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Hepatoprotective Properties of methanolic extract of *Canscora decussata* (Schult) against paracetamol induced liver toxicity in rabbits

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ABSTRACT: Liver dysfunction is a major health problem .Excessive drug therapy, free radicals, environmental pollutants, and alcoholic intoxicants are the main causes of liver disorders. The present investigation was designed to evaluate its *in vivo* hepatoprotective properties of *Canscorra decussata* (family Gentianaceae) whole plant methanolic extract against paracetamol toxicity in rabbits. Hepatoprotective activities of methanolic extracts of *C. decussata* were examined against paracetamole induced liver damage in rabbits using silymarin as control. Enzyme activities of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), and Alkaline Phosphatase (ALP), bilirubin total and direct billiribin were analyzed. Oral administration of methanolic extract exhibited significant hepatoprotective activity.

KEYWORDS: *Canscorra decussata,* Silymarin, Hepatoprotective, Intoxicants, Free redicals.

1 BACKGROUND

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification of the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism.[1] Many traditional practitioners have claimed that numerous medicinal plants and their formulations can be effectively used for the alleviation of different types of liver diseases [2]. Plant-derived natural products have received considerable attention in recent years due to their diverse pharmacological actions including antioxidant and hepatoprotective activity [3],[4]. Diverse homeostatic mechanisms are affected if liver function is impaired, with potentially serious consequences. About 20, 000 deaths occur every year due to liver diseases. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2, 50,000 new cases each year. Although viruses are the main cause of liver diseases, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon. Liver disease is a worldwide problem; Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs .[5]Modern drugs have very little to offer for alleviation of hepatic ailments, whereas most important representatives of phytoconstituents used for liver diseases chiefly on regional basis include drugs like silymarine (*Silybum marianum*) and catechin (*Anacardium occidentalis*) in Europe, Glycyrrhizin (*Glycyrrhiza glarbra*) in Japan and chizandrins(*Schizandra chinesis*) in China.[6]

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2 MATERIALS AND METHODS

2.1 CHEMICALS

Methanol, Ethanol, Paracetamol, Formalin, Diagnostic kits (SGPT, SGOT, ALP, DB and TB), Xylene, Paraffin wax, Eosin, Hematoxylin and were purchased from Merck, Darmstadt, Germany. Silymarin and Pentothal sodium were acquired from Abbott Laboratories, Pakistan. Olive oil was purchased from the local market prepared by P. Sasso, Italy. All the chemicals were of analytical grade.

2.2 PLANT MATERIAL AND EXTRACTION PROCEDURE

The whole fresh plants were collected in sufficient amount from the Raiwind Lahore, Pakistan. These were identified and authenticated by the taxonomist of University of Sargodha, Sargodha. The plants were shade dried and powdered finaly with a Chinese herbal grinder. The powder was be stored in well closed cellophane bags in the refrigerator at 4 $^{\circ}$C. [7] The coarse powder (3000 g) of plant material was macerated in 9 L of methanol for approximately 3 days with frequent shaking. The extract was filtered and marc left behind. Extract was concentrated under reduced pressure on Rotary evaporator until a semisolid residue is obtained. Marc was further extracted under the same conditions twice. The semisolid residues, collected after extraction, were combined and evaporated to dryness by vacuum at temperature below 60 °C. At the end, a dark brown solid residue was obtained (yield; 20.7%). Silymarin (a standardized extract from *Silybum marianum*) was used as a reference hepatoprotective agent, which has been administered previously from 50-200 mg/kg in various animal studies. [8],[9].

2.3 ANIMALS

Healthy rabbits of either sex (local breed), weighing from 1.5-2 kg were purchased from local market. They were kept in the animal house of Faculty of Pharmacy, University of Sargodha. The animals were maintained at standard housing conditions and fed a standard pellet diet and water *ad libitum* [10] All procedures were performed as per approval of the Institutional Animal Ethics Committee.

