of oxidative products that lead to cell necrosis and cell death[44]. The current findings showed a significant increase in serum ALT, AST, and ALP levels in group II rats intoxicated with CCl_4 indicating a significant liver cell injury. But subsequent failure of these hepatic markers, both in the treatment groups, and the silymarin groups pointed towards stabilization of liver cell functions by re-establishing serum ALT, AST, and ALP.

These results of tissue repair are further substantiated by our histopathological analysis (Fig. 3). The study revealed extensive fatty changes, necrosis, fibrosis, and nuclear degeneration upon CCl_4 exposure that cause oxidative stress in the liver (Fig. 3B). These symptoms were diminished in the treatment groups (Group III–V), Fig. 3C, Fig. 3E, where AMACCC administration showed restoration of hepatocytes with a mild degree of tissue injury. The histological indications seen in this investigation are compatible with those reported by other authors[45,46].

Conclusions

According to our findings the medicinal plants namely *A. racemosus, C. cajan, C. fistula,* and *C. spinarum* all have combined therapeutic benefits. The phyto-constituents found in these plants have a remarkable potential to ameliorate CCl_4-induced liver lesions while also avoiding oxidative damage.

This finding might pave the way for more investigation into these plants as potential novel liver disease treatments.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

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Conflict of Interest

All authors have read and approved the manuscript. The authors declare that they have no conflict of interests.

Author Contributions

SA and CL made an idea or hypothesis for research and/or manuscript and planned methodology to reach the conclusion; SA, AS, MH and EPL took the responsibility in execution of the experiments, data management and reporting; DI, SA, and DCR took the accountability in logical interpretation and presentation of the results; SA, MTR and IJ wrote of the whole or body of the manuscript; CL, SA and DCR reviewed the article before submission not only for spelling and grammar but also for its intellectual content. All authors have read and approved the manuscript.

References

1 Chiang J, Liver physiology: MetaboLism and detoxification, *Pathobiol Hum Dis A Dyn Encycl Dis Mech*, 2014 1770-1782.

2 Ma Z, Zhang B, Fan Y, Wang M, Kebebe D, *et al*., Traditional Chinese medicine combined with hepatic targeted drug delivery systems: A new strategy for the treatment of liver diseases, *Biomed Pharmacother*, 117 (2019) 109128.

3 Staff M clinic, Toxic hepatitis - Symptoms and causes - Mayo Clinic, 2021.

4 Mitchell S J & Hilmer S N, Drug-induced liver injury in older adults, *Ther Adv Drug Saf*, 1 (2) (2010) 65.

5 Sevindik M, Akgul H, Pehlivan M & Selamoglu Z, Determination of therapeutic potential of *Mentha longifolia* ssp. Longifolia, *Fresenius Environ Bull*, 26 (7) (2017) 4757-4763.

6 Phaniendra A, Jestadi D B & Periyasamy L, Free radicals: Properties, sources, targets, and their implication in various diseases, *Indian J Clin Biochem*, 30 (1) (2015) 11.

7 Pham-Huy L A, He H & Pham-Huy C, Free radicals, Antioxidants in disease and health, *Int J Biomed Sci*, 4 (2) (2008) 89.

8 Rastogi S, Pandey M M, Rawat A K S, Kushwaha V & Murthy P K, *In vitro* antifilarial activity, antioxidant potential and phenolic constituents of *Quisqualis indica* L., *Indian J Tradit Know*, 18 (4) (2019) 648-654.

9 Mohammed F S, Akgul H, Sevindik M & Khaled B M T, Phenolic content and biological activities of Var., (April) 2018.

10 Akgül G, Yılmaz N, Celep A, Celep F & Çakılcıoğlu U, Ethnobotanical purposes of plants sold by herbalists and folk bazaars in the center of Cappadocica (Nevsşhir, Turkey), *Indian J Tradit Know*, 15 (1) (2016) 103-108.

11 Pezzani R, Salehi B, Vitalini S, Iriti M, Zuñiga F A, *et al*., Synergistic effects of plant derivatives and conventional chemotherapeutic agents: An update on the cancer perspective, *Med*, 55 (4) (2019).

