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Research Article

Hepatoprotective Activity of Some Indigenous Plants of Northern India Against Chronic Paracetamol Intoxication in Rats

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ABSTRACT
Liver diseases have become a global concern worldwide. Liver injury or its dysfunction is a major health problem. The principal causative factors for liver injury are the availability of hepatotoxic drugs, alcohol consumption, infection, malnutrition, anemia etc. In the present study the hydroalcholic extract of some plants of northern India was evaluated for hepatoprotective activity against chronic paracetamol induced hepatotoxicity in rats. The liver injury was induced by Paracetamol orally at a dose of 500 mg/kg, b.w for entire duration of study. The rats were divided in nine groups. Rats of group I served normal control and received distilled water. Rats of group II served as toxic control and received Paracetamol orally at a dose of 500 mg/kg, b.w of rats. Group III received Silymarin at a dose of 100 mg/kg b.w, and served as standard. The animals of groups IV, V, VI, VII, VIII and IX served as test control groups. Protective effect of the hydroalcholic extract was assessed by measuring the levels of serum biomarkers such as SGOT, SGPT, ALP, Bilirubin, serum albumin and total protein. Results of this study showed that the treatment of the hydroalcholic extract of plants at 400 mg/kg b.w, showed significant (*** $P< 0.001$) reduction in elevated serum enzyme levels compared to paracetamol induced toxic group, indicating the protective role of plants extract against paracetamol induced chronic liver toxicity. Among the all plants *Calotropis procera* at a dose of 400 mg/kg, b.w showed the most significant hepatoprotective activity.

Keywords: Hepatoprotective activity, Liver, Hepatotoxicity, Serum Enzymes, Silymarin, plants of Northern India.

INTRODUCTION

Liver is the largest and vital organ in the vertebrate body and the site for intense metabolism[1]. It is the key organ for maintenance of homeostasis, detoxification of toxic substances and disposition of endogenous substances like xenobiotics, drugs etc, most importantly, the liver is considered to be the center of metabolic transformation of drugs and other toxins entering from the gastrointestinal tract[2]. Chronic liver diseases stand as one of the foremost health troubles worldwide. Liver disease is one of the major causes of death. According to the National Center for Health Statistics (NCHS) and the Centers for Disease Control and Prevention (CDC), chronic liver disease and cirrhosis is the 12th leading cause of death, claiming 30,000 lives annually in the United States[3].

Mechanism of Hepatic Injury by Paracetamol

Paracetamol is a widely used analgesic and antipyretic drug which produces acute or chronic liver damage if overdoses are consumed. It is believed that selective inhibition of the enzyme Cox-3 in the brain and spinal cord explains the effectiveness of Paracetamol in relieving pain and reducing fever. However its mechanism of action is not fully understood, but it is generally accepted that Paracetamol is a centrally acting drug. Paracetamol (acetaminophen) is mainly metabolized in liver to excretable glucuronide and sulphate conjugates[4,5]. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450[6] to a highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI)[7]. NAPQI is initially detoxified by conjugation with reduced glutathion (GSH) to form mercapturic acid[8]. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH. Liver damage is detected initially by elevated levels of biochemical parameters like SGOT (Serum glutamic oxaloacetatic transaminase), SGPT (Serum glutamic pyruvic transaminase), ALP (Alkaline phosphatase) and total bilirubin levels. Bilirubin may be elevated in hepatitis or cholestasis. In chronic liver disease a rising bilirubin may indicate a deteriorating liver function[9].

MATERIALS AND METHODS

Chemicals

Silymarin was obtained from local medical store (Silybon-140, Micro Labs Ltd, H.P. India). Paracetamol (API) was obtained from Cipla Ltd, Vill Juddikalan, Baddi, H.P. The

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Table 1: Effect of hydroalcholic leaves extract of some plants of Northern India against Paracetamol toxicity on Biochemical parameters.

