

EVALUATION OF HEPATOPROTECTIVE EFFICACY OF RHIZOMES *CURCUMA CAESIA* IN PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

*SATYENDRA SINGH BAGHEL, YOGENDRA MAVAI, RAJENDRA SINGH BAGHEL, INDU SIKARWAR, NIKHIL SHRIVASTAVA

Department of Pharmacology, Shriram College of Pharmacy, S.R.G.O.C Campus, AB Expressway, Banmore, M.P., India.
Email: satyendra2401@gmail.com

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ABSTRACT

Objective: To evaluation of hepatoprotective efficacy of rhizomes *Curcuma caesia* in paracetamol induced hepatotoxicity in rats.

Methods: Wistar rats were divided into five groups (n=6). Ethanolic extract of *Curcuma caesia* rhizomes was prepared and evaluated for its hepatoprotective efficacy against paracetamol (PCM) (2g/kg B. wt.) induced hepatotoxicity in rats. Silymarin (100mg/kg b. wt., oral) was used as standard. Organ to body weight indices (OBWI), Levels of Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), Alkaline phosphatase (ALP), bilirubin, total protein, Reduced and Oxidized glutathione in tissue and lipid peroxidation was evaluated along with histopathological investigation in various experimental groups of rats.

Results: Paracetamol has enhanced the OBWI, levels of SGPT, SGOT, SALP, bilirubin, tissue and serum MDA and tissue GSSG levels, whereas plasma protein, tissue GSH levels are decreased significantly ($P < 0.05$) as compared to normal control. Pretreatment with ethanolic extract of *Curcuma caesia* (250mg/kg B. wt. and 500 mg/kg B. wt.) has brought back the altered levels of biochemical markers to the near normal levels.

Conclusion: *Curcuma caesia* extract (CCE) acts in the liver as a potent scavenger of free radicals to prevent the toxic effects of Paracetamol both in the biochemical and histopathological parameters.

Keywords: *Curcuma caesia*, Hepatoprotective, Paracetamol induced hepatotoxicity.

INTRODUCTION

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injuries induced by various hepatotoxins have been recognized as a major toxicological problem for years [1]. Treatment and management of liver disease still pose a significant challenge to the modern medicine due to the lack of rational therapy. In this context a number of medicinal plant preparations whose usage have been in vogue since centuries recommended by the Ayurvedic system of medicine for the treatment of liver disorders hold promise [2]. Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates [3,4]. 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 [5] to a highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI) [6].

The genus *CURCUMA* is a well known spice of India. It is also called haldi and more than 200 species and subspecies of it is found all across the world. One of which is *Curcuma caesia* Family: Zingiberaceae. It is also known as "Kali Haldi". It is an erect rhizomatous herb with large leaves. Fresh rhizomes are aromatic with intense camphoraceous odor and are applied externally to sprain and bruises [7].

Curcuma caesia is native to North-East and Central India. It is also sparsely found in Papi hills of East Godavari, the root hills of the Himalayas and North Hill forest of Sikkim. The rhizomes of Black Turmeric have a high economical importance owing to its putative medicinal properties. The rhizomes are used in the treatment of hemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting, menstrual disorder, anthelmintic, aphrodisiac, inflammation, gonorrheal discharges, etc. [8,9]. Almost all species of *Curcuma* contains antioxidant activity and the pharmacological effects and prospects for future clinical use had been tried so far [10].

MATERIALS AND METHODS

Plant Material

The rhizomes of *C. caesia* were collected from the Dindori District, M.P., India. Plant was identified and authenticated by Dr. Anil Mangal (Research officer Ayurveda) National Research institute for Ayurveda

HRD, Gwalior (M.P.). The voucher specimen no: srpc/2012/004 has been deposited at the Herbarium unit of the Department of Pharmacognosy, Shriram college of pharmacy, Banmore, Morena (M.P.).

Preparation of Extract

Rhizomes were cut into small pieces (5cm), shade dried and grounded by hand grinder to fine powder. The powdered plant material was sieved and 450 g was extracted with ethanol (750 ml) by using a Soxhlet apparatus at a temperature of 55 to 60°C for a period of 16 hrs. The crude extract obtained was filtered and evaporated to dryness in vacuo (at 35°C and 0.8 MPa) in a Buchi evaporator. The residues was weighed and stored at 4 °C until use.

