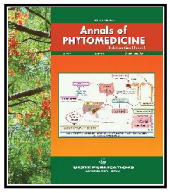
Annals of Phytomedicine 13(1): 539-547, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.56

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Protective effects of different parts of *Cassia fistula* L. against ethanol-induced oxidative damage in rat liver and kidney

Dinesh Kumar Sharma*◆, Suresh Kumar Sharma, Rajendra Damu Patil*** and Milind Mitra Lonare****

** Department of Veterinary Pharmacology and Toxicology, DGCNCOVAS, CSKHPKV, Palampur-176062, Himachal Pradesh, India*
*** Department of Veterinary Pharmacology and Toxicology, GADVASU, Ludhiana-141004, Punjab, India*
**** Department of Veterinary Pathology, DGCNCOVAS, CSKHPKV, Palampur-176062, Himachal Pradesh, India*

Article Info

Article history
Received 21 January 2024
Revised 8 March 2024
Accepted 9 March 2024
Published Online 30 June 2024

Keywords
Total phenolic contents
Antioxidant
Cassia fistula L.
Ethanol
Rats

Abstract

The present study investigated the total phenolic contents (TPC), *in vitro* antioxidant activity and ameliorative effects of ethanolic extracts of leaf, bark, flower and fruit pulp of *Cassia fistula* L. against ethanol-induced oxidative injury in the liver and kidney of wistar rats. Fourty-two male wistar rats received 0.5% carboxymethyl cellulose (0.5 ml/day), 50% ethanol (0.5 ml/day), 50% ethanol (0.5 ml/day) plus ethanolic extracts of *C. fistula* leaf, bark, flower and fruit pulp (100 mg/kg bw/day) and 50% ethanol (0.5 ml/day) plus silymarin (50 mg/kg bw/day) with 6 animals per group for two weeks. *In vitro* studies revealed that bark extract contained the highest TPC (43.10 ± 1.68 mg of gallic acid equivalent /g of extract). Bark extract also showed strongest ABTS scavenging activity (IC_{50} = 90.94 µg/ml /ml), DPPH scavenging activity (IC_{50} = 177.55 µg /ml) and superoxide radical scavenging activity (IC_{50} = 241.03 µg/ml) as compared to other parts. *C. fistula* extracts especially bark and leaf significantly improved the liver function enzymes (AST, ALT, and ALP), decreased erythrocyte MDA levels and raised plasma SOD and CAT activities. The reduced microscopic changes further confirmed the protective action of *C. fistula* extracts on liver and kidney tissues in rats.

1. Introduction

For ages, plants and their parts have been used by human civilization for the prevention and treatment of diseases. Currently, more than 100 active compounds isolated from plants are in use. About one-fourth of the drugs prescribed nowadays have plants as their origin. WHO has put 252 drugs on the list of essential medicines and 11% of these drugs have their origin from plants. In India, the majority of the rural population (>75%) is dependent on the traditional system of medicine (Sahoo *et al*., 2010). In recent years, the use of different types of medicinal plants has been increased by traditional medical practitioners (Deka *et al*., 2021). The majority of the drugs currently in use are synthetic drugs. However, they have drawbacks in the form of side effects, toxicity, and high cost. Herbal medicines have emerged as alternatives to synthetic drugs because of their fewer side effects and low cost (Che *et al*., 2017). Ethanol toxicity mainly affects the liver (Loguercio *et al*., 1996). Ethanol administration for a prolonged time leads to the generation of excessive amounts of reactive oxygen species, which eventually cause cell death and tissue damage in the liver. The reactive oxygen species and acetaldehyde formed from ethanol also produce a nephrotoxic effect by causing lipid peroxidation of epithelial cells of renal tubules (Rodrigo *et al*., 2015). The plants rich in antioxidants prevent the cell death and tissue damage caused by chronic alcohol consumption. Phytoconstituents of herbal origins were claimed and possess effective alternative therapy for hepatoprotection (Nadeem *et al*., 2022).

Due to their worldwide medicinal use in traditional medicine, the *Cassia* species have been investigated by Pharmacologists throughout the world (Kumar *et al*., 2021). *Cassia fistula* (*C. fistula*) is an average-sized tree. Its height may range up to 25 m and its girth may be up to 1.5-2.0 m. It has greenish-gray bark, compound leaves, and golden-yellow color flowers. The plant is cultivated almost all over the world and has been extensively used in all forms of traditional medicine. Almost all the parts of *C. fistula* have medicinal use. The main phytoconstituents in *C. fistula* are: rhein, triterpenes, kaempferol, lupeol, β-sitosterol, and hexacosanol. *C. fistula* is also a rich source of soluble sugars, calcium, potassium, linoleic, oleic, stearic, palmitic acids, caprylic and myristic acids (Rahmani, 2015). Previous studies conducted on *C. fistula* have demonstrated that different parts of *C. fistula* are effective in the treatment of various disease conditions their antimicrobial and hepatoprotective effects have also been reported (Panda *et al*., 2011; Das, 2008). However, after going through literature, we find that no comparative study has been conducted reporting the hepatoprotective and nephroprotective effects of different parts of the *C. fistula*. Keeping in view the medicinal properties and lack of comparative studies, this study was planned to explore the protective effects of different parts of *C. fistula* against ethanol-induced injury in the liver and kidney of wistar rats.

2. Materials and Methods

2.1 Preparation of *C. fistula* extract

The parts of *C. fistula* used in the present study were its leaves, flowers, bark, and fruits which were collected from different places

Corresponding author: Dr. Dinesh Kumar Sharma
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, DGCNCOVAS, CSKHPKV Palampur-176062, Himachal Pradesh, India
E-mail: dineshcovas79@gmail.com
Tel.: +91-9418477233

in and around District Ludhiana (Punjab), India. The plant parts were identified at CSIR-IHBT, Palampur (H.P) India (Voucher No. PLP 15392). Different parts were then dried in shade and after drying converted to powder form by using a grinder. Ethanolic extracts were prepared by maceration technique and stored at 4°C (Abd Hamid *et al*., 2017).

2.2 Experimental study

The present study was conducted on male wistar albino rats procured from LUVAS, Hisar, Haryana, India. The animals were reared as per the guidelines for the rearing of animals. An acclimatized period of two weeks was given before starting the experiment. The study protocol was approved by IAEC Vide No. IAEC/2018/1216-1250. The rats were divided into groups (n = 6 per group) as follows: Group 1: Control (vehicle control) group, receiving 0.5 ml/day of 0.5% carboxymethyl cellulose (CMC) by gavage; Group 2: Model group, receiving 0.5 ml/day of 50% ethanol (3 g/kg bw) by gavage; Groups 3-6: Treatment groups, receiving ethanolic extracts of *C. fistula* leaves, bark, flowers and fruit pulp (100 mg/kg bw) in 0.5 ml/day of 0.5% CMC plus 0.5 ml/day of 50% ethanol (3 g/kg bw) by oral gavage. Group 7: Positive control group, receiving silymarin (50 mg/kg/day bw) in 0.5 ml/day of 0.5% CMC plus 0.5 ml/day of 50% ethanol (3 g/kg bw) by oral gavage. Treatments continued daily for 14 days.