Parameters	Normal Control	Toxic Control	Silymarin (100 mg/kg)	*Prunus persica* Extract (200 mg/kg)	*Prunus persica* Extract (400 mg/kg)	*Calotropis procera* Extract (200 mg/kg)	*Calotropis procera* Extract (400 mg/kg)	*Canscora decussate* Extract (200 mg/kg)	*Canscora decussate* Extract (400 mg/kg)
SGOT (IU/L)	88.36±2.23	310.98±5.43##	101.08±9.82*	237.5±7.33**	196.3±6.62**	227.69±3.42**	131.9±2.75**	241.4±5.47**	198.4±5.43**
SGPT (IU/L)	60.33±7.98	208.83±4.50##	76.33±3.25*	169.6±4.01**	95.63±2.20**	146.7±4.14**	82.73±5.20**	176.6±3.14**	96.43±2.54**
SALP (IU/L)	137.73±4.61	329.14±7.52##	141.18±4.16**	191.6±5.19**	153.76±2.98**	188.36±6.28**	147.47±3.66**	202.37±4.56**	157.56±5.56**
Sr. Bilirubin (mg/dl)	0.66±0.01	3.15±0.03##	0.85±0.02*	1.79±0.04**	1.01±0.09**	1.07±0.03**	0.93±0.02**	1.86±0.09**	1.10±0.06**
Sr. Albumin (g/dl)	5.78±0.07	1.08±0.03##	4.88±0.19**	3.45±0.06**	4.23±0.08*	3.96±0.05**	4.78±0.06*	3.37±0.15**	4.12±0.05*
Sr. Total Protein (g/dl)	8.13±0.28	3.55±0.23##	7.33±0.16**	5.15±0.24**	6.44±0.06**	6.28±0.32**	7.24±0.33***	5.01±0.02**	5.94±0.03**

All values represent Mean ± S.E.M. of n=6/group; ## p<0.01 when compared with normal control & *p<0.05, **p<0.01 as compared with toxic control group.

kits for biochemical estimation were purchased from Merk Ltd, Kalyan Badlapur Road, Ambernath (INDIA). The solvents and other chemicals were obtained from local dealers.

Plants Collection and Authentication

The leaves of *Prunus persica* and *Calotropis procera* were collected from the garden of Aman Vihar Colony, Krishna Nagar, Roorkee Road Meerut and leaves of *Canscora decussate* were purchased from Khari Baoli street, Chandni chowk, New Delhi in the month of July and then authenticated by Dr. R.S. Saxena, Reader & Head Botany Department, Meerut College, Meerut (U.P.) 250001, deposited to T.I.P.E.R. for future reference.

Preparation of Leaves Extract

The coarsely powdered leaves (500 g) were extracted with soxhlet apparatus using petroleum ether for about 24 hrs. After defatting, the marc was dried in hot air oven at 50^0C, packed in soxhlet apparatus, and further extracted with 1 L of 95% (V/V) ethanol and water mixture by percolation method. The solvent were removed from the extracts under reduced pressure by using rotary vacuum evaporator[10].

Phytochemical Screening

The hydroalcholic extract of plants were screened for the presence of various phytoconstituents by adopting standard procedures[11,12,13].

Animals

After prior approval from the Institutional Animal Ethical Committee (IAEC approval No. IAEC/Ph-16/TIPER/057), the rats were obtained from animal house facility of T.I.P.E.R. for evaluation of hepatoprotective activity of *Calotropis procera*, *Canscora decussate* & *Prunus persica* against paracetamol induced hepatic injury in rats.

The healthy albino rats of Wistar strain of either sex weighing about 170-200 gm were kept at temperature 23±2 ºC and humidity (50-55 %) with 12 hrs light and dark cycles. They were caged with a maximum of three animals in each polypropylene cage and were fed with standard pellet diet and water *ad libitum*.

Selection of Doses

Doses were selected on the basis of maximum tolerated safe doses found from toxicity studies. The doses were selected $1/10^{th}$ & $1/5^{th}$of the maximum tolerated safe dose 2000 mg/kg, *i.e.* 200 mg/kg & 400mg/kg respectively and were administered orally once daily for twenty eight days as per Debjit, B *et al*., 2010.

Induction of Hepatotoxicity

Paracetamol (PCM) was used as hepatotoxin. The paracetamol induced hepatotoxicity model described by Parmar *et al*., (2010) was used with slight modifications. Rats were fasted over night and hepatotoxicity was induced by administration of paracetamol in pure form (API) in distilled water at the dose rate of 500 mg/kg body weight by gastric gavage to the rats once daily for the entire duration of study.

Experimental Designs

Assessment of hepatoprotective activity was carried out on wistar albino rats. The animals were segregated into nine groups of six animals each.

Group 1: Normal Control Group: animals of this group received distilled water p.o. for 28 days.