2.4 PARACETAMOL INDUCED HEPATOTOXICITY

Thirty six healthy rabbits used in the study were randomly divided into six groups (n = 6 in each group). Group I (control) animals were administered with distilled water (1 ml/Kg) daily for seven days. Group II (paracetamol) received distilled water (1 ml/Kg) once daily for seven days and received paracetamol (2 g/kg) on day 7. Group III received standard drug silymarin (100 mg/kg) orally once daily for seven days. Test group animals (group IV-VI) were administered orally doses of 150, 300 and 500 mg/kg of aq MeOH extract, respectively, in the form of aq suspension, once daily for seven days. Groups III-VI was simultaneously administered paracetamol: distilled water (1:1, 2 g/kg) on day seven after 30 min of administration of silymarin and extracts. Animals were sacrificed 48 h after the last treatment. Blood was collected, allowed to clot and serum was separated by centrifugation at 2500 rpm at 37°C for 15 min and analyzed for various biochemical parameters [11].

2.5 BIOCHEMICAL DETERMINATIONS

The biochemical parameters like Serum Glutamate Oxaloacetate transaminase, Serum Glutamate Pyruvate Transaminase, alkaline phosphate total billirubin and direct bilirubin were assayed using standard assay kits [9] [12].

2.6 HISTOPATHOLOGICAL STUDIES

One animal from the treated group showing maximal activity as indicated by improved biochemical parameters from each group were utilized for this purpose. The animals were sacrificed and the abdomen was cut open to removal the liver. The livers were quickly preserved in neutral buffered formalin. Histological liver sections were prepared as described previously by Luna et al., 1968. 5 mm thick pieces of the liver were fixed in different concentrations of EtOH, then embedded in paraffin, and stained, using haematoxylin eosin dye and finally observed under microscope for histopathological changes in liver architecture and their photographs were taken to check the inflammation and necrosis of hepatocytes [13], [14].

2.7 DATA ANALYSIS

All data are expressed as means ± SD. ANOVA was used to evaluate the difference between multiple groups. P< 0.05 was considered to be significant.

3 RESULTS

3.1 PHYTOCHEMICAL ANALYSIS

Extracts subjected for phytochemical study showed the presence of flavonoids, alkaloids, carbohydrates, proteins, amino acids, phenolic compounds and cardiac glycosides [15]

Table 1. Effect of methanolic extract of C. decussata and Silymarin on biochemical parameters of the rabbits treated with paracetamol

S. No	Groups	ALT [IU/L]	AST [IU/L]	ALP [IU/L]	DB (g/dl)	TB (g/dl)
1.	Normal Control	61.05 ± 1.85	57.55 ± 2.49	138.31 ± 4.57	0.91 ± 0.03	0.18 ±0.07
2.	Paracetamol Control	160.43 ± 8.60	109.61 ± 5.12	363.50 ± 6.56	3.27 ± 1.11	1.23±0.21
3.	Silymarin + Paracetamol	46.45 ± 2.34**	67.85 ± 3.22**	121.70 ± 3.06**	0.53 ± 0.09**	0.33±0.07**
4.	Mehanolic extract (150 mg/kg) + Paracetamol	106.79 ± 4.94*	98.15 ± 4.61*	159.86 ± 2.59**	1.46 ± 0.11*	0.87±0.17**
5.	Mehanolic extract (300 mg/kg) + Paracetamol	93.42 ± 1.58**	90.79 ± 2.13*	192.41 ± 2.51**	1.08 ± 0.07**	0.69±0.11**
6	Mehanolic extract (500 mg/kg) + Paracetamol	79.11 ± 4.04**	72.37 ± 2.56**	164.35 ± 4.33**	0.84 ± 0.02**	0.36±0.09**

Where, DB= Direct bilirubin and TB= Total bilirubin

Data are presented as mean ±SEM (n=6). Means were compared using one-way analysis of variance (ANOVA). *p < 0.05, and **p < 0.01 vs. paracetamol control group.