2.3 Total phenolic content (TPC)

Folin-ciocalteu reagent (FCR) based assay was used to determine the total phenolic content in the extracts of *C. fistula*. In this method, to 50 µl of plant extract, 950 µl of distilled water was added and mixed. After mixing, 500 µl of FCR with distilled water (1:1) and 2.5 ml of the 20% sodium carbonate solution were added. The mixture was then kept for 40 min at room temperature and absorbance was recorded at 725 nm with a spectrophotometer (Shimadzu UV-VIS Spectrophotometer, Shimadzu, Japan). The standard curve of gallic acid was prepared for the determination of TPC (Gulcin *et al*., 2002) (Figure 1).

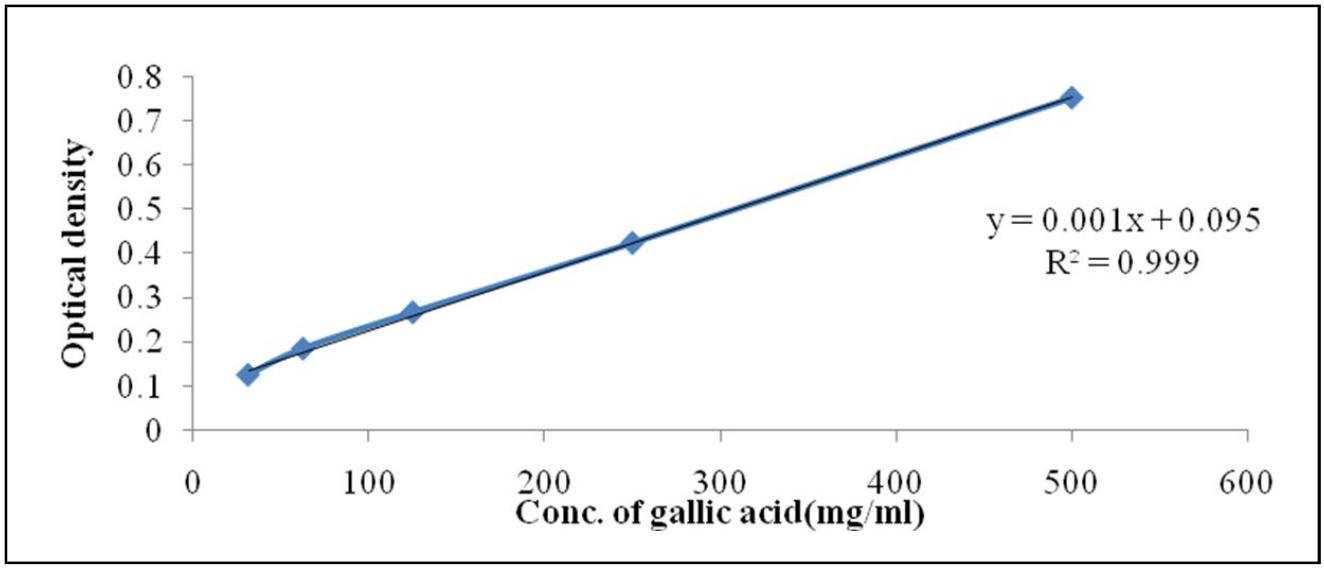


Figure 1: Standard curve of gallic acid for estimation of total phenols.

2.4 Antioxidant activities

2.4.1 ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging assay

ABTS radical scavenging activity was performed as per the standard method (Re *et al*., 1999). A 2 mM ABTS solution was prepared in distilled water. ABTS radical cations (ABTS•+) were produced by mixing 50 ml of the ABTS stock solution with 200 µl of 70 mM potassium persulfate. To ensure complete oxidation of ABTS, the mixture was held at room temperature in the dark for 16 to 20 h prior to analysis. The resulting ABTS•+ solution was diluted with phosphate buffer (about four fold) to give an absorbance reading of 0.5 ± 0.05 at 734 nm. The antioxidant activity of *C. fistula* extracts and trolox as standard were evaluated in concentration ranging from 25 to 1000 µg/ml. Radical scavenging analysis was performed by mixing 30 µl of the sample solution into 3 ml of ABTS•+ solution and reading the absorbance at 734 nm after 3 minutes. A control solution of 30 µl distilled water in 3.0 ml of ABTS•+ solution was prepared and analyzed.

% ABTS•+ inhibition = [1 – (A734 nm test/ A734 nm control)] x 100

IC_{50} values were determined by a graph between the per cent of inhibition against concentration.

2.4.2 DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity

DPPH radical scavenging activity was performed by a standard method (Hsu *et al*., 2006). A 100 µM solution of DPPH in methanol was prepared. The free radical scavenging action of *C. fistula* extracts was seen in concentration ranges from 25 to 1000 µg/ml. BHT (Butylated hydroxytoluene) was taken as standard antioxidant. 0.5 ml sample solution was added to 2.0 ml of DPPH in a 20 ml test tube. A control solution was prepared by adding 0.5 ml of methanol to 2 ml DPPH solution. Samples were vertexed for 10 to 15 s and held at room temperature in the dark for 30 min. The absorbance of the sample and control solutions was determined at 517 nm.

2.4.3 Superoxide anion radical scavenging activity

This activity was determined by a standardized method (Gulcin *et al*., 2004). Superoxide anion radicals were generated in PMS-NADH

(phenazinemethosulfate-nicotinamide adenine dinucleo-tide) system by oxidation of NADH and assayed by the reduction of NBT (Nitro blue tetrazolium). In this test, superoxide anion radicals were generated in 2.5 ml of Tris-HCl buffer (16 mM, pH 8.0) containing 0.5 ml of NBT (300 µM) solution, 0.5 ml of NADH (468 µM) solution and 1 ml of plant extract sample in concentration ranging from 25 to 1000 µg/ml. The reaction was started by adding 0.5 ml of PMS (60 µM) solution to the mixture. The reaction mixture was incubated at room temperature for 5 min and the absorbance was read at 560 nm. The per cent radical scavenging activity and IC_{50} were calculated similarly to ABTS scavenging activity.

2.5 Biochemical analysis

On the 15th day after completion of the experimental trial, the animals were sacrificed. Blood and tissue samples were collected. Blood samples were used for the estimation of liver enzymes; aspartate transaminases (AST), alanine transaminases (ALT), and alkaline phosphatase (ALP) using standard diagnostic kits (Erba Diagnostics Manheim, Germany).