Drugs & Chemicals

Paracetamol (Sigma chemicals, USA), Silymarin (Micro labs, Tamilnadu), Ethyle alcohol (Kidneycare fluids, Noida), disodium hydrogen phosphate (Na_2HPO_4), Dihydrogen potassium phosphate anhydrous, thiobarbituric acid and Tri Chloro acetic acid were purchased from Merck India Ltd, Mumbai, India. All other chemicals and other biochemicals used in the experiments were of analytical grade from different firms.

Animals

Male wistar rats weighing between 200-220 gm were used for this study. The animals were obtained from central animal facility of Shri Ram College of Pharmacy, Banmore, M.P., India [891/AC/05/CPCSEA] and was maintained in polypropylene cages on rodent pellet condition of controlled temperature ($22 \pm 2^\circ\text{C}$) and acclimatized to 12/12 h light/dark cycle. Free access to food and water was allowed until 2h before the experiment. The care and maintenance of the animals was as per the approved guidelines of the "Committee for the purpose of control and supervision of experiments on animals (CPCSEA)". All experiments on animals were conducted according to the guidelines of establishment's ethical committee on animal experimentation (Approval no. SRCP/M.Pharm/IAEC/34/11-12).

Experimental designs

Group-I: Normal control (0.5% CMC 1 ml/kg b.wt., oral)

Group -II: PCM (2g/kg b.wt., oral)

Group -III: CCE (250mg/kg b.wt., oral) + PCM (2g/kg b.wt., oral)

Group -IV: CCE (500mg/kg b.wt., oral) + PCM (2g/kg b.wt., oral)

Group -V: Silymarin (100mg/kg b.wt., oral) + PCM (2g/kg b.wt., oral)

Animals were divided into five groups of 6 animals each. The first group received 0.5% CMC 1 ml/kg for one week (control). The group II received 0.5% CMC 1 ml/kg for one week (positive control). The groups III, IV and V received 250 mg/kg and 500 mg/kg[11] of ethanolic extract of rhizomes *C. caesia* and silymarin (100 mg/kg p.o.) respectively once a day for seven days. On the fifth day, after the administration of the respective treatments, all the animals of groups II, III, IV and V were administered with paracetamol 2 g/kg orally. The body weights of the animals were also recorded daily up to 7 days. After 7 days animals were anaesthetized with ether for collection of blood from retro orbital plexus, and then sacrificed under ether anesthesia for the removal of liver. Various haematological and biochemical analysis were carried out.

Organ to Body Weight Indices (OBWI)

After sacrificing the animals, liver will be removed and the washed free of extraneous materials and weighed. The organ to body weight indices (OBWI) will be calculated as per the formula given below:

$$OBWI = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

Biochemical Evaluation in Serum

Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), Alkaline phosphatase (ALP), Total protein concentration and total bilirubin was estimated by using commercial kits as per the manufacturer instructions.

MDA assay

The reaction mixture containing 1ml 0.67% thiobarbituric acid (TBA), 1 ml 20% trichloro acetic acid (TCA), and 100 µl serum was incubated at 100°C for 20 min and centrifuged at 12,000 rpm for 5 min. The absorbance of the supernatant was read at 532 nm and MDA concentration was determined by using a molar extinction coefficient of 1.56×10^5 M/cm and the values was expressed as mM[12].

Biochemical Evaluation in Liver Tissue

A portion of the liver was used for biochemical estimation. Liver lipid peroxidation was determined by measuring the level of MDA

according to the method of Ohkawa *et al.*, 1979[13]. Tissue GSH was determined by the method of Sedlak and Lindsay (1968)[14] and Oxidized glutathione (GSSG) was measured according to the method described by Aseni *et al.* (1999) based on the principle of glutathione reductase enzyme reducing GSSG to GSH with the concomitant oxidation of NADPH to NADP+[15].

