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Hepatoprotective Activity of Methanol Extract of Some Medicinal Plants Against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats

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Abstract: The methanol extracts of plant materials of some plants like *Casuarina equisetifolia*, *Cajanus cajan*, *Glycosmis pentaphylla*, *Bixa orellana*, *Argemone mexicana*, *Physalis minima*, *Caesalpinia bonduc*, belonging to the different family were studied for hepatoprotective activity against Swiss albino rats with liver damage induced by carbon tetrachloride(CCl_4). It was found that the methanol extract of *B. orellana*, *C. cajan*, *G.pentaphylla* and *C. equisetifolia* at a dose of 500 mg/kg body weight exhibited moderate protective effect by lowering the serum levels of alanine aminotransferase(ALT) or Serum Glutamate Pyruvate Transaminase (SGPT), aspartate aminotransferase (AST)or Serum Glutamate Oxaloacetate Transaminase (SGOT) and cholesterol to a significant extent. Other methanol extracts of *A. mexicana*, *P. minima* and *C. bonduc* had no effect of lowering blood serum level rather than produced toxicity at the above specified dose. The highest activity of observed for methanol extract of *B. orellana* at a dose of 500 mg/kg body weight (b.wt.) and the reduction of serum level of ALT, AST and cholesterol were 52.08%, 57.37% and 52.90% respectively. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Since results of biochemical studies of blood samples of carbon tetrachloride treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by CCl_4 and blood samples from the animals treated with the methanol extracts of *B. orellana*, *C. cajan*, *G.pentaphylla* and *C. equisetifolia* showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells, the extracts of four above plants could afford significant dose-dependent protection against CCl_4 induced hepatocellular injury.

Key words: *Bixa orellana* · Hepatoprotective activity · Carbon tetrachloride

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. The major functions of the liver are carbohydrare, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailment like hepatitis, cirrhosis and alcoholic liver disease [2-3]. Thus liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drug available for the treatment of liver disorders [4-5] Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl_4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts [6-7].

The plant *Casuarina equisetifolia* Forst (locally known as Jhau gachh, Hari) belongs to the family Casuarinaceae. Extracts of leaves exhibit anticancer properties [8]. Bark is astringent and in stomacheache,

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diarrhea, dysentery and nervous disorders [9]. Seeds are anthelmintic, antispasmodic and antidiabetic [10]. Leaf juice of *Cajanus cajan* (Linn.) Huth (local name Arhar) belonging the family Papilionaceae, is useful in jaundice and disease of the mouth. Infusion of leaves of *Glycosmis pentaphylla* Corr. (vernicular name: tooth-brush plant and Family: Rutaceae) is used in fever, liver complaints, cough and jaundice [11]. The plant *Bixa orellana* Linn. of which local name is lotkan, shidhur, belongs to the family Bixaceae. Seeds are used in fever, apetising agent and stimulant. Extracts of the plant *Argemone mexicana* Linn., locally named as prickly poppy (English), belonging to the family Papaveraceae possesses tonic, anthelmintic, diuretic and hypnotic properties. Latex and extract of plants are used in jaundice, tumors, cancers and eye diseases [12-13]. Alkaloid of the plant *Physalis minima* Linn. (local name, Tepari or Patka in Bengali) belonging to the family Solanaceae may have potential use for leukemia chemotherapy [14]. Leaves and fruits are tonic, diuretic and purgative and used in gonorrhoea and spleen disorders [10, 15]. *Caesalpinia bonduc* (Linn.) Roxb. locally named as Natakaranja in Bengali belonging to Caesalpiniaceae family is used to treat fevers and roasted seeds are used to treat diabetes [10]. Powder of this plant is an effective in blood dysentery [16]. Thus the objective of the present study was designed to test the hepatoprotective activity of the methanol extracts of plant material of above specified plants against carbon tetrachloride induced liver damage in rats.