2.6 Estimation of lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activities

The spectrophotometric method was used to measure LPO, SOD, and CAT activities. LPO was measured by the method of Stocks and Dormandy, (1971). To 2 ml of 33 % packed erythrocyte, 1 ml of 40 mM H_2O_2 was added, and then 0.1 ml sodium azide were added in the test tube and the mixture was incubated at 37°C for 1 h. After incubation, the total volume was made up to 4 ml with PBS in each tube and 2 ml of ice chilled TCA was added to stop the reaction. The tubes were centrifuged at 3000 rpm for 15 min. Now, 4 ml of supernatant was collected and sediment was discarded. To 4 ml of supernatant, 1 ml of 1 per cent TBA was added and it was kept in boiling water bath for 10 min. Blank was made by adding the reagents except the packed erythrocytes which were substituted with equal volume of distilled water. Finally, the optical density was measured at 535 nm against blank, after cooling the contents to room temperature. Superoxide dismutase was analyzed by the standard method (Marklund and Marklund, 1974). To a cuvette, 1.5 ml of 100 mM Tris-HCl buffer, 0.5 ml of 6 mM EDTA and 1 ml of 0.6 mM pyrogallol solution were added. The rate of auto-oxidation of pyrogallol was measured by the increase in absorbance at 420 nm in a spectrophotometer every min, after a lag of 30 s up to 4 min. For the test, 20 µl of erythrocyte lysate was added to inhibit the auto-oxidation of pyrogallol to about 50%. A unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of the auto-oxidation of pyrogallol observed in blank. The values were expressed as units/mg Hb. Catalase levels were analyzed by the standard method (Aebi, 1983). To 2 ml of phosphate buffer in quartz cuvette, 20 µl of erythrocyte lysate was added and mixed well. The reaction was started by the addition of 1 ml of 30 mM H_2O_2 and the decrease in absorbance was recorded at every 10 sec interval for 1 min at 240 nm in a U.V. spectrophotometer. The results were expressed as µmol H_2O_2 decomposed per min per mg Hb using 36 as molar extinction coefficient of H_2O_2.

2.7 Histopathological investigation

A systematic necropsy examination was conducted (Luna, 1968). Microphotography was performed using an Olympus Microscope (BX-40, Japan).

2.8 Histopathological lesion score

The intensity of microscopic changes in the tissues was scored as mild (1), moderate (2), and severe (3). The total of individual lesion scores (vascular, degenerative, necrotic, and inflammatory lesions) was calculated as the sum of the products of each lesion score of a particular type of lesion with the number of rats showing that particular lesion and severity. The mean was determined by dividing the total score by the total number of rats in that group. The total lesion score for the liver and kidneys of each rat was determined by the total of intensity score of each alteration.

2.9 Statistical analysis

IBM SPSS version 20.0. software was used for statistical analysis The Kruskal-Wallis non-parametric test was employed for the analysis of microscopic lesions in the liver and kidney tissues using Graph Pad InStat software (San Diego, USA).

3. Results

3.1 Total phenolic content (TPC) and percent recovery of *C. fistula* extracts

The total phenolic content of the ethanolic extract of *C.fistula* bark was found to be the highest (43.1 ± 1.68) mg of GAE/gm of extract, followed, by leaf, flower, and fruit pulp. The per cent recovery was highest in bark (4.2%) followed by leaf, fruit pulp, and flower (Table 1).

Table 1: Total phenolic content (TPC) (Mean ± SE) in leaves, bark, flowers and fruit pulp of *C. fistula* extracts (n=3) measured as gallic acid equivalent (mg of GAE/g of extract) and per cent recovery of extracts.

Extract	TPC (mg of GAE/g of extract)	% Recovery
Leaf (Ethanolic)	11.12 ± 0.22	3.7
Bark (Ethanolic)	43.10 ± 1.68	4.2
Flower (Ethanolic)	09.09 ± 0.31	2.0
Fruit pulp (Ethanolic)	02.53 ± 0.28	2.4

3.2 ABTS scavenging activity

On comparing IC_{50} values of *C. fistula* leaves, bark, flower, fruit pulp extracts and standard antioxidant trolox (water-soluble vitamin E analog) the ABTS scavenging activity in decreasing order was bark (90.94 µg/ml) > leaf (152.81 µg/ml) > trolox (195.43 µg/ml) > flower (206.95 µg/ml) > fruit pulp (396.23 µg/ml). ABTS scavenging activity of *C. fistula* bark and leaves was found better than the standard antioxidant trolox.

3.3 DPPH radical scavenging activity

On comparing IC_{50} values of *C. fistula* leaves, bark, flower, fruit pulp ethanolic extracts, and standard antioxidant BHT, the DPPH scavenging activity in decreasing order was bark (177.55 µg/ml) > leaf (236.99 µg/ml) > BHT (289.02 µg/ml) > flower (378.87 µg/ml) > fruit pulp (674.55 µg/ml). The free radical scavenging activity of extracts of *C. fistula* bark and leaf was found better than the standard antioxidant BHT.

3.4 Superoxide radical scavenging assay

The order of superoxide radical scavenging assay and standard antioxidant in decreasing order was ascorbic acid (90.37 µg/ml) > bark (241.03 µg/ml) > leaf (692.99 µg/ml) > flower (733.2µg/ml) > fruit pulp (858.27 µg/ml).

3.5 Effects of *C. fistula* extract on biochemical parameters in ethanol-treated rats

The level of liver enzymes AST, ALT, and ALP was found significantly (p<0.05) increased in ethanol treated group as compared to the normal group. The enzymes significantly (p<0.05) decreased in all the *C. fistula* extracts treated groups. The maximum decrease in levels of these enzymes was observed in the *C.fistula* bark-treated group (Figure 2).

3.6 Effect of *C. fistula* extract on serum LPO, CAT, and SOD levels in ethanol-treated rats

The levels of serum SOD and CAT significantly (p<0.01) decreased in ethanol-treated rats. However, leaf and bark extract of *C.fistula* significantly increased SOD and CAT levels. LPO levels increased significantly (p<0.01) in the group of ethanol treated rats which decreased significantly (p<0.01) in *C.fistula* leaf and bark extract treated rats (Figure 3).