Group 2: Toxic Control Group: animals orally received Paracetamol (500 mg/kg body weight) for 28 days.

Group 3: Standard Group: animals of this group received 100 mg/kg body weight of standard drug silymarin and

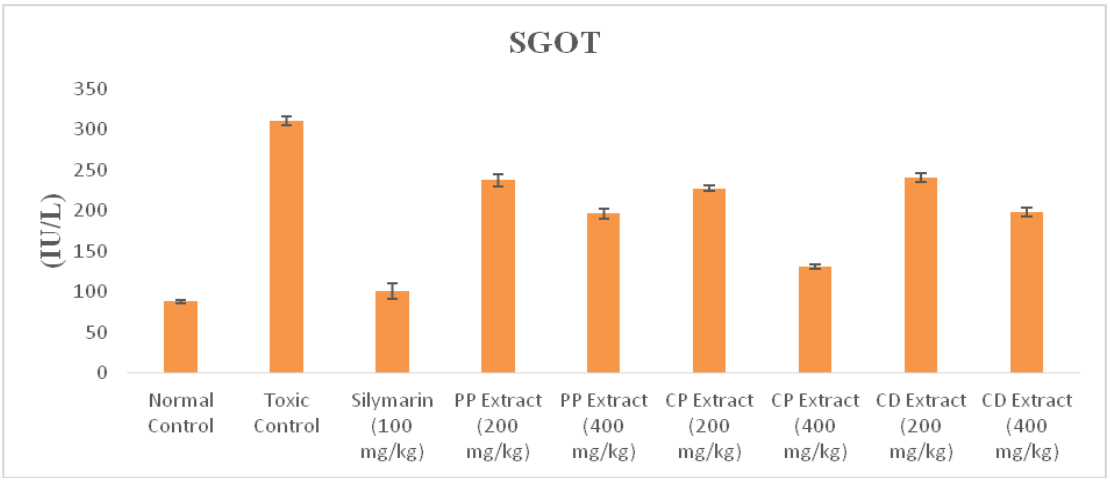

Figure 1: Effect of silymarin & hydroalcholic extracts pretreatment on SGOT level in PCM induced chronic hepatotoxicity in rats

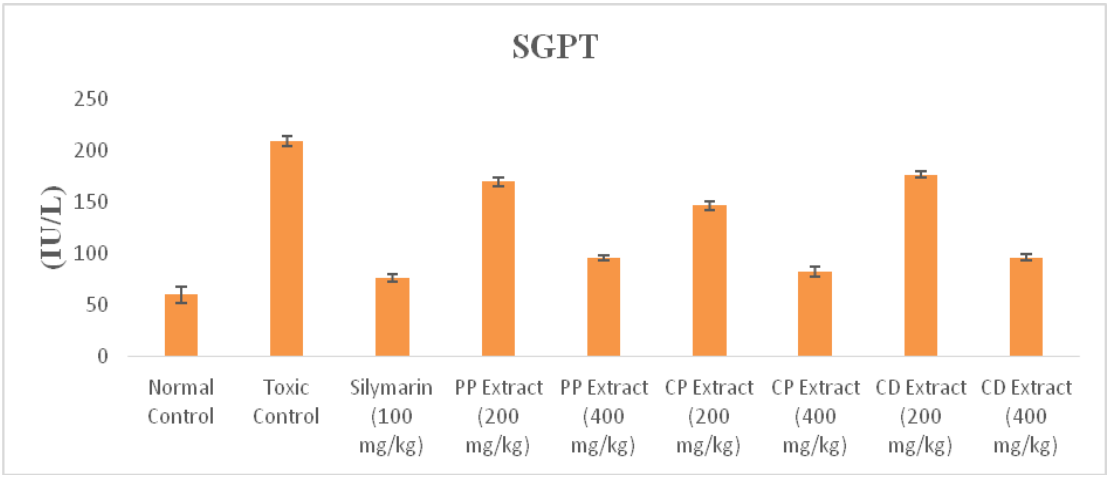

Figure 2: Effect of silymarin & hydroalcholic extracts pretreatment on SGPT level in PCM induced chronic hepatotoxicity in rats