Histopathology of liver

Liver tissues of rat was removed and washed with normal saline. The cleared tissue was fixed in 10% natural buffered formalin solution (pH 7.0-7.2). After proper fixation tissue was processed for dehydration in ascending grade of ethanol, clearing with toluene, followed by impregnation in paraffin wax, then sections of 5 µ in thickness was cut with help of semi-automatic rotary microtome. Sections were stained with hematoxyline. All the sections of the tissues were examined under microscope for the analyzing the altered architecture due to the liver tissue due to paracetamol challenge and improved liver architecture due to pretreatment with test extracts and standard drug. These were examined under the microscope for histopathological changes such as congestion, hemorrhage, necrosis, inflammation, Infiltration, kuffer cells and sinusoids and photographs were taken.

Statistical Analysis

Statistical evaluations were made using one-way ANOVA followed by Dunnett's t test. A probability of 0.05 and less was taken as statistically significant. The analyses were carried out using sigma stat for windows version 2.03 (SPSS Inc.USA).

RESULTS

The paracetamol-induced liver damage was treated with *C. caesia* for seven days continuously. Paracetamol has enhanced the OBWI, levels of SGPT, SGOT, SALP, bilirubin (both total and direct bilirubin levels), tissue and serum MDA and tissue GSSG levels, whereas plasma protein, tissue GSH levels are decreased significantly ($P < 0.05$) as compared to normal control. This clearly indicates that there is a significant hepatic damage due to paracetamol. Treatment with silymarin, 250mg/kg and 500mg/kg of *C. caesia* rhizomes (ethanolic extract) has significantly ($P < 0.05$) brought down the elevated OBWI, levels of SGPT, SGOT, SALP, bilirubin, tissue and serum MDA and tissue GSSG levels as compared to PCM treated group, also significantly ($P < 0.05$) enhanced the decreased levels of tissue GSH and plasma protein as compared to PCM treated group. Results are reported in figures.

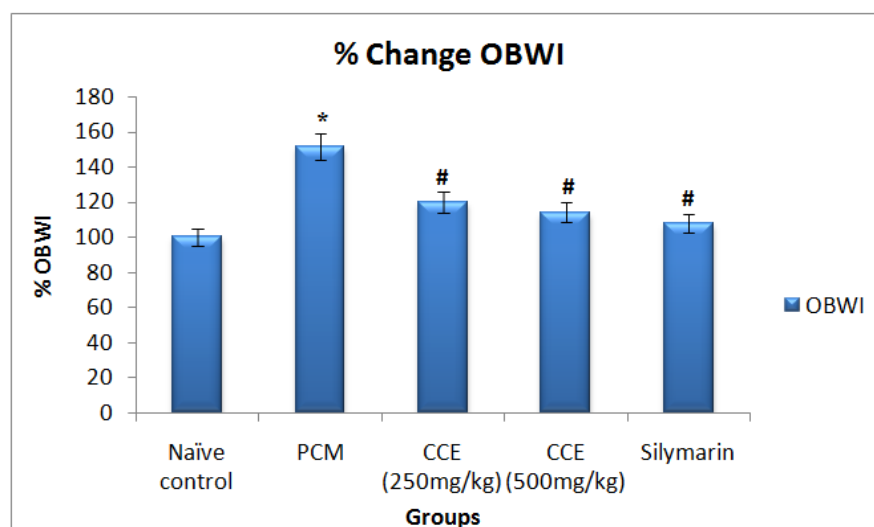


Fig. 1: Effect of pretreatment of CCE and Silymarin on liver organ body weight indices (OBWI).

* $P < 0.05$, compared to control group (One way ANOVA followed by Dunnett's t test).

$P < 0.05$, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for liver OBWI: 2.98 ± 0.18 .

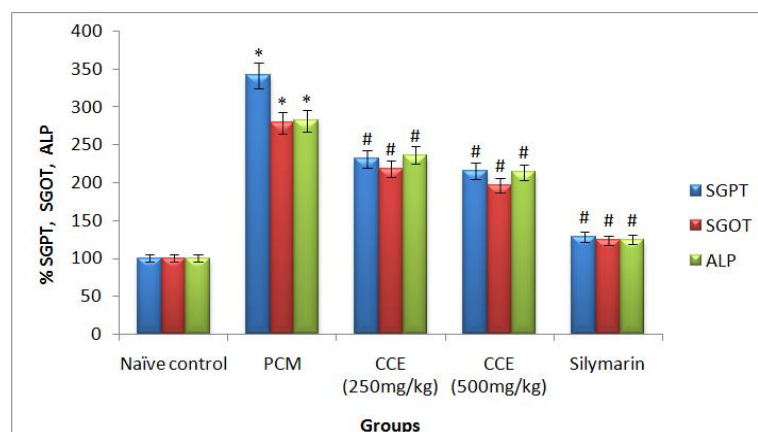


Fig. 2: Effect of pretreatment of CCE and Silymarin on %SGPT, SGOT and ALP levels in paracetamol induced hepatotoxicity in rats.