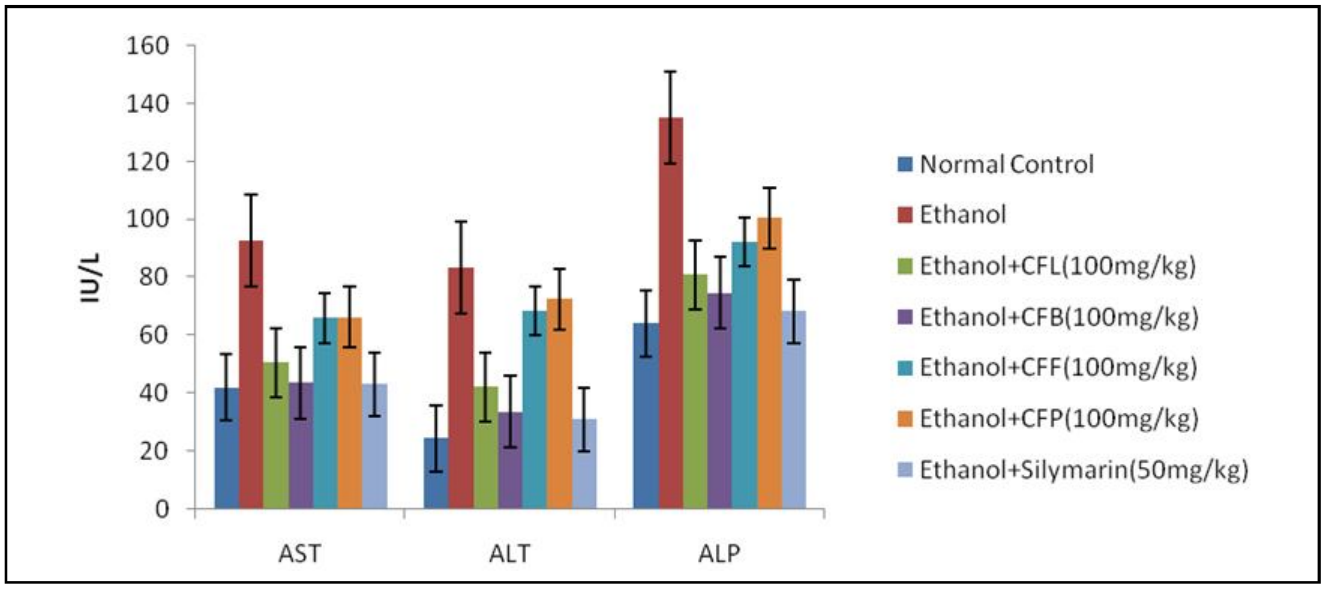


Figure 2: Serum activity of AST, ALT and ALP levels after treatment with *C. fistula* extracts (mean ± SE).

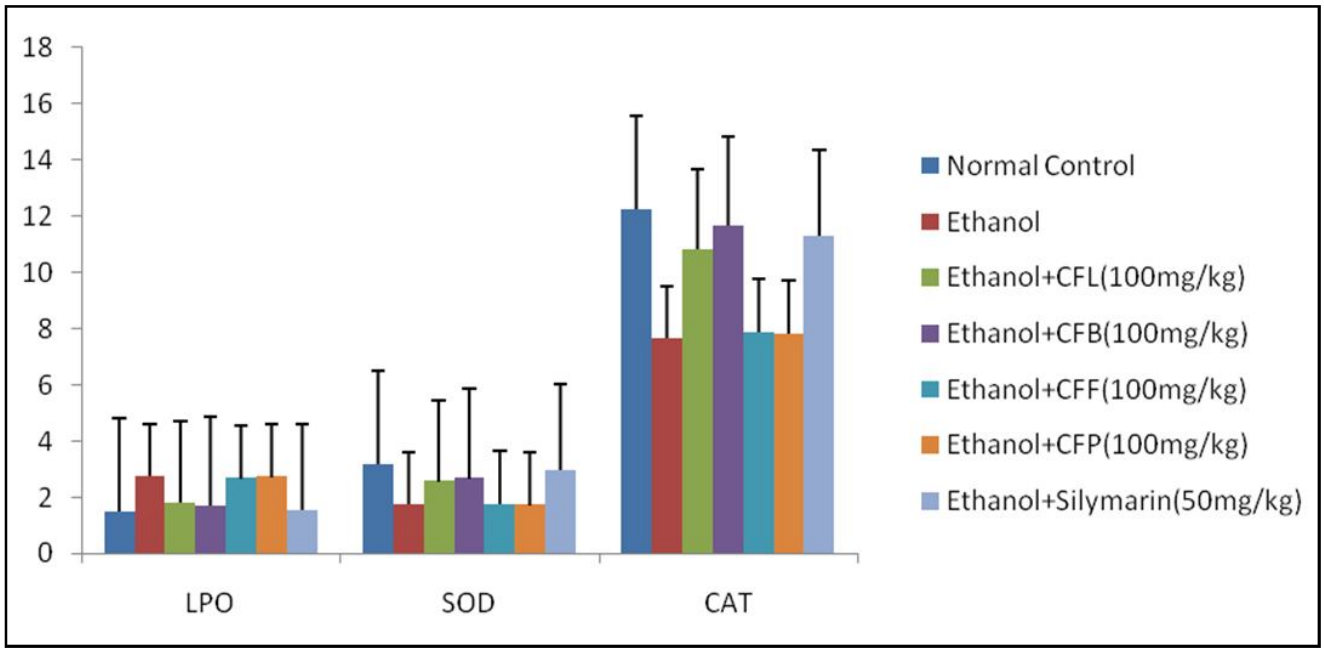


Figure 3: Serum levels of LPO (nmolMDA/ml), SOD (U/ml) and CAT (uM/min/ml) (mean ± SE); LPO: lipid peroxidation; SOD: superoxide dismutase; CAT: catalase.

3.7 Histopathological findings

The total mean lesion score for histopathological changes comprising vascular changes, degenerative changes, necrotic changes, and cellular infiltration in the liver and kidneys was found to be significantly (p<0.05) lower in *C. fistula* bark extract-treated group animals, and the values were presented in Tables 2,3 and illustrated in Figures 4, 5.

Table 2 : Mean lesion scores (mean ± SE) and mean total lesion scores (mean ± SE) of the liver tissues in rats (n=6)

