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Amelioration of Paracetamol-Induced Hepatic Damage by Aqueous Extract of *Curcuma Caesia* in Rat

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ABSTRACT

Man's inventiveness is demonstrated by the usage of plants to heal specific illnesses. There are numerous reports of *Curcuma caesia*, sometimes referred to as kali haldi in Hindi, being utilized as a treatment for a variety of illnesses. The aim of this study was to assess the potential of a flavonoid-rich extract of the entire *Curcuma caesia* plant to mitigate the hepatotoxic effects of paracetamol on mice. Total phenolics were determined using phytochemical screening and extraction in a variety of solvents. The aqueous extract of *Curcuma caesia* was utilized to investigate the preventive effect on hepatic damage caused by paracetamol. 7.97%w/v of the aqueous extract and 12.36%w/v of the methanol extract were produced. The aqueous extract was found to contain alkaloids, glycosides, phenolics, flavonoids, carbohydrates, saponins, sterol, and terpenes, according to the phytochemical screen. It was discovered that the methanolic and aqueous extracts had total phenolic contents of 63.17 and 78.54 GAE mg/g of dry extract, respectively. In contrast to the group that received PCM, the biochemical analysis unequivocally shows that the hepatic cells in the group treated with an aqueous extract of *Curcuma caesia* (50 and 100 mg/kg, p.o.) are normal. Because it may restore the liver function that has been damaged by PCM, the aqueous extract of *Curcuma caesia* can be regarded as an effective hepatoprotective in nature. Therefore, it was determined that *Curcuma caesia* extract has hepatoprotective properties

Keywords: *Curcuma caesia , hepatotoxicity, flavonoid, biochemical parameters, histopathology*

Abbreviations

PCM- Paracetamol; CMC – carboxymethylcellulose; ALP- Alkaline phosphatase; SGOT-Serum Glutamic-oxaloacetic Transaminase; SGPT-Serum Glutamic Pyruvic Transaminase; TG-Triglycerides; TB- Total bilirubin; TC- Total Cholesterol

1. INTRODUCTION

Man's inventiveness is demonstrated by the usage of plants to heal specific illnesses. An extensive account of plant-derived medicines can be found in ancient Chinese and Indian writings, and these plants have been used as medicine from the beginning of human history (Solecki, 1977; Ahmad et al., 2006). By serving as a link between natural goods and medicine, these traditional medicines have helped to isolate a number of bioactives, supporting the idea that medicinal plants are beneficial (Phillipson, 2001).

Many organic and inorganic substances and medications that enter the body through the respiratory system, the gastrointestinal tract, or injections are metabolized primarily by the liver. By means of the P-450 cytochrome and cytochrome reductase enzyme system, the primary drug metabolism system is located in the microsomal fraction of the liver cells' smooth endoplasmic reticulum. Drug and chemical-induced toxic liver injury can almost exactly resemble any type of liver disease that occurs naturally. The most prevalent type of iatrogenic disease is hepatotoxicity caused by medications and chemicals. Iron, phosphorus, arsenic, and cooper are some of the different inorganic substances that cause hepatotoxicity. Some naturally occurring plant toxins, such as pyrrolizidine alkaloids, mycotoxins, and bacterial toxins, are classified as organic agents. Pre-existing liver disease, aging, female sex, and genetic incapacity to conduct a certain biotransformation are among the risk variables that predispose an individual to hepatic drug injury (Paliwal et al., 2017).

The perennial rhizomatous plant *Curcuma caesia* , also called kali haldi in Hindi, erectly grows to a height of 0.5 to 1.0 m

. It features broad, vertical oblong leaves, a pale yellow flower with a reddish border, and a huge tuberous rhizome (Pandey & Gupta, 2014). People from all throughout India, including tribal people, have utilized the plant for a variety of biological purposes. Scientific research has investigated the antibacterial (Kaushik et al., 2017), anti-tumor (Ghosh et al., 2013), anti-arthritic (Reenu et al., 2015), and antioxidant (Behar et al., 2013) properties of several sections of *Curcuma caesia* . The plant's