MATERIALS AND METHODS

Plant Materials and Preparation of Extracts: Fresh plant materials (Table 1) of *Casuarina equisetifolia, Cajanus cajan, Glycosmis pentaphylla, Bixa orellana, Argemone mexicana, Physalis minima* and *Caesalpinia bonduc* were collected from Botanical Garden of Dept. of Pharmacy, Rajshahi University, Rajshahi, Bangladesh. Their botanical identities were determined and authenticated by Dr. Ashik Mossaddik, Associate Professor, dept. of Pharmacy, Rajshahi University, Rajshahi-6205, Bangladesh. Some voucher specimen numbers were submitted to the authority for future references. The plant materials were washed with water, cut into pieces, sun dried for 5 days and then dried in an oven below 60°C. The dried plant materials were then pulverized into coarse powder in a grinding machine. 10 gm of each (7 samples) plant sample was extracted separately in cold methanol. Solvent from each sample was filtered, squeezed off and evaporated off under reduced pressure in a rotary evaporator to obtain crude extract.

Table 1: Plant names and used parts with their percent of yield

Scientific Name	Used Part	Percent Yield
Casuarina equisetifolia	Leaf and bark	15
Cajanus cajan	Whole Plant	11
Glycosmis pentaphylla,	Leaf and bark	14
Bixa orellana	Seed	41
Physalis minima	Whole Plant	12
Argemone mexicana	Leaf and Flower	18
Caesalpinia bonduc	Leaf and bark	12

Animals: Swiss albino rats weighing between 80 and 90 gm were used in this evaluation. These rats aged between 2 and 2.5 months were procured from animal house located at Rajshahi city, Bangladesh. They were housed in well ventilated stainless-steel cages at room temperature (24±2°C) in hygienic condition under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given *ad libitum*.

Experimental Design for Hepatoprotective Activity: The rats were divided randomly into nine groups of six rats each. The hepatoprotective activity of the plant extracts was tested using CCl_4 model. Group I (normal control) received neither the plant extract nor Ccl_4 for 72 hours that is they receive only food and water only; Group II (induction control) was given a single intraperitoneal dose (3ml/kg) [17]. Group III-VI was subdivided into further two subgroups such as Group 3A, Grpup B and so on. Group IIIA, Gr. IVA, Gr. VA, Gr. VIA received crude MeOH-extract of plant materials of *Casuriana equisetifolia*, *Cajanus cajan*, *Glycosmis pentaphylla*, *Bixa orellana*, *Argemone mexicana*, *Physalis minima*, *Caesalpinia bonduc* respectively at an intraperitoneal dose of 500mg/kg b. wt as a fine suspension made by adding sorbitol and a single dose of CCl_4. But each B subgroups of the same groups received the extract suspension at an intraperitoneal dose of 250mg/kg b. wt and a single dose of CCl_4. Remaining three groups Gr. VII to Gr. IX received only an intraperitoneal dose of 500mg/kg b. wt and a single dose of CCl_4. The suspensions of test samples were administered to rats 1hr, 24 hrs and 48 hrs after CCl_4 injection.

Assessment of Hepatoprotective Activity: In the present study the hepatoprotective activity was evaluated biochemically and histopathologically. After 72 hours of

drug treatment, the animals were dissected under ether anesthesia. Blood from each rat was withdrawn from carotid artery at the neck and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 minurtes. The separated serum were used for the estimation of some biochemical parameters like Alanine aminotransferase(ALT/SGPT), Aspartate aminotransferase (AST/SGOT), cholesterol, bilirubin and glucose. The substrate and the buffer solution used in the measurement of serum levels were supplied by Randox, UK. The present research had used the chemical analyzer apparatus, named RA-50, chemical analyzer, manufactured by Technocon, United kingdom which was an automatic machine to measure the amount of the required enzymes. For histopathological study, liver from each animal was removed after dissection and preserved in 10% formalin. Then representative blocks of liver tissues from each lobe were taken and possessed for paraffin embedding using the standard microtechnique [18]. Sections (5 μm) of livers stained with hemotoxylin and eosin, were observed microscopically for histopathological studies.