3.2 BIOCHEMICAL OBSERVATIONS

In biochemical analysis tests were performed to assess hepatocellular membrane integrity and liver injury and the results were given in Table 1 and Fig 1. It is well known that carbon tetrachloride is used as a hepatotoxic agent on various animals [16] Administration of paracetamol to rabbits produced hepatotoxicity showed by significant increase in the serum levels of SGOT, SGPT, ALKP TB and D.Bil in comparison to control group. The aqueous methanolic extract of *C.decussata* showed a significant decrease in the serum enzymes when compared to the paracetamol compared control groups. Standard drug, silymarin (100 mg/kg) also exhibited similar results. The rabbits treated with methanolic extract of *C.decussata* showed (Table 1) significant reduction in all the biochemical parameters elevated by paracetamol. This reduction in biochemical parameters is similar to silymarin. There was also a significant dose-dependent difference in the activities of the high-dose and the low-dose groups.The elevations in liver function parameters as found in this work are not such that should discourage or indicates its non-hepatotoxic effect.

3.3 HISTOPATHOLOGICAL OBSERVATIONS

The histopathological profile of the rabbits treated with aq methanolic extract showed no visible changes, confirming the safety of the extract at selected dose (Figure 1). The control group (group I) also showed normal cellular architecture with well brought out central vein, well presented cytoplasm and prominent nucleus (Figure 2). The liver sections of paracetamol treated animals showed hepatic cells with severe toxicity characterized by disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis (Figures 3). Rabbits treated with silymarin and intoxicated with paracetamol showed less disarrangement and degeneration of hepatocytes, indicating marked regeneration activity.

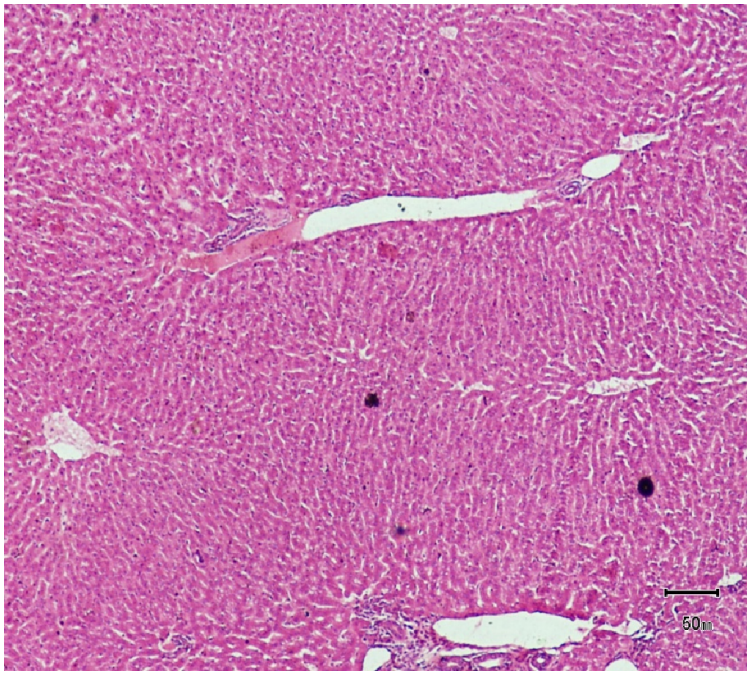

Figure 1. Liver of rabbit treated with aq methanolic extract of C.decussata.