12 Om F R, Rupesh K M, Tamizh M T, Mohamed N K, Satya K B, *et al.,* Hepatoprotective activity of "*Asparagus racemosus* root" on liver damage caused by paracetamol in rats, *Indian J Nov Drug Deliv*, 3 (2) (2011) 112-117.

13 Rege N N, Thatte U M & Dahanukar S A, Adaptogenic properties of six rasayana herbs used in ayurvedic medicine, *Phyther Res*, 13 (4) (1999) 275-291.

14 Pandey S K, Sahay A, Pandey R S & Tripathi Y B, Effect of *Asparagus racemosus* rhizome (Shatavari) on mammary gland and genital organs of pregnant rat, *Phyther Res*, 19 (8) (2005) 721-724.

15 Ghosh T, Ghosh S & Maity T K, Evaluation of hepatoprotective activity of ethanolic extract of *Cajanus cajan* against paracetamol induced hepatotoxicity in rats, *Int J Nat Prod,* 4 (2) (2014) 47-53.

16 Sahreen S, Khan M R & Khan R A, Hepatoprotective effects of methanol extract of *Carissa opaca* leaves on CCl_4-induced damage in rat, *BMC Complement Altern Med*, 11 (2011) 48.

17 Jabeen A, Khan M A, Ahmad M, Zafar M & Ahmad F, Indigenous uses of economically important flora of Margallah Hills National Park, Islamabad, Pakistan, *Afr J Biotechnol*, 8 (5) (2009) 763-784.

18 I A S, A A A, S M, M A K, S J Q, *et al.,* Ethnobotanical studies of Chikar and its allied areas of District Muzaffarabad, *J Biol Sci*, 1 (12) (2001) 1165-1170.

19 Abbasi A M, Khan M A, Ahmad M, Zafar M, Khan H, *et al.,* Medicinal plants used for the treatment of jaundice and hepatitis based on socio-economic documentation, *Afr J Biotechnol*, 8 (8) (2009) 1643-1650.

20 Ahmed D, Saeed R, Shakeel N, Fatima K & Arshad A, Antimicrobial activities of methanolic extract of *Carissa opaca* roots and its fractions and compounds isolated from the most active ethyl acetate fraction, *Asian Pac J Trop Biomed*, 5 (7) (2015) 541-545.

21 Ilavarasana R, Mallika M & Venkataraman S, Anti-inflammatory and antioxidant activities of *Cassia fistula* Linn bark extracts, *Afr J Tradit Complement Altern Med*, 2 (1) (2005).

22 Siddhuraju P, Mohan P S & Becker K, Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp, *Food Chem*, 79 (1) (2002) 61-67.

23 Nan W, Kuang F, Yu-Jie F, Yuan-Gang Z, Fang-Rong C, *et al*., Antioxidant activities of extracts and main components of pigeonpea [*Cajanus cajan* (L.) Mill sp.] leaves, *Molecules*, 14 (3) (2009) 1032-1043.

24 Eze E, Iweala J, Evbakhavbokun W O & Maduagwu E N, Scientia pharmaceutica antioxidant and hepatoprotective effect of *Cajanus cajan* in N-Nitrosodiethylamine-induced liver damage.

25 Luximon-Ramma A, Bahorun T, Soobrattee M A & Aruoma O I, Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of *Cassia fistula*, *J Agric Food Chem*, 50 (18) (2002) 5042-5047.

26 Shimada K, Fujikawa K, Yahara K & Nakamura T, Antioxidative properties of Xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, *J Agric Food Chem*, 40 (6) (1992) 945-948.

27 Tundis R, Menichini F, Bonesi M, Conforti F, Statti G, *et al*., Antioxidant and hypoglycaemic activities and their relationship to phytochemicals in *Capsicum annuum* cultivars during fruit development, *LWT - Food Sci Technol*, 53 (1) (2013) 370-377.

28 OECD, Test guideline 425: Acute oral toxicity - Up-and-Down Procedure, *Guidel Test Chem*, (12) (2001) 26.

29 Bal C, Akgul H, Sevindik M, Akata I & Yumrutas O, Determination of the anti-oxidative activities of six =mushrooms, *Fresenius Environ Bull*, 26 (10) (2017) 6246-6252.

30 Sevindik M, Antioxidant and antimicrobial capacity of *Lactifluus rugatus* and its antiproliferative activity on A549 cells, *Indian J Tradit Know*, 19 (2) (2020) 423-427.