RESULTS

The present study had been attempted to demonstrate the role of hepatoproctective activity of crude methanol extracts of plant materials of *Casuarina equisetifolia*, *Cajanus cajan*, *Glycosmis pentaphylla*, *Bixa orellana*, *Argemone mexicana*, *Physalis minima*, *Caesalpinia bonduc*, belonging to the different family in carbon tetrachloride induced hepatotoxicity at different doses. The results of hepatoprotective activities of crude methanol extracts of these plants at a dose of 250 mg/kg b.wt and 500mg/kg b.wt. on rats intoxicated with carbon tetrachloride were illustrated in the Table 2. The table also showed the comparison of effects among the

Table 2: Effects of methanol extract of plant materials of Casuarina equisetifolia, Cajanus cajan, Glycosmis pentaphylla, Bixa orellana, Argemone mexicana, Physalis minima and Caesalpinia bonduc on various biochemical parameters in rats with carbon tetrachloride induced hepatotoxicity

Gr.	Treat-ment	SGPT Level (U/L)	SGOT Level (U/L)	Bilirubin (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)
I	Control	17.11 ± 0.57	30.81± 0.62	0.77 ± 0.35	108.66 ± 1.11	92.33 ± 3.48
II	CCl4	35.50 ± 1.17 (107.5% △)	70.40± 0.61 (128.49 %△)	1.04 ± 0.01 (35.06%△)	229.67 ± 0.75 -111.30%	90.66 ± 2.45 (1.80%▼)
III A	CCl4 + 500 CE	28.16 ± 0.94 (20.67%△)	51.68 ± 0.59 (26.59%△)	0.77 ± 0.04 (25.96%▼)	174.75±1.17 (23.91%▼)	93.5±2.60 -3.13%
III B	CCl4 + 250 CE	24.33±0.76 (31.46%▼)	57.29± 1.31 (18.62%▼)	0.85 ± 0.02 (18.26%▼)	167.29± 0.73 (27.16%▼)	89.08 ± 1.20 (1.74%▼)
IV A	CCl4 + 500 CC	17.67 ± 1.64 (50.22%▼)	30.60 ± 1.38 (56.53%▼)	0.78 ±0.03 (25.0%▼)	127.33±0.71 (44.55%▼)	93.50±2.99 (3.13%▼)
IV B	CCl4 + 250 CC	18.75±0.77 (47.18%▼)	58.00±1.39 (17.61%▼)	0.87±0.01 (16.34%▼)	115.50±5.79 (49.715%▼)	92.50±2.09 (2.04%▼)
V A	CCl4 + 500 GP	22.34±0.88 (37.07%▼)	42.91±1.51 (39.04%▼)	0.82±0.01 (21.15%▼)	143.60±0.95 (37.47%▼)	89.75±1.61 (1.00%▼)
V B	CCl4 + 250 GP	25.00±0.85 (29.57%▼)	42.67±5.56 (39.38%▼)	0.82±0.00 (21.15%▼)	118.66±2.15 (48.33%▼)	90.83±1.95 (0.18%▼)
VI A	CCl4 + 500 BO	17.01±0.49 (52.08%▼)	30.01±1.15 (57.37%▼)	0.82±0.01 (21.15%▼)	108.17±4.18 (52.90%▼)	92.66±0.98 (2.20%▼)
VI B	CCl4 + 250 BO	27.33±0.49 (23.01%▼)	47.08v±1.24 (33.12%▼)	0.82±0.01 (21.15%▼)	141.66±1.94 (38.32%▼)	89.16±1.22 (1.65%▼)
VII	CCl4 + 500 AM	63.68±0.557 (79.77%△)	73.50±1.176 (4.40%△)	ns	ns	ns
VIII	CCl4 + 500 PM	63.33±0.76 (84.02%△)	71.83±1.70 (2.03%△)	ns	ns	ns
IX	CCl4 + 500 CB	61.83± 0.70 (74.16%△)	71.00±1.317 (0.85%△)	ns	ns	ns