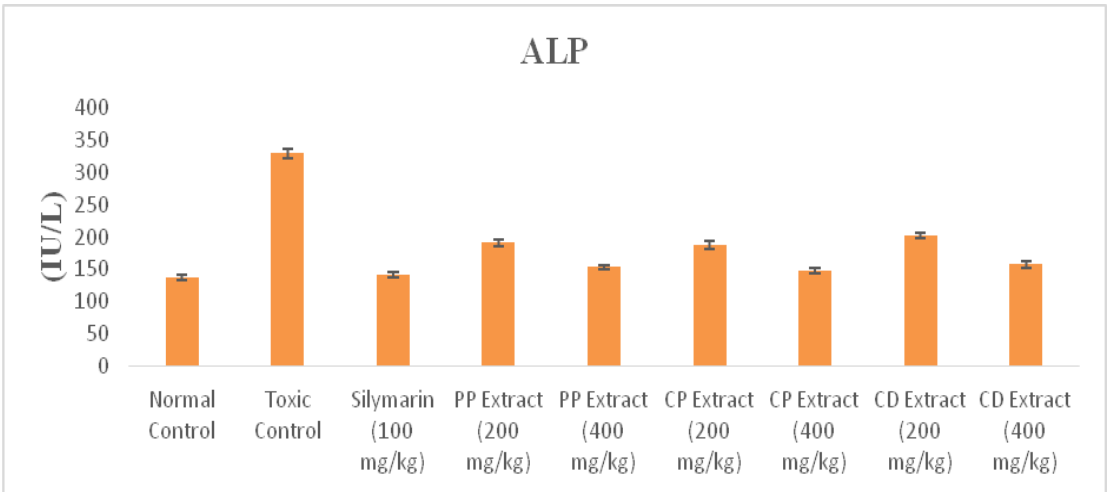

Figure 3: Effect of silymarin & hydroalcholic extracts pretreatment on Serum ALP level in PCM induced chronic hepatotoxicity in rats

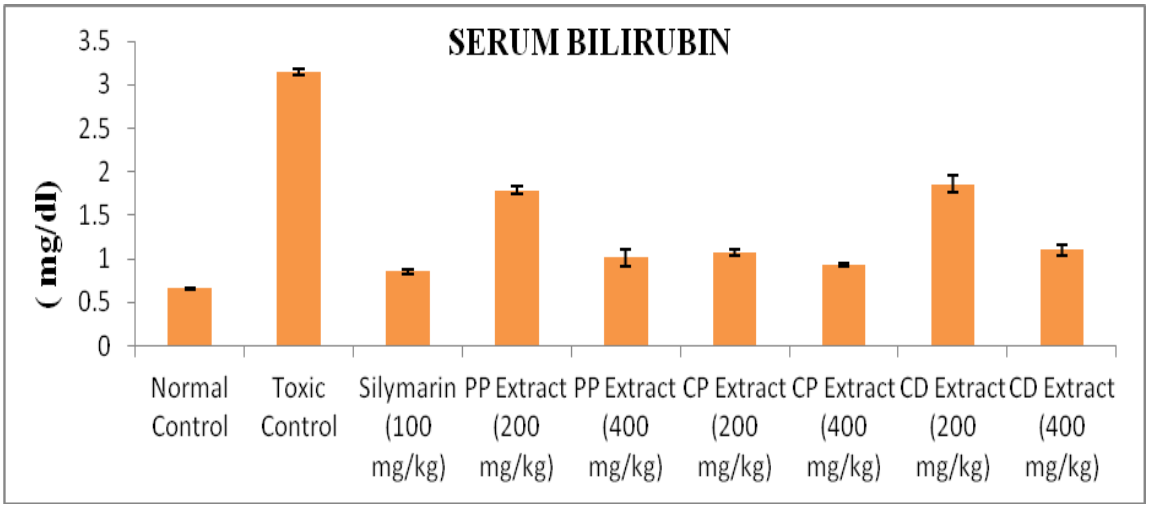

Figure 4: Effect of silymarin & hydroalcholic extracts pretreatment on Serum Bilirubin level in PCM induced chronic hepatotoxicity in rats