31 Osweiler G D, Carson T L, Buck W B & van Gelder G A, Clinical and diagnostic veterinary toxicology, 1985, xiv, pp. 494.

32 Wresdiyati T, Stephany S, Handharyani E, Sa'diah S & Astawan M, Acute toxicity test of pigeon pea leaves extract (*Cajanus cajan*) in rats, *E3S Web Conf*, 151 (2020) 1-4.

33 Moh F S, Gunal S, Sabik A E, Akgul H & Sevindik M, Antioxidant and antimicrobial activity of *Scorzonera papposa* collected from Iraq and Turkey.

34 Mohammed F S, Karakas M, Akgul H & Sevindik M, Medicinal properties of *Allium calocephalum* collected from Gara Moutain (Iraq), *Fresenius Environ Bull*, 28 (10) (2019) 7419-7426.

35 Gaur P, Bhatia S, Andola H C & Gupta R K, *In vitro* radical scavenging activity and antimicrobial potential of *Berberis asiatica* Roxb. ex DC. fruit extracts in four different processed forms, *Indian J Tradit Know*, 16 (4) (2017) 706-713.

36 Jadid N, Hidayati D, Hartanti S R & Arraniry B A, Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl, In: *Using DPPH Assay American Institute of Physics Conference Proceedings*, (2017) 20019.

37 Phongpaichit S, Nikom J, Rungjindamai N, Sakayaroj J, Hutadilok-Towatana N, *et al*., Biological activities of extracts from endophytic fungi isolated from *Garcinia* plants, *FEMS Immunol Med Microbiol*, 51 (3) (2007) 517-525.

38 Muthulingam M & Chaithanya K K, Total antioxidant capapcity, ferric reducing power and iron chelating activities of methanolic leaf extract of *Rhizophora apiculata* Blume, *Drug Invent Today*, 2018.

39 Chung Y-C, Chang C-T, Chao W-W, Lin C-F & Chou S-T, Antioxidative activity and safety of the 50 ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1, *J Agric Food Chem*, 50 (8) (2002) 2454-2458.

40 Jaishree V & Badami S, Antioxidant and hepatoprotective effect of swertiamarin from *Enicostemma axillare* against d-galactosamine induced acute liver damage in rats, *J Ethnopharmacol*, 130 (1) (2010) 103-106.

41 Mahadevan N, Goyal M, Rajalakshimi V, Khan N A & Chidambaram K, Hepatoprotective effect of combined extracts of *Andrographis paniculata*, *Boerhavia diffusa*, *Eclipta alba* and *Picrorhiza kurroa* on carbon tetrachloride and paracetamol-induced hepatotoxicity in rats, *Indian J Tradit Know*, 21 (3) (2022) 545-551.

42 Li L, Li W, Kim Y H & Lee Y W, *Chlorella vulgaris* extract ameliorates carbon tetrachloride-induced acute hepatic injury in mice, *Exp Toxicol Pathol*, 65 (1-2) (2013) 73-80.

43 Karakus E, Karadeniz A, Simsek N, Can I, Kara A, *et al*., Protective effect of *Panax ginseng* against serum biochemical changes and apoptosis in liver of rats treated with carbon tetrachloride (CCl_4), *J Hazard Mater*, 195 (2011) 208-213.

44 Shyamal S, Latha P G, Shine V J, Suja S R, Rajasekharan S, *et al*., Hepatoprotective effects of *Pittosporum neelgherrense* Wight & Arn., a popular Indian ethnomedicine, *J Ethnopharmacol*, 107 (1) (2006) 151-155.

45 Gnanaraj C, Shah M D, Emdadul H A T M, Makki J S & Iqbala M, Hepatoprotective and immunosuppressive Effect of *Synedrella nodiflora* L. on carbon tetrachloride (CCl_4)-intoxicated Rats, *J Environ Pathol Toxicol Oncol*, 35 (1) (2016) 29-42.

46 Shah M D, Gnanaraj C, Khan M S & Iqbal M, *Dillenia suffruticosa* L. impedes carbon tetrachloride & minus; induced hepatic damage by modulating oxidative stress and inflammatory markers in rats, *J Environ Pathol Toxicol Oncol*, 34 (2) (2015) 133-152.