△=Increase in mean serum level;Control= normal food and water only; Gr.= Group; ns =Not significant
▼= decrease in mean serum level
CCl_4 = Single dose of CCl_4, 3mg/kg b.wt.
500 CE = thrice dose of 500 mg/kg b.wt of methanol extract of *Casuarina equisetifolia*
250 CE = thrice dose of 250 mg/kg b.wt of methanol extract of *Casuarina equisetifolia*
500 CC = thrice dose of 500 mg/kg b.wt of methanol extract of *Cajanus cajan*
250 CC = thrice dose of 250 mg/kg b.wt of methanol extract of *Cajanus cajan*
500 GP = thrice dose of 500 mg/kg b.wt of methanol extract of *Glycosmis pentaphylla*
250GP = thrice dose of 250 mg/kg b.wt of methanol extract of *Glycosmis pentaphylla*
500 BO = thrice dose of 500 mg/kg b.wt of methanol extract of *Bixa orellana*
250 BO = thrice dose of 500 mg/kg b.wt of methanol extract of *Bixa orellana*
500 AM = thrice dose of 500 mg/kg b.wt of methanol extract of *Argemone mexicana*
500 PM = thrice dose of 500 mg/kg b.wt of methanol extract of *Physalis minima*
500 CB = thrice dose of 500 mg/kg b.wt of methanol extract of *Caesalpinia bonduc*