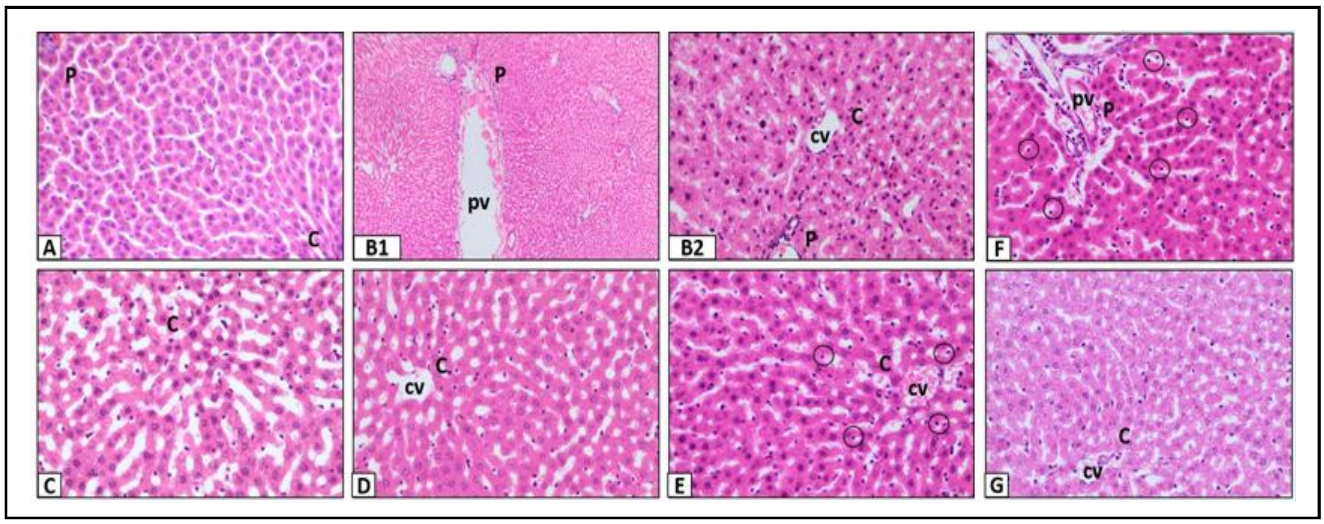
Group	Dose (mg/kgbw)	*Microscopic lesion score				**Total lesion score
		Vascular changes	Degenerative changes	Necrotic changes	Cellular infiltration	
Control	-	0.33 ± 0.21^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.33 ± 0.21^{a}
Ethanol (50%)	3000	2.33 ± 0.33^{c}	2.00 ± .37^{c}	1.50 ± 0.22^{b}	1.50 ± 0.22^{b}	7.33 ± 1.09^{c}
Ethanol (50%) + Leaves of *C. fistula*	100	2.00 ± 0.37^{bc}	1.00 ± 0.00^{bc}	0.67 ± 0.21^{a}	0.83 ± 0.17^{a}	4.50 ± 0.22^{bc}
Ethanol (50%) + Bark of *C. fistula*	100	1.00 ± 0.00^{ab}	0.83 ± 0.17^{a}	0.67 ± 0.21^{a}	1.00 ± 0.00^{b}	3.50 ± 0.34^{ab}
Ethanol (50%) + Flower of *C. fistula*	100	1.67 ± 0.21^{abc}	1.00 ± 0.00^{bc}	0.50 ± 0.22^{a}	0.83 ± 0.17^{a}	4.00 ± 0.26^{ac}
Ethanol (50%) + Fruit pulp of *C. fistula*	100	1.33 ± 0.21^{abc}	1.00 ± 0.00^{bc}	0.50 ± 0.22^{a}	0.83 ± 0.17^{a}	3.67 ± 0.21^{ab}
Ethanol (50%) + Silymarin	50	1.00 ± 0.37^{ab}	0.83 ± 0.17^{a}	0.67 ± 0.21 ^{a}	0.67 ± 0.21^{a}	3.17 ± 0.65^{ab}

Means bearing superscripts $^{a, b, c}$ differ significantly ($p\leq0.05$) in a column.*Mean microscopic lesion scores were obtained for each group by dividing the sum of the products of intensity scores (Mild=1; Moderate=2; Severe=3) and the number of rats showing that particular intensity by number of total rats at that interval.**Mean total lesion scores were obtained for each group by dividing the sum of the total lesion scores (vascular changes, degenerative changes, necrotic changes and cellular infiltration) by the total number of rats of the corresponding group.

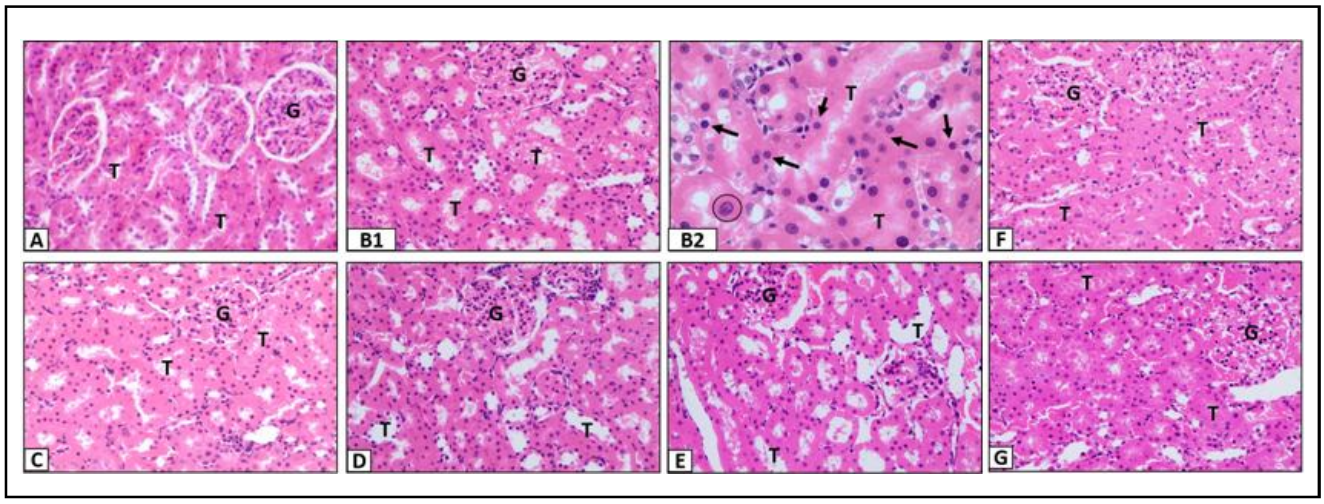
Table 3: Mean lesion scores (mean ± SE) and mean total lesion scores (mean ± SE) of the kidney tissue in rats(n = 6)

Group	Dose (mg/kgbw)	*Microscopic lesion score				**Total lesion score
		Vascular changes	Degenerative changes	Necrotic changes	Cellular infiltration	
Control	-	0.17 ± 0.17^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.17 ± 0.17^{a}
Ethanol (50%)	3000	2.00 ± 0.26^{b}	1.83 ± 0.31^{b}	1.50 ± 0.22^{b}	1.33 ± 0.21^{c}	6.67 ± 0.88^{c}
Ethanol (50%) + Leaves of *C. fistula*	100	1.33 ± 0.21^{a}	1.17 ± 0.31^{b}	0.67 ± 0.21^{a}	1.00 ± 0.26^{ac}	4.17 ± 0.54^{bc}
Ethanol (50%) + Bark of *C. fistula*	100	1.00 ± 0.00^{a}	0.83 ± 0.17^{a}	0.50 ± 0.22^{a}	0.33 ± 0.21^{ab}	2.67 ± 0.49^{ab}
Ethanol (50%) + Flower of *C. fistula*	100	1.00 ± 0.37^{a}	1.00 ± 0.00^{a}	0.67 ± 0.21^{a}	0.50 ± 0.22^{ac}	3.17 ± 0.70^{ac}
Ethanol (50%) + Fruit pulp of *C. fistula*	100	1.50 ± 0.22^{a}	0.83 ± 0.17^{a}	0.50 ± 0.22^{a}	0.50 ± 0.22^{ac}	3.33 ± 0.33^{ac}
Ethanol (50%) + Silymarin	50	1.33 ± 0.21^{a}	1.17 ± 0.31^{b}	0.67 ± 0.21^{a}	1.00 ± 0.26^{ac}	4.17 ± 0.54^{bc}

Means bearing superscripts a, b, c differ significantly (p<0.05) in a column. Data are means ± S.E (n=6).*Mean lesion scores were obtained for each group by dividing the sum of the products of intensity scores (Mild=1; Moderate=2; Severe=3) and the number of rats showing that particular intensity by number of total rats at that interval.**Mean total lesion scores were obtained for each group by dividing the sum of the total lesion scores (vascular changes, degenerative changes, necrotic changes and cellular infiltration) by the total number of rats of the corresponding group.