*P<0.05, compared to control group (One way ANOVA followed by Dunnett's t test).

#P<0.05, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for: SGPT = 78.57±5.4 U/L, SGOT = 128.26±8.7 U/L, ALP = 123.76 ± 5.6 U/L.

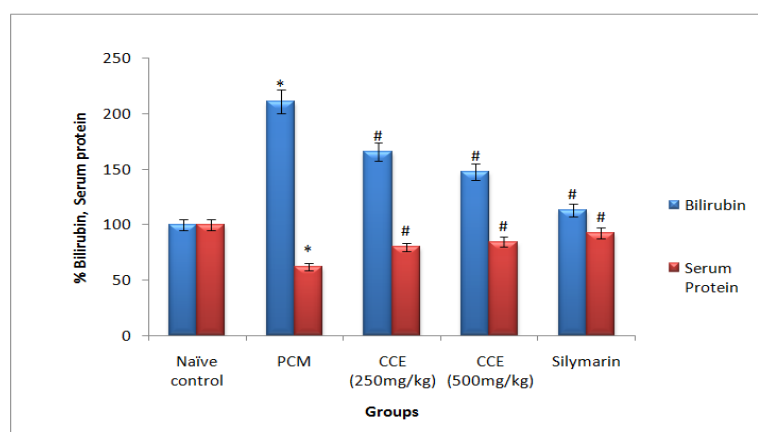


Fig. 3: Effect of pretreatment of CCE and Silymarin on % serum bilirubin and protein levels in paracetamol induced hepatotoxicity in rats.

*P<0.05, compared to control group (One way ANOVA followed by Dunnett's t test).

#P<0.05, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for: Total Bilirubin (mg/dl) = 0.785 ± 0.03, Total Protein (mg/dl) = 7.3 ± 0.6.

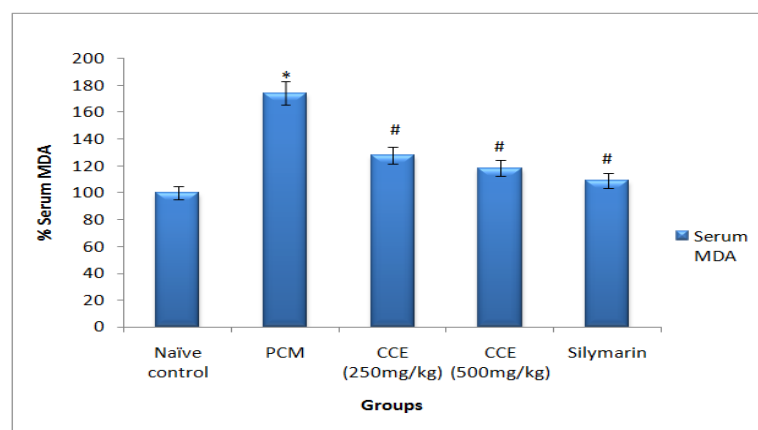


Fig. 4: Effect of pretreatment of CCE and Silymarin on % serum MDA level in paracetamol induced hepatotoxicity in rats.

*P<0.05, compared to control group (One way ANOVA followed by Dunnett's t test).

#P<0.05, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for Serum MDA: 0.22± 0.07× 10⁻³ mM.