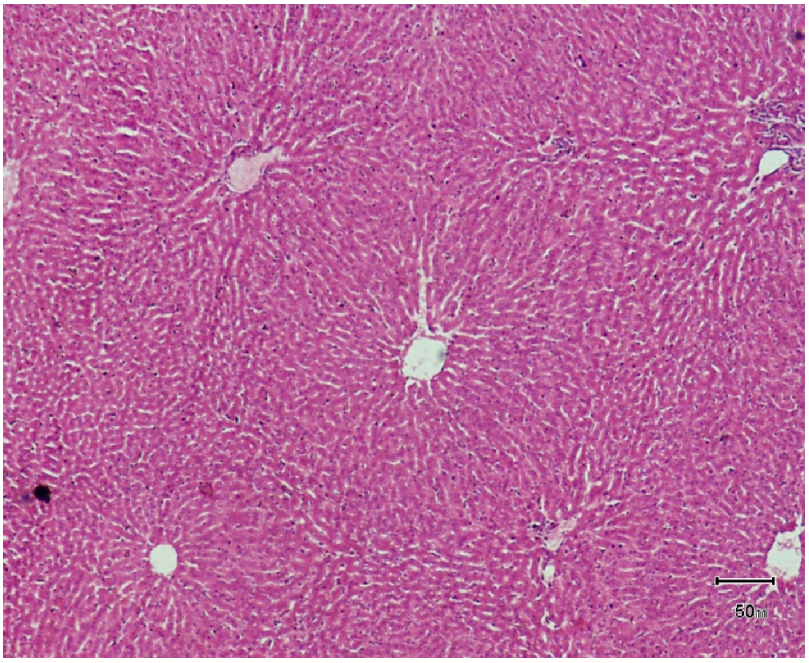

Figure 2. Liver of rabbit treated with normal diet

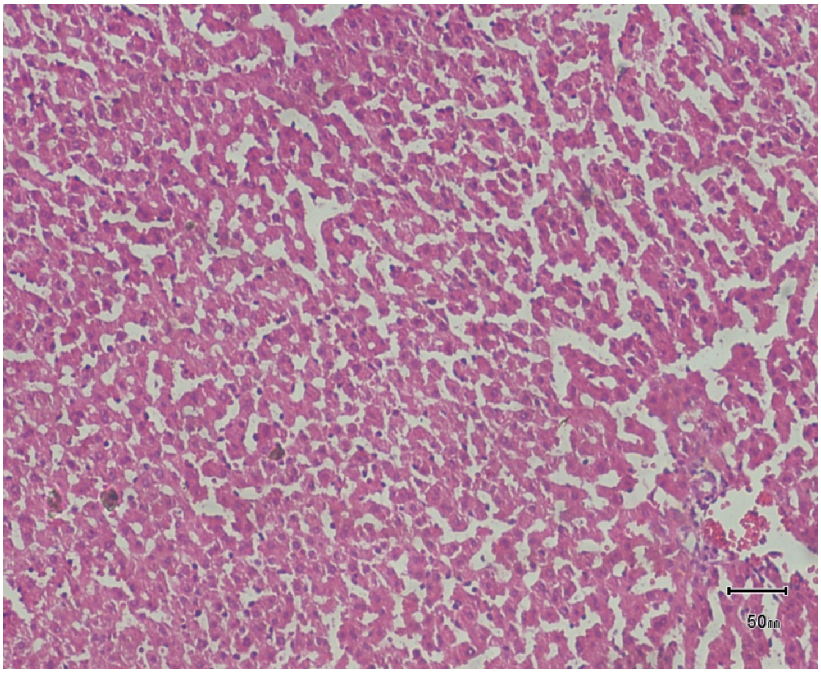

Figure 3. Liver of rabbit treated with paracetamol.

4 DISCUSSION

Liver injury can be caused by toxic chemicals, drugs, and viral infiltration from infection by ingestion. Paracetamol an analgesic and antipyretic is assumed be safe in low doses [17] Poisoning due to paracetamol intoxication is very common worldwide. In the United Kingdom and the United States it is the most widespread cause of acute liver injury [18]. Small doses are eliminated by conjugation followed by excretion but increased doses produce liver necrosis [19]. Liver injury induced by paracetamol is the best characterized system of xenobiotics induced hepatotoxicity in human being. Paracetamol intoxication in normal rabbits elevated the levels of SGPT, SGOT, ALKP T.Bllirubin and D.Billirubin significantly, indicating acute centrilobular necrosis. The rabbits treated with methanolic extract of *C.decussta* showed (Tables 1) significant reduction in all the biochemical parameters elevated by paracetamol. This reduction in biochemical parameters is similar when compared with that of silymarin. Since the phytochemical analysis of the extracts had shown the presence of flavonoids and phenolic compounds, which had been known for their antioxidant and hepatoprotective activities [20],thus it can be concluded that possible mechanism of hepatoprotective activity may be due to the presence of flavonoids and phenolic compounds in the extracts. More work needs to be conducted to elucidate viable potentials of *C.decussata*.

5 CONCLUSION

Methanolic extract of the *C.decussata* offers protective effect against paracetamol induced hepatotoxicity in experimental rabbits. The mechanism of action is yet to be investigated but may be due to the antioxidant effects of flavonoids found to be present in the methanolic extract.