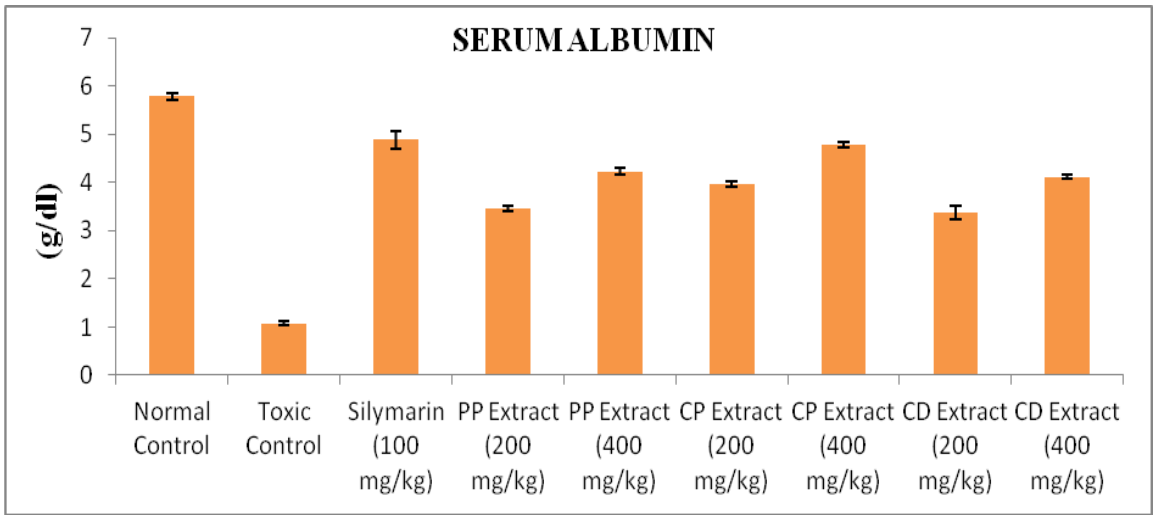

Figure 5: Effect of silymarin & hydroalcholic extracts pretreatment on Serum Albumin level in PCM induced chronic hepatotoxicity in rats

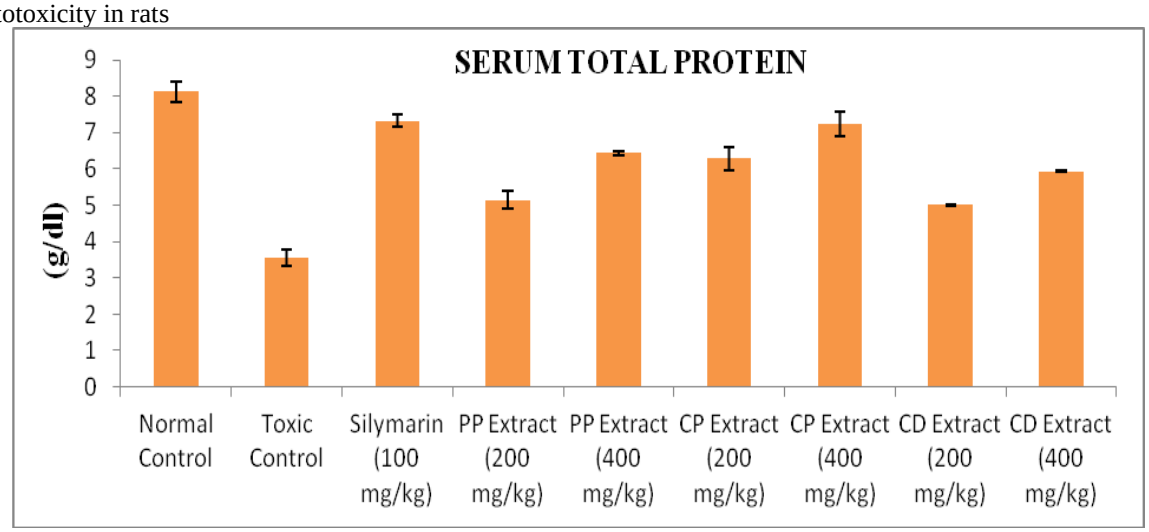

Figure 6: Effect of silymarin & hydroalcholic extracts pretreatment on Serum Total Protein level in PCM induced chronic hepatotoxicity in rats

Paracetamol (500 mg/kg body weight) for 28 days.
Group 4: Test Group: animals of this group received hydroalcholic leaves extract of *Prunus persica* (200 mg/kg body weight) along with 500 mg/kg body weight of Paracetamol for 28 days.