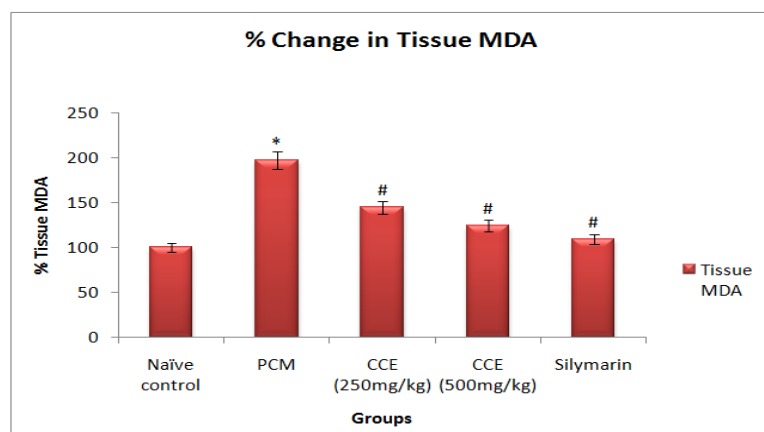


Fig. 5: Effect of pretreatment of CCE and Silymarin on % Tissue MDA levels in paracetamol induced hepatotoxicity in rats.

* $P < 0.05$, compared to control group (One way ANOVA followed by Dunnett's t test).

$P < 0.05$, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for Tissue MDA = 93.2 ± 0.2 nmol/gm of tissue.

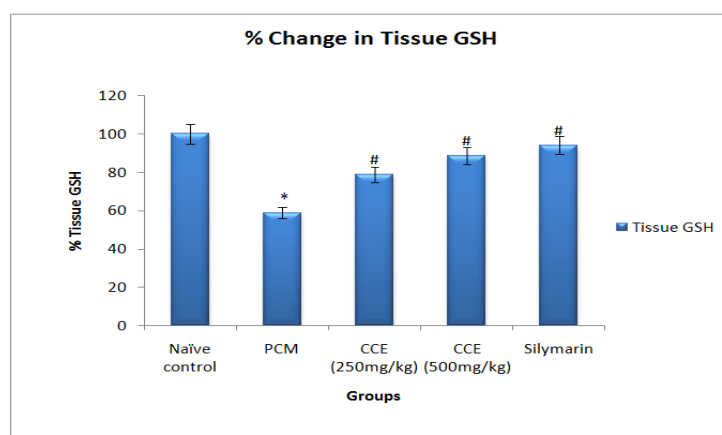


Fig. 6: Effect of pretreatment of CCE and Silymarin on %Tissue GSH levels in paracetamol induced hepatotoxicity in rats.

* $P < 0.05$, compared to control group (One way ANOVA followed by Dunnett's t test).

$P < 0.05$, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for Tissue GSH = 7.8 ± 0.8 μ moles/gm of tissue.

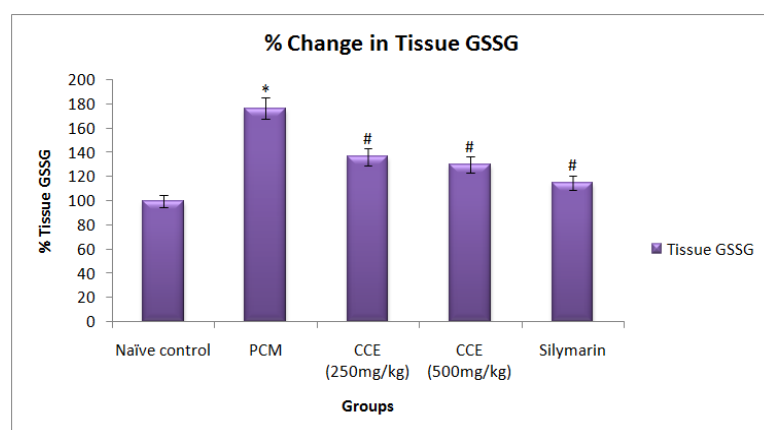
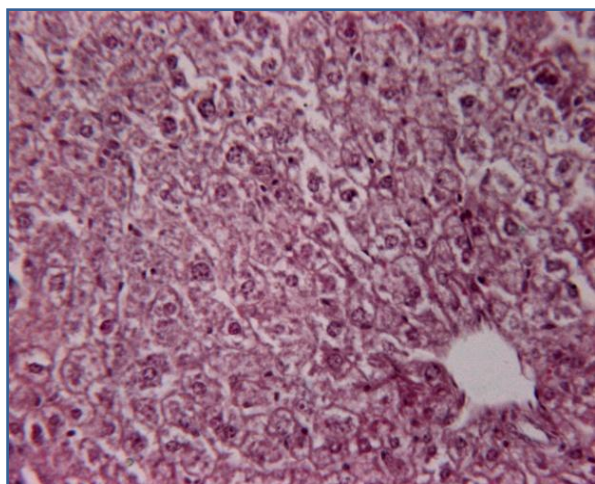


Fig. 7: Effect of pretreatment of CCE and Silymarin on Tissue GSSG levels in paracetamol induced hepatotoxicity in rats.