Figures 4a-g: **Histopathological results of liver in rats. A: The control group showing normal structures of sinusoids, portal area (P), centrilobular area (C) and well-arranged hepatocytes. B1: Ethanol treated group showing congested portal vein (pv) in the portal region (P) and sinusoids. B2:Degenerated hepatocytes with increased eosinophilic cytoplasmic granularity and cytoplasmic vacuolations and the presence of pyknotic nuclei in most of the hepatocytes around the central vein (cv). C: Leaf treated group showing engorged and dilated sinusoids and degeneration of hepatocytes in the centrilobular region. D: Bark treated group showing degeneration of hepatocytes in the centrilobular region. E: Flower treated group showing hepatocytic degeneration with infiltration of inflammatory cells mainly lymphocytes (encircled). F: Fruit pulp treated group showing infiltration of lymphocytes (encircled) around the portal region. G: Silymarin treated group showing hepatocytic degeneration with increased cytoplasmic granularity. {A, B2, C, D, E, F, G, H&E 200x} {B1 H&E 40x}.**



Figures 5a-g: **Histopathological results of kidney in rats. A: The control group showing normal glomeruli (G) and tubules (T) in the cortex. B1: Ethanol treated group showing severely degenerated tubular epithelium and the presence of pyknotic nuclei in the renal tubules. B2: kidney showing degenerated tubular (T) epithelium with increased eosinophilic cytoplasmic granularity and the nuclear changes including pyknosis (arrow) and karyomegaly (encircled). C: Leaf treated group showing degenerative changes in the tubular (T) epithelium. D: Bark treated group showing degeneration of tubular (T) epithelium. E: Flower treated group showing dilatation of renal tubules and degenerated tubular epithelium. F: Fruit pulp treated group showing degenerated tubular (T) epithelium. G: Silymarin treated group showing degenerated tublular (T) epithelium with increased eosinophilic cytoplasmic granularity {A, B1, C, D, E, F, G, H&E 200x} {B2 H&E 400x}.**

4. Discussion

Phenols have good antioxidant activity. These are responsible for neutralizing the oxidative stress caused in the body due to the generation of free radicals (Falak *et al*., 2023). In our study, percent recovery was found to be highest in bark (4.2%), followed by leaf, fruit pulp, and flower. Total phenolic content in decreasing order among different parts of *C. fistula* are bark followed by leaf, flower, and fruit pulp. Our results are in consonance with an earlier study conducted by Siddhuraju and Becker (2003), who found similar results. Tzekiat and Chiang (2013), also found higher contents of

total phenol, tannin, and antioxidant activity in *C. fistula* bark extracts as compared to other parts. In our study, a substantial concentration of phenols was detected in *C. fistula* bark extract. The higher the phenolic content greater the antioxidant activity of plants.The ethanolic extract of *C. fistula* bark possesses a good ability to scavenge the ABTS radicals. At the highest concentration of 1 mg/ml, the ethanolic bark extract of *C. fistula* showed 84.2% $ABTS^{+}$ radical Scavenging, which is at par with standard trolox showing 87% inhibition. The phenolic compounds because of their high mol. wt. have more ability to scavenge the free radicals (Hagerman *et al*., 1998). The high antioxidant activity of *C. fistula* bark extract might be due to its high mol. wt. phenolic compounds. DPPH radical scavenging activity of plant extracts is observed as a color change of DPPH solution from purple to yellow which may be due to donation of hydrogen ions (Kumar *et al*., 2008). In the present study, all the *C. fistula* plant parts used showed DPPH scavenging activity. However, there is a difference in the scavenging activity of different plant parts which may be due to the different concentrations of polyphenols and flavonoids. In the present study, DPPH scavenging activity was found to be dose-dependent which may be due to the difference in concentration of hydrogen ions. More is the concentration of hydrogen ions lighter the solution will be (Silva *et al*., 2005; Villano *et al*., 2007). Superoxide anion radicals are mainly released by monocytes, macrophages, eosinophils, and neutrophils (Kumar *et al*., 2008). These radicals were generated in the PMS-NADH (phenazinemethosulfate-nicotinamide adenine dinucleotide) system by oxidation of NADH and assayed by the reduction of NBT (Nitro blue tetrazolium) (Oktay *et al*., 2003). In our study, the IC_{50} value of ascorbic acid was found less than *C. fistula* extracts suggesting that ascorbic acid is a better scavenger of superoxide radicals than *C. fistula*. Among the extracts, the bark extract of *C. fistula* has better scavenging activity. Better superoxide radical scavenging activity might be due to higher polyphenolic content in *C. fistula* bark extract.