Group 5: Test Group: animals of this group received hydroalcholic leaves extract of *Prunus persica* (400 mg/kg body weight) along with 500 mg/kg body weight of Paracetamol for 28 days.

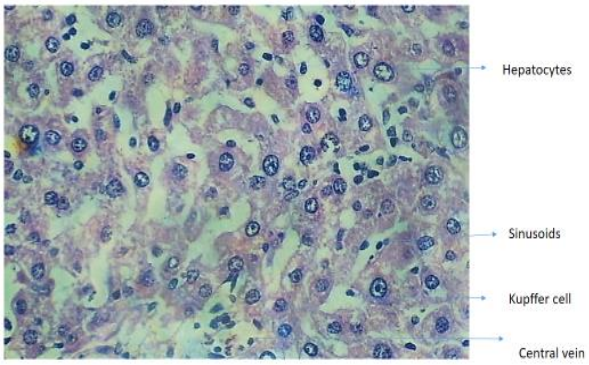

Figure 7: normal control group, History showed there is no changes in liver.

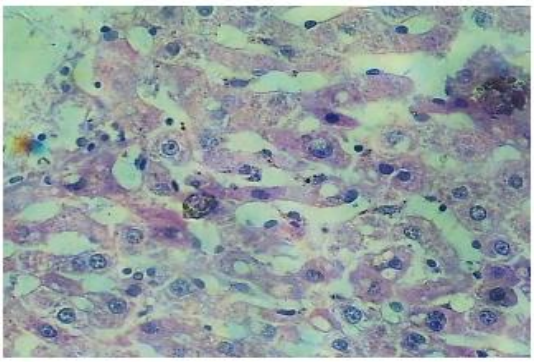

Figure 8: toxic control group.

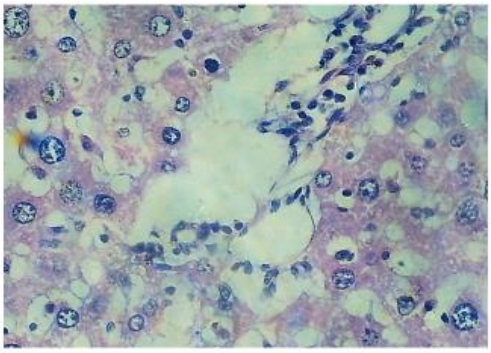

Figure 9: Standard group, shows there is no change in structure: only less acidophilic bodies, hypocytosis.(hyperplasia of hepatocytes) seen.

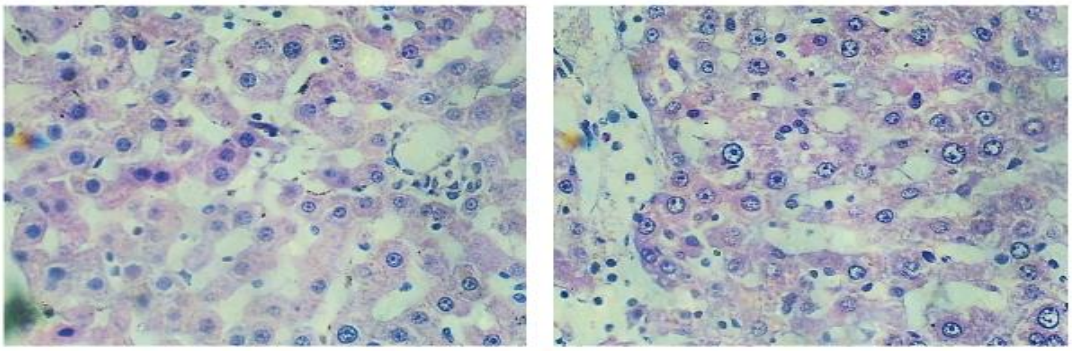

Figure 10 & 11: Test group (Prunus persica (200 mg/kg & 400 mg/kg)). Histology of liver showed there is less intense infiltration of neutrophils, and lymphocytic infiltrate and acidophilic bodies and also there is no damage in central portal vein.