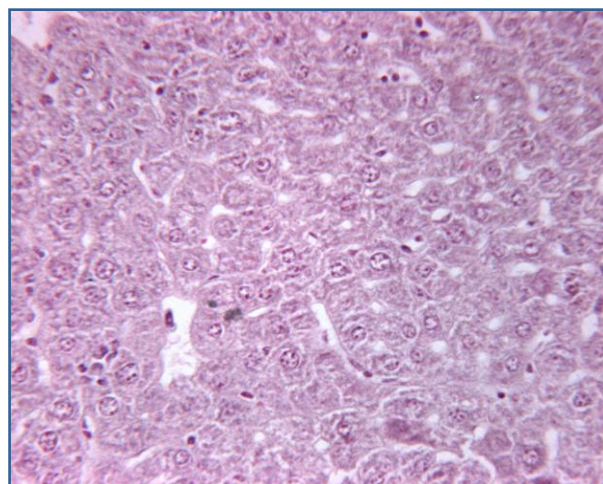
* $P < 0.05$, compared to control group (One way ANOVA followed by Dunnett's t test).

$P < 0.05$, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

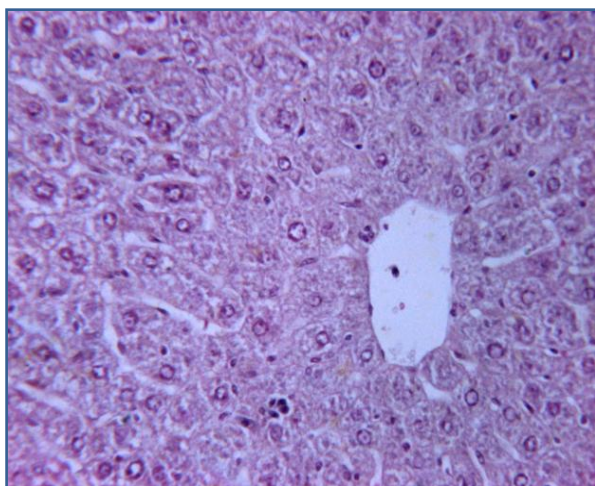
Control value for Tissue GSSG = 0.04 ± 0.01 μ g/gm of tissue.



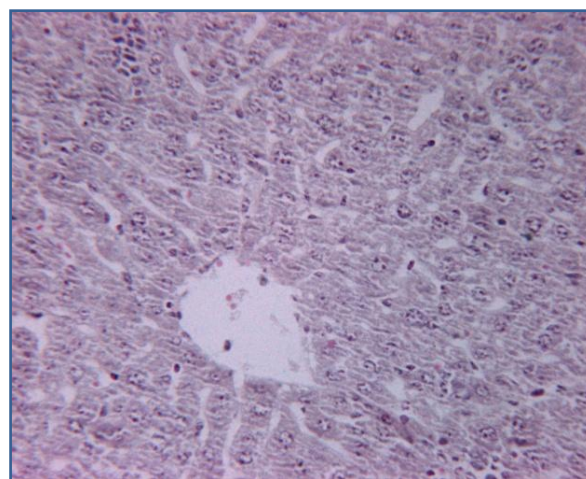
(A) Control



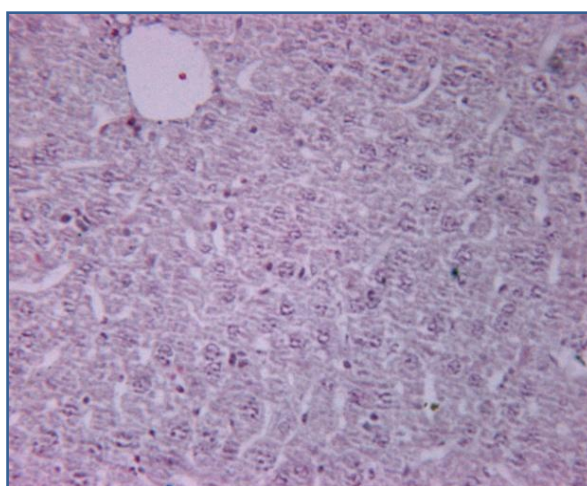
(B) PCM



(C) CCE(250mg/kg)+PCM



(D) CCE(500mg/kg)+PCM



(E) Silymarin+PCM

Fig. 8(A-E): Photomicrographs of liver tissue of rats treated with PCM, CCE & other.