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REFERENCES

[1] Bhardwaj A, P Khatri. Soni P M.L, Ali D.J. Potential herbal hepatoproteci. *J.Adv.Sci.Res.* ve sdrug- a reviw, 2(2), 15-20. 2011.

[2] Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam D, Sengupta P, Maiti BC, Maity TK. Evaluation of hepatoprotective and antioxidant activity of Ichnocarpus frutescens (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. Trop J Pharm Res, 6(3), 755-765. 2007.

[3] Wang BJ, Liu CT, Tseng CY, Wu CP, Yu ZR. Hepatoprotective and antioxidant effects of *Bupleurum Kaoi* Liu. (Chaoet Chuang) extract and its fractions fractionated using supercortical CO2 on CCl4 induced liver damage. *Food Chem Toxicol,* 42, 609-17. 2004.

[4] DeFeudis FV, Papadopoulos V, Drieu K. *Ginkgo biloba* extracts and cancer: a research area in its infancy. *Fundam Clin Pharmacol*, 17, 405-17. 2003.

[5] Chattopadhyay RR, Bhattacharyya SK. *Terminalia chebula*: An update, Pharmacog, 1(1), 439–45. 2007.

[6] Hikino H, Kiso Y. Natural products for liver diseases. Economic and Medicinal Plant Research. Vol. II, Academic Press, London p, 39-67. 1988.

[7] Akhtar MS, Amin M, Maqsood M and Alamgeer.*Hepatoprotective* effect of *Rheum* emodi roots (Revand chini) and Akseer-e-Jigar against paracetamol-induced hepatotoxicity in rats. Ethnobotanical Leaflets. 13, 310-315. 2009.

[8] Arshad AN, Kausar M and Savita DP. Hepatoprotective effect of Cocculus hirsutus linn. against carbon tetrachoride induced liver damage in albino wistar rats. *IJPI's J Pharmacol and Toxicol*, **1**(1): 1-7. 2010.

[9] Eminzade S, Uras F and Izzettin FV.Silymarinprotects liver against toxic effects of anti-tuberculosis drugs in experimental animals. *Nutr Metab* (Lond), **5**, 18-25. 2008.

[10] National Institute of health.Guide for the care and use of laboratory animals. NIH contact no. NOI-RR-2-2135. NIH, Bethesda, MD, 11-28. 1985.

[11] Prochezian E, Ansari SH.Hepatoprotective activity of Abutilon indicum on experimental liver damage in rats. Phytomed, 12, 62-64. 2005.

[12] Kind PRN, King EJ.1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Pathol, 7, 322-326. 2005.

[13] Luna LG. Manual of Histology, Staining Methods of Armed Forces, Institute of Pathology, 3rd ed., McGraw Hill Book Co., New York, 43. 1968.

[14] Yasmin S, Kashmiri M A and Anwar K. Screening of aerial parts of *Abutilon bidentatum* forhepatoprotective activity in rabbits. *J of Medicinal Plants Research* 5(3), 349-353. 2011.

[15] Khandelwal KR. Practical Pharmacognosy, XIV ed. Nirali Prakashan, Pune, pp, 149-153.

[16] Recknagel RO. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci, 33, 401-408. 1983.

[17] Recknagel RO, Glende EA, Jr Dolak JA, Waller RL. Mechanism of carbon tetrachloride toxicity. Pharmacol. Ther, 43, 139-154. 1989.

[18] Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, Reisch JS, Schiødt FV, Ostapowicz G, Shakil AO and Lee WM. Acute Liver Failure Study Group. Acetaminophen- induced acute liver failure: results of a United States multicenter, prospective study. Hepatology Baltimore, Md, 42 (6), 1364–1372. 2005.

[19] Dahlin DC, Miwa GT, Lu AY, Nelson SD. N-acetyl-pbenzoquinone imine: a cytochrome P- 450 mediated oxidation product of acetaminophen. Proc. Natl. Acad. Sci, 81: 1327-1331. 1984.

[20] Di Carlo G, Mascolo N, Izzo AA, Capasso F.Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci, 65, 337-353. 1999.