Hepatic dysfunctions are commonly screened by liver function tests. Liver function tests are determined by the level of liver enzymes in the circulation. The more severe the liver damage more will be the enzymes released into the circulation (Mahar *et al*., 2022). In the current study, oral administration of ethanol (50%) @ 3 g/kg b.wt. for two weeks significantly ($p<0.01$) raised AST, ALT, and ALP enzymes. Treatment with extracts of *C. fistula* significantly ($p<0.01$) decreased these liver enzymes. Standard drug silymarin @ 50 mg/kg also significantly ($p<0.01$) decreased the level of these enzymes. The maximum decrease in levels of these enzymes was observed in bark treated group. However, the decrease was less than in the silymarin-treated group. Our results are in agreement with an earlier study on *C. fistula* in which the elevated level of plasma enzymes by CCl_4 was reduced by the aqueous extract of leaves and bark (Pradeep *et al*., 2005). Previous studies with CCl_4, thioacetamide, and diethyl nitrosamine (DEN) reported liver damage accompanied by a rise in levels of liver enzymes (AST, ALT, bilirubin, and alkaline phosphatase) and an increase in oxidative stress marker LPO and fall in the activities of SOD, CAT, and glutathione reductase levels. *C. fistula* leaves ethanolic extract @ 500 mg/kgb.w for 7 days and n-heptane extract @ 400 mg/kg restored normalcy by reducing the elevated level of liver enzymes, LPO levels and increasing levels of catalase and glutathione reductase (Bhakta *et al*.,1999; Pradeep *et al*., 2007; Pradeep *et al*., 2010; Kaur *et al*., 2019). In the liver of the ethanol-treated group, the sections of the liver showed moderate to severely engorged and dilatation of blood vessels and sinusoids. The Kupffer cell activity was consistently increased. The degenerative changes such as increased eosinophilic cytoplasmic granularity and cytoplasmic vacuolations in the hepatocytes were the common findings. Occasional, fat vacuoles were seen in the centrilobular hepatocytes. Nuclear changes such as the frequent presence of pyknotic nuclei, karyomegaly, karyorrhexis, or dissolution of nuclei and/or apoptotic hepatocytes were also noted especially in the hepatocytes of the centrilobular region. Variable infiltration of inflammatory cells including lymphocytes along with a few neutrophils also noted inside the parenchyma (Figures 4 b1-b2). Treatment with *C. fistula* extracts of leaf, bark, flower, fruit pulp, and silymarin restored the normal architecture of ethanol-intoxicated damaged liver (Figures 4c-g). The mean lesion score for vascular changes in the liver significantly ($p<0.05$) declined in *C. fistula* (bark and pulp extracts) treated group animals. The mean lesion score for degenerative changes in the hepatocytes was significantly ($p<0.05$) decreased in *C. fistula* (bark, flower, and pulp extracts) treated group animals. The mean lesion score for necrotic changes was significantly ($p<0.05$) decreased in *C. fistula* (leaf, bark, flower, and pulp extracts) treated group animals. The mean lesion score for cellular infiltration in the liver was significantly ($p<0.05$) reduced in *C. fistula* (bark and flower extracts) treated group animals.

In the kidneys of the ethanol-treated group, the sections of kidneys showed moderate to severely engorged and dilatation of blood vasculature in the cortex and medullary region. The glomerular tufts were engorged in the renal cortex. The moderate to severely degenerated renal tubular epithelium revealed increased eosinophilic cytoplasmic granularity and cytoplasmic vacuolations. The hyaline/ epithelial casts were also seen inside the tubular lumen. The nuclear changes such as pyknosis, karyomegaly, and fragmented or dissolution of nuclei were also seen in the renal epithelium (Figures 5b1-b2). Treatment with *C. fistula* extracts of leaf, bark, flower, fruit pulp, and silymarin restored the normal architecture of ethanol-intoxicated damaged kidneys (Figures 5c-g). The mean lesion score for vascular changes in kidneys significantly ($p<0.05$) decreased in *C. fistula* (leaf, bark, flower, and pulp extracts) treated group animals. The mean lesion score for degenerative changes in kidneys was significantly ($p<0.05$) decreased in *C. fistula* (bark, flower, and pulp extracts) treated group animals. The mean lesion score for necrotic changes in kidney tissues was significantly ($p<0.05$) decreased in *C. fistula* (leaf, bark, flower, and pulp extracts) treated group animals. The mean lesion score for cellular infiltration in kidneys was significantly ($p<0.05$) decreased in *C. fistula* (bark) treated rats.

The hepatoprotection of *C. fistula* leaves/fruit/bark extracts promisingly improved histo-architecture of the liver by reducing the microscopic lesions in several studies suggesting its primary hepatoprotective nature in experimental animals (Bhakta *et al*., 2001; Jehangir *et al*., 2010, Kalantri *et al*., 2011; Patwardhan *et al*., 2009). In one of the earlier studies, histologic examination of kidney of mice revealed that hydro-alcoholic *C. fistula* fruit extract @ dose range from 200 to 800 mg/kg b.w followed by 460 mg/kg bromobenzene significantly restored bromobenzene-induced toxicity (Kalantri *et al*., 2011). In this study, histological examination revealed that the ethanolic extract of *C. fistula* bark significantly restored the nephrotoxic effect of ethanol.

5. Conclusion

In our study, it was found that the ethanolic extract of all *C. fistula* plant parts has good antioxidant activity and protective effects in the liver and kidney against ethanol. Among the different parts bark and leaves of *C. fistula* showed the best results. The protective effects on the liver and kidney might be due to the antioxidant properties. However, the compounds producing these effects are still to be identified and tested before their clinical usage.

Acknowledgements

The authors are thankful to Dean Postgraduate Studies Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India for providing necessary facilities to carry out the research work.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abd Hamid, H.; Mutazah, R.; Yusoff, M.M.; Abd Karim, N.A. and Razis A.F. (2017). Comparative analysis of antioxidant and antiproliferative activities of *Rhodomyrtus tomentosa* extracts prepared with various solvents. Food and Chemical Toxicology, **108**:451-457.

Aebi, H.E. (1983). Catalase. Methods of Enzymatic Analysis, pp:273-286.

Bhakta, T., Banerjee, S.; Mandal, S.C.; Maity, T.K., Saha, B,P. and Pal M. (2001). Hepatoprotective activity of *Cassia fistula* leaf extract. Phytomedicine, **8**(3):220-224.

Bhakta, T.; Mukherjee, P.K.; Mukherjee, K.; Banerjee, S.; Mandal, S.C.; Maity, T.K.; Pal, M. and Saha, B.P. (1999). Evaluation of hepatoprotective activity of *Cassia fistula* leaf extract. Journal of Ethnopharmacology, **66**(3):277-282.

Che, C.T.; George, V.; Ijinu, T.P.; Pushpangadan, P. and Andrae-Marobela, K. (2017). Traditional medicine. In: Pharmacognosy, pp:15-30. Academic Press.

Das, S.; Sarma, G. and Barman, S. (2008). Hepatoprotective activity of aqueous extract of fruit pulp of *Cassia fistula* (AFCF) against carbon tetrachloride (CCL_4) induced liver damage in albino rats. Journal of Clinical and Diagnostic Research, **2**(5):1133-1138.

Deka, N. J.; Nath, R.; Shantanu Tamuly, M.; Pegu, S. R. and Deka, S. M. (2021). Green synthesis and characterization of silver nanoparticles using leaves extract of Neem (*Azadirachta indica* L.) and assessment of its *in vitro* antioxidant and antibacterial activity. Ann. Phytomed., **10**(1):171-177.

Falak Bamne, Nikhat Shaikh, Munira Momin, Tabassum Khan and Ahmad Ali (2023). Phytochemical analysis antioxidant and DNA nicking protection assay of some selected medicinal plants. Ann. Phytomed., **12**(2):406-413

Gulcin, I.; OKTAY, M.; Kufrevioglu, O. and Aslan A. (2002). Determination of antioxidant activity of lichens *Cetraria islandica* (L) Ach. Journal of Ethnopharmacology, **79**(3):325-329.

Gulcin, I.; ªat, I.G.; Beydemir, S.; Elmastaº, M. and Kufrevioglu OI. (2004). Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). Food Chemistry, **87**(3):393-400.