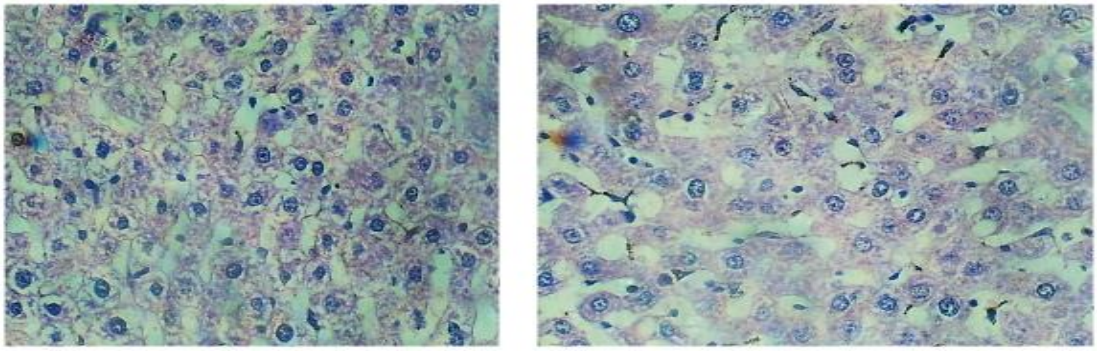

Figure 12 & 13: Test group (*Calotropis procera* (200 mg/kg & 400 mg/kg)).both slides showed there is less acidophilic bodies, hyperplasia of kupffer cells and sinusoidal damage.

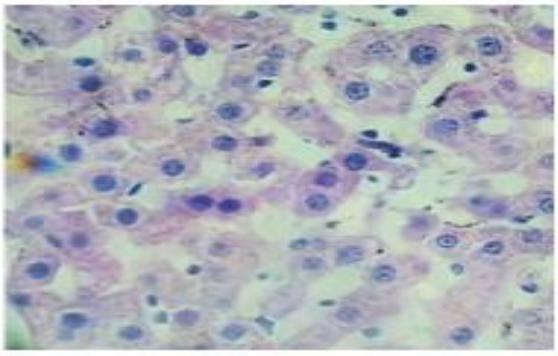
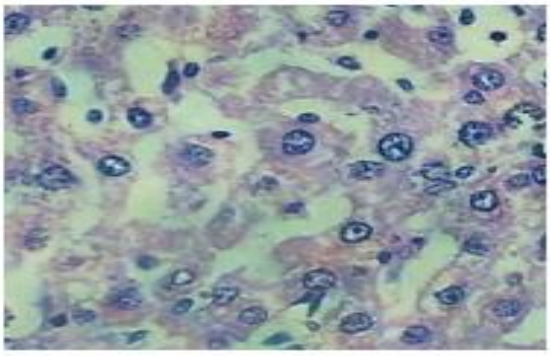

Figure 14 & 15: Test group (*canscora decussate* (200 mg/kg & 400 mg/kg) both slides showed there is less abnormality of liver architecture.

Group 6: Test Group: animals of this group received hydroalcholic leaves extract of *Calotropis procera* (200 mg/kg body weight) along with 500 mg/kg body weight of Paracetamol for 28 days.
Group 7: Test Group: animals of this group received hydroalcholic leaves extract of *Calotropis procera* (400 mg/kg body weight) along with 500 mg/kg body weight of Paracetamol for 28 days.
Group 8: Test Group: animals of this group received hydroalcholic leaves extract of *Canscora decussate* (200 mg/kg body weight) along with 500 mg/kg body weight of Paracetamol for 28 days.
Group 9: Test Group: animals of this group received hydroalcholic leaves extract of *Canscora decussate* (400 mg/kg body weight) along with 500 mg/kg body weight of Paracetamol for 28 days[14].

Estimation of Biochemical Parameters

After 24 hours of Paracetamol administration (Day 29th) animals of all groups were sacrificed by cardiac puncture. Blood sample of each group was collected separately into sterilized dry centrifuge tubes. The collected blood was allowed to clot at room temperature and serum was separated by centrifugation at 2500 rpm for 15 min. Then serum was used for the estimation of biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP).The biochemical parameters were estimated as per the standard procedure prescribed by manufacturer's instruction manual provided in the standard kit using autoanalyser[15].

Histopathological Observations

Liver was dissected out and the liver samples were excised from the experimental animals of each group and washed with the normal saline. Liver was sliced and pieces were preserved in 10% formalin for proper fixation. These tissue samples were embedded in paraffin and processed as per standard procedures. These tissue samples were sectioned at 3-5µ thickness and were stained with Mayer's haematoxylin and eosin for histopathological examination[16].