DISCUSSION

Paracetamol is normally eliminated mainly as sulfate and glucuronide. Only 5% of the paracetamol is converted into N-acetyl-p-benzoquinimine. However, upon administration of toxic doses of paracetamol the sulfation and glucuronidation routes become saturated and hence, higher percentage of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquinimine (NAPQI)

by cytochrome-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI, can covalently binds to macromolecules of cellular membrane and increase the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently, leads to increased lipid peroxidation and liver damage [16].

In case of toxic liver, liver weight and liver volume is increased. In this case water is retained in cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass. Fig 1 represents the organ bodyweight indices of the different groups of rats. The OBWI of Paracetamol induced group II rats has been increased significantly ($p < 0.05$) comparing with naive control. OBWI were changed significantly by pretreatment with *Curcuma caesia* and silymarin in group III, IV and group V as compare to PCM treated group.

During hepatic damage, cellular enzymes like SGPT, SGOT and ALP present in the liver cells leak into the serum, resulting in increased concentrations[17]. In the assessment of liver damage by paracetamol the determination of enzyme levels such as SGPT and SGOT is largely used. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver[18]. Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of alkaline phosphatase in the blood serum is related to the increased synthesis of it by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure[19]. There was a significant increase ($p < 0.05$) of these serum markers were observed in PCM treated group compared to naive control group. Pretreatment with CCE and silymarin show significant changes in SGPT, SGOT and ALP activities compared to PCM treated rats.

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes[20]. Administration of *Curcuma caesia* decreased the level of bilirubin and increased the level of protein suggesting that it offered protection.

Determination of GSH, concomitant to its reduced and oxidized fraction, is a key factor to show the amount antioxidant reserve in the organism [21,22,23]. Induction of cytochrome P450 and/or depletion of liver GSH is needed for paracetamol induced liver toxicity [24]. NAPQI, paracetamol reactive metabolite, can conjugate with GSH and cause its depletion which leads to the cellular necrosis[25]. In the present study, the content of GSH in the paracetamol treated group was changed significantly as compared to naive control group. Prereatment with CCE and silymarin restored GSH levels which further highlights their role against paracetamol induced hepatotoxicity.

Among the cellular molecules, lipids that contain unsaturated fatty acids with more than one double bond are particularly susceptible to action of the free radicals. The resulting reaction, known as lipid peroxidation, disrupts biological membranes and is thereby highly deleterious to their structure and function. Lipid peroxidation is assessed indirectly by the measurement of the secondary products, such as malondialdehyde (MDA) [26,27]. In the present study, the MDA level was increased significantly in the paracetamol treated group. Prereatment with CCE and silymarin were changed MDA levels significantly as compared to paracetamol treated group, which further highlights their role against paracetamol induced hepatotoxicity.

Paracetamol causes centrilobular hepatic necrosis in rats, mice, guinea pigs, hamsters, rabbits, cats, dogs and pigs[28]. Histological profile of control animals showed normal hepatocytes [figure 8 (A)]. The section of the liver of the toxic control group of animals exhibited severe intense congestion, hydropic degeneration, pyknosis and occasional necrosis [figure 8 (B)]. The liver section of the silymarin treated animals showed normal hepatic architecture with few fatty globules [figure 8 (E)]. The liver section of the animals treated with CCE showed normal hepatic cords and absence of severe congestion, pyknosis and occasional necrosis [figure 8 (C, D)] indicating pronounced protection of hepatocytes by paracetamol induced hepatic damage.

CONCLUSION

The alcoholic extract of rhizomes of *C. caesia* has shown the ability to maintain the normal functional statuses of the liver. From the above

preliminary study, we conclude that the alcoholic extract of *Curcuma caesia*, is proved to be one of the herbal remedies for liver ailment.

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