Hagerman, A.E.; Riedl, K.M.; Jones, G.A.; Sovik, K.N.; Ritchard, N.T.; Hartzfeld, P.W. and Riechel, T.L. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. Journal of Agricultural and Food chemistry, **46**(5):1887-1892.

Hsu, B.; Coupar, I.M. and Ng, K. (2006). Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. Food Chemistry, **98**(2):317-328.

Jehangir, A.; Nagi, A.H.; Shahzad, M. and Zia, A. (2010). The hepatoprotective effect of *Cassia fistula* (amaltas) leaves in isoniazid and rifampicin induced hepatotoxicity in rodents. Biomedica, **26**(1):25-29.

Kalantari, H.; Jalali, M.; Jalali, A.; Mahdavinia, M.; Salimi, A.; Juhasz, B.; Tosaki, A. and Gesztelyi, R. (2011). Protective effect of *Cassia fistula* fruit extract against bromobenzene-induced liver injury in mice. Human and Experimental Toxicology, **30**(8):1039-1044.

Kalantari, H.; Jalali, M.; Jalali, A.; Salimi, A.; Alhalvachi, F.; Varga, B.; Juhasz, B.; Jakab, A.; Kemeny-Beke, A.; Gesztelyi, R. and Tosaki, A. (2011). Protective effect of *Cassia fistula* fruit extract on bromobenzene-induced nephrotoxicity in mice. Human and Experimental Toxicology, **30**(10):1710-1715.

Kaur, S.; Sharma, D.; Singh, A.P. and Kaur, S. (2019). Amelioration of hepatic function, oxidative stress, and histopathologic damages by *Cassia fistula* L. fraction in thioacetamide-induced liver toxicity. Environmental Science and Pollution Research, **26**(29):29930-29945.

Kumar, K.S.; Ganesan, K. and Rao, P.S. (2008). Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty: An edible seaweed. Food Chemistry, **107**(1):289-295.

Kumar, S.; Singh, R.; Nagar, P. S. and Dwivedi, M. (2021). Morphological, anatomical characterization and profiling of laxative principles sennosides in fifteen species from genus *Cassia*, Chamaecrista and Senna. Ann. Phytomed., **10**(1):33-44.

Loguercio, C.; Piscopo, P.; Guerriero, C.; Girolamo, V.D.; Disalvo, D. and Del Vecchio Blanco C. (1996). Effect of alcohol abuse and glutathione administration on the circulating levels of glutathione and on antipyrine metabolism in patients with alcoholic liver cirrhosis. Scandinavian Journal of Clinical and Laboratory Investigation, **56**(5):441-447.

Luna, L.G. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd Edition, McGraw-Hill, New York.

Mahar, J.B.; Sharma, S.; Kaur, R.; Grover, K.; Dharni, K. and Nara, U. (2022). Ameliorative effect of lemongrass (*Cymbopogon flexuosus* Nees ex Steud.) W. Watson and celery (*Apium graveolens* L.) against CCl_4. Ann. Phytomed., **11**(1):351-358.

Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, **47**(3):469-474.

Nadeem Bari, M.; Rizwan Ansari, M. and Anwar, M. (2022). Bioactive compounds from medicinal plants in liver disease treatment : A review. Ann. Phytomed., **11**(1):215-220.

Oktay, M.; Gülçin, I.; and Kufrevioglu, O.I. (2003). Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT-Food Science and Technology, **36**(2):263-271.

Panda, S.K.; Padhi, L.P. and Mohanty, G.; (2011). Antibacterial activities and phytochemical analysis of *Cassia fistula* (Linn.) leaf. Journal of Advanced Pharmaceutical Technology and Research, **2**(1):62-67.

Patwardhan, S.; Vadnal, P.; Singhai, A.K. and Somani, R. (2009). Studies on hepatoprotective activity of ethanolic extract of *Cassia fistula* bark against CCl_4 induced hepatic damage in wistar rats. Pharmacology Online, **2**:50-63.

Pradeep, K.; Mohan, C.; Anand, K. and Karthikeyan, S. (2005). Effect of pretreatment of *Cassia fistula* Linn. leaf extract against subacute CCl_4 induced hepatotoxicity in rats. Indian Journal of Experimental Biology, **43**(6):526-530.

Pradeep, K.; Mohan, C.V.; Gobianand, K. and Karthikeyan, S. (2007). Effect of Cassia *fistula* Linn. leaf extract on diethylnitrosamine induced hepatic injury in rats. Chemico-Biological Interactions, **167**(1):8-12.

Pradeep, K.; Raj Mohan, C.V.; Gobianand, K. and Karthikeyan, S. (2010). Protective effect of *Cassia fistula* Linn. on diethylnitrosamine induced hepatocellular damage and oxidative stress in ethanol pretreated rats. Biological Research, **43**(1):113-125.

Rahmani, A.H. (2015). Cassia fistula Linn: Potential candidate in the health management. Pharmacognosy Research, **7**(3):217-224

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M. and Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, **26**(9-10):1231-1237.

Rodrigo, R.; Thielemann, L.; Olea, M.; Muñoz, P.; Cereceda, M. and Orellana, M. (2015). Effect of ethanol ingestion on renal regulation of water and electrolytes. Archives of Medical Research, **29**(3):209-218.

Sahoo, N.; Manchikanti, P. and Dey, S. (2010). Herbal drugs: Standards and regulation. Fitoterapia, **81**(6):462-471.

Siddhuraju, P. and Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. Journal of Agricultural and Food chemistry, **51**(8):2144-2145.

Silva, G.N.; Martins, F.R.; Matheus, M.E.; Leitao, S.G. and Fernandes, P.D. (2005). Investigation of anti-inflammatory and antinociceptive activities of *Lantana trifolia*. Journal of Ethnopharmacology, **100**(3):254-259.

Stocks, J. and Dormandy, T.L. (1971). The autoxidation of human red cell lipids induced by hydrogen peroxide. British Journal of Haematology, **20**(1):95-111.

Tzekiat, L.A. and Chiang, L.K. (2013). Total phenolics, total tannins and antioxidant activity of *Cassia fistula* L. extracts of bark, stem, leaf and root under different age classes. Asian Journal of Pharmaceutical Research and Health Care, **5**(2):52-57.

Villano, D.; Fernandez-Pachon, M.S.; Moya, M.L.; Troncoso, A.M. and García-Parrilla M.C. (2007). Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta, **71**(1):230-5.

Citation **Dinesh Kumar Sharma, Suresh Kumar Sharma, Rajendra Damu Patil and Milind Mitra Lonare (2024). Protective effects of different parts of *Cassia fistula* L. against ethanol-induced oxidative damage in rat liver and kidney. Ann. Phytomed., 13(1):539-547. http://dx.doi.org/10.54085/ap.2024.13.1.56.**