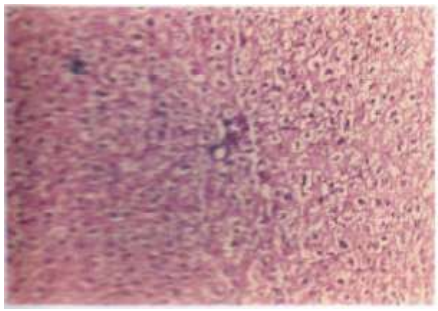

Fig. 1: Micros view of liver tissue of normal rat

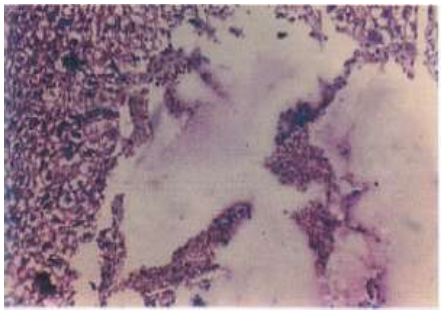

Fig. 2: Micros view of liver tissue of CCl_4 induced rat

untreated (normal control) and carbon tetrachloride treated (induction control or standard) group with the drug treated group of rats. The results were represented as Mean± Standard Error of Mean (M±SEM). The statistical significance was computed using student's't' test and Graph Pad Prism statistical program. Carbon tetrachloride group significantly increased the serum level of SGPT (107.50%), SGOT (128.49%), Bilirubin (35.06%) and cholesterol (111.30%) shown in Table 2. The't' value obtained for exceeded the limit for significance. That is in all cases,'p' was less than 0.0001. The plant extracts of *Argemone mexicana*, *Physalis minima*, *Caesalpinia bonduc*, at a dose of 500 mg/kg b.wt. showed very insignificant changes rather than produced toxicity compared to normal group. That is percent of increase of SGPT and SGOT for *Argemone mexicana* were 79.77% and 4.4% respectively; for *Physalis minima*, 84.02% and 2.03% respectively; for *Caesalpinia boduc* 74.16% and 0.85% respectively. So, no more study was done with these three extract for heptoprotective screening. Another four plant extract namely, *Casuarina equisetifolia*, *Cajanus cajan*, *Glycosmis pentaphylla*, *Bixa orellana* exhibited significant protection against CCl_4-induced liver injury as manifested by the reduction in toxin mediated rise in SGPT, SGOT and cholesterol level of rats. The methanol extract of *Bixa orellana* showed highest percent of recovery of SGPT, (52.08%) followed by *Cajanaus cajan* (50.22%), *Glycosmis pentaphylla* (37.07%) and *Casuarina equisetifolia* (20.67%) compared to CCl_4 treated grops at a dose of 500 mg/kg b.wt. Interestingly, at a dose of 250mg/kg *Cajanus cajan* extract showed the highest percent of recovery of SGPT (47.18%) than *Bixa orellana* (23.01%). In case of SGOT, among the four plants extracts, *Bixa orellana* extract exhibited the highest percent of recovery at both 250 mg/kg b.wt and 500 mg/kg b.wt. doses. On the other hand, *Casuarina equisetefolia* showed the lowest percent of recovery in SGOT at 500 mg/kg b.wt compared to CCl_4 treated group ($P<0.0001$). At 250mg/kg b.wt. dose *Cajanus cajan* extract showed highest percent of reduction in the cholesterol level (49.71%) followed by *Glycosmis pentaphylla* (48.33%), *Bixa orellana* (38.32%) and *Casuarina equisetifolia* (21.16%) compared to CCl_4 treated rats. But at 500 mg/kg b. wt. dose *Bixa orellana* extract showed highest percent of recovery in cholesterol level among the four tested extracts. Such results indicate that the activity of *Bixa orellana* extract may be dose specific and more work is needed to know the mechanism of action of its anti-cholesterol effect. One important parameter, bilirubin level was not found significant changes in all cases because its alteration depends on three to four weeks experiment. It should be noted that in all cases CCl_4 and plant extracts could not significantly changes the blood glucose level of rats (Table 2).

Results of histopathological studies provided supportive evidence for biochemical analysis. Histology of liver section of normal control animal (group 1) exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus and nucleolus and well brought out central vein (Figure 1), whereas that of Ccl_4 intoxicated group animal showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids, kupffer cell hyperplasia, crowding of central vein and apoptosis (Figure 2). Treatment with methanol extract of four plants, *Casuarina equisetifolia*, *Cajanus cajan*, *Glycosmis pentaphylla*, *Bixa orellana* at a dose of 500 mg/kg b.wt. showed moderate to weak activity in protecting the liver cells from CCl_4-injury (Figure 3 to 6). Among these plant extract, treatment with *Bixa orellana* extract returned the injured liver to quite normal. Now, it could be decided that the hepatoprotective activity was dose and time dependent. Out of four plant extracts, the crude methanol extract of *Bixa orellana* had shown very potential heptoprotective activity at a dose of 500 mg/kg b.wt.

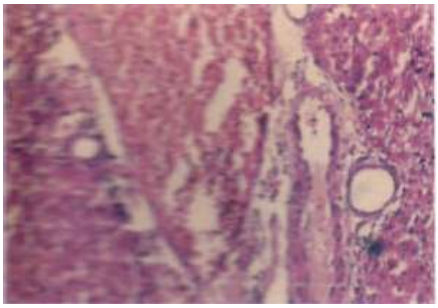

Fig. 3: Micros view of liver tissue of methanol extract of *Cajanus cajan*

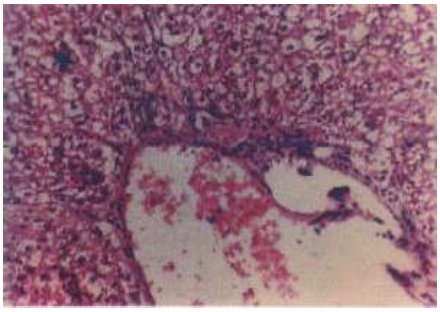

Fig. 4: Micros view of liver tissue of methanol extract of *Glycosmis pentaphylla*