Statistical analysis

The results are expressed as mean ± SEM of six animals from each group. The data were evaluated by one-way ANOVA followed by using Graph Pad Prism software. A probability of less than 5% ($p<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

The preliminary phytochemical screening confirmed the presence of alkaloids, glycosides, saponins, flavonoids, phenols and proteins in the hydroalcholic leaves extract of *Prunus persica*. After phytochemical screening of the hydroalcoholic extract of *calotropis procera*, it was found that the extract contain alkaloids, glycosides, saponins, phytosterols, flavonoids and the hydroalcoholic extract of *Canscora decussate* showed the presence of alkaloids, carbohydrates, glycosides, saponins, flavonoids, phenols and proteins.

Present study was aimed to investigate the protective effect of some indigenous plants of Northern India against Paracetamol induced chronic liver injury. The extent of hepatic damage is assessed by the level of release of cytoplasmic biomarkers in the circulation. It is well documented that Paracetamol is metabolized by Cytochrome P450 enzymes to a reactive metabolite N-acetyl-p-benzo-quinoneimine (NAPQI) which covalently binds to the cellular macromolecules and initiates cell damage[17]. Hepatic cellular damage may result in leakage of enzymes like SGOT, SGPT, ALP which can be measured as indicators of cell damage. Their levels are markedly elevated in hepatitis and other chronic liver damage[18]. However till date allopathic medical practice do not have satisfactory liver protective drug. Herbal preparation play important role in the management of liver disorders.

As exhibited from Table 1 there was statistical significant ($p<0.05$) increase in hepatic function biomarkers (*viz* SGOT, SGPT, SALP, Sr. Bilirubin) in serum of rats of toxic control group, indicating the extensive hepatic cellular damage caused by PCM when compared to that of normal control group. The once daily oral pre-treatment with standard drug, silymarin (100 mg/kg) and hydroalcholic extracts (200 & 400 mg/kg) of *Prunus persica, Calotropis procera* & *Canscora decussate* leaves significantly alleviates the toxic effect of PCM, evidenced by decrease in SGOT, SGPT, SALP, Sr. Bilirubin of rats of Group-III, IV,V,VI,VII,VIII & IX when compared to toxic control group (Group-II).

Also, there was statistically significant decrease in albumin and total protein in serum of experimental rats of PCM treated group whereas the animals of groups pretreated with silymarin (100 mg/kg) and hydroalcholic extracts (200 & 400 mg/kg) showed less decrease in serum albumin and total protein when compared to that of normal control which is an indicative of hepatoprotective effect of *Prunus persica*, *Calotropis procera* & *Canscora decussate* leaves extract.

Histopathological studies

Histopathological observations of liver tissue confirmed the hepatic toxicity in toxic control group and healing or preventive potential of the test, plant extracts & other treatment group.

Histopathological observations of normal control animals (Group I) shows normal hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus, and well brought out central vein were observed (Figure 7). The liver sections of toxic control group (Group II) rats showed small aggregates of chronic inflammatory cells. There is intense infiltration of neutrophils, hyperplasia of hepatocytes and kupffer cells and acidophilic bodies seen. Total loss of hepatic architecture with centrilobular hepatic necrosis (Figure 8). Groups pretreated with silymarin and hydroalcholic extracts of *Prunus persica*, *Calotropis procera* and *Canscora decussate* leaves showed decreased abnormality of liver architecture as sign of protection against damage induced by paracetamol (Figure 9, 10, 11, 12, 13, 14, 15).

CONCLUSION

The present study reports the hepatoprotective activity of some indigenous plants of northern India against chronic paracetamol intoxication in rats. Phytochemical screening revealed the presence of glycosides, flavonoids, alkaloids, phytosterols, saponins and phenolic compounds in the hydroalcholic extracts of plants. Several investigators have shown that plant extract containing flavonoids are responsible for hepatoprotective potential in various experimental animal models. Thus, it can be interpreted that the hepatoprotective effect may be due to the presence of flavonoids[19].

On the basis of results obtained, it can be concluded that the hydroalcholic extracts of *Prunus persica, Calotropis procera* & *Canscora decussate* leaves seems to have hepatoprotective activity. The further studies are needed to evaluate potential usefulness of hydroalcholic extract in clinical condition associated with liver damage. Among the all plants *Calotropis procera* at a dose of 400 mg/kg, b.w showed the most significant hepatoprotective activity.

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