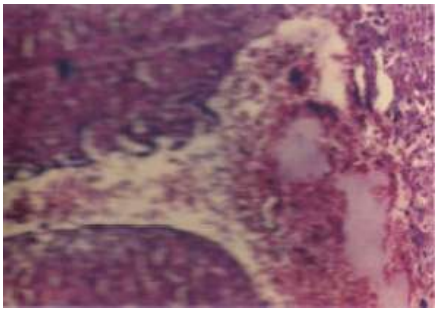

Fig. 5: Micros view of liver tissue of methanol extract of *C. equisetifolia*

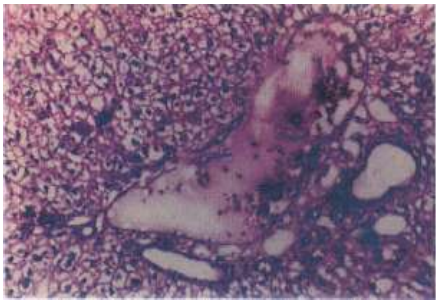

Fig. 6: Micros view of liver tissue of methanol extract of *Bixa orellana*

DISCUSSION

Literature review revealed that various chemical and biological investigations were carried out with these plants. A protein fraction (CI-1) isolated from *Cajanus cajan* leaf extract reduced the serum level of SGPT, SGOT compared to the CCl_4 treated animal at a dose of 50-60 µg/ml for a period of 7, 14 or 21 days treatment [19]. Again the extracts of leaf and stem bark of *Glycosmis pentaphylla* showed a good hepatoprotection at a dose of 750 mg/kg b.wt. in CCl_4 induced rat [20]. A water extract of the root and seed of *Bixa orellana* had been demonstrated hypotensive acitivity in rat. The same extract demonstrated smooth muscle-relaxant activity in guinea pigs and lowered gastric secretions in rats which explain its usage as a digestive aid and for stomach disorder [21]. *Bixa orellana* seed extracts had been documented to raise blood glucose level in some species of animals [22] and leaves were reported to possess aldose reductase inhibitory actions, a process implicated in the advancement of diabetic neuropathy [23]. But in the present study the elevation blood glucose level was not significant by the treatment with *B. orellana* seed extract. Literature review also revealed that no research work have been done on hepatoprotective investigation of seed methanol extract of *Bixa orellana*. But the present histopathological and biochemical analysis of said extract showed a good development in the carbon tetrachloride-damaged liver cells (Figure 6).

Liver damage induced by CCl_4 is commonly used model for the screening of hepatoprotective drugs [24]. The rise in serum levels of AST, ALT and cholesterol has been attributed to the damaged structureal integrity of he liver, because they are cytoplasmic in location and released into circulation after celluar damages [25]. When rats were treated with carbon tetrachloride it induces hepatotoxicity by metabolic activation, therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P-450 dependent mixed oxidease in the endoplasmic reticulum to form trichloromethyl free radical (CCl_3) which combined with cellular lipids and proteins in the presence of oxygen to induce lipid per-oxidation [26-28]. These result in changes of structures of the endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver injury [29-32]. Treatment with *Bixa orellana* methanol seed extract recovered the injured liver to normal after

72 hrs at a dose of 500 mg/kg b.wt. which indicate that *Bixa orellana* has antihepatotoxic effect. In addition, the possible antihepatotoxic mechanism of *Bixa orellana* have not been reported yet. It is assumed that the effect of *Bixa orellana* extract on liver protection is related to glutathione-mediated detoxification as well as free radical suppressing activity. In conclusion, from the overall result of the biochemical and histopathological examinations, it could be inferred that *Bixa orellana* showed the highest hepatoprotective activity among the four tested plant extracts. The result could also be expressed in the order of *Bixa orellana> Cajanus cajan > Glycosmis pentaphylla > Casuarina equisetifolia*. Further study on the plants could be extended for the isolation and structure determination of the hepatoprotective principle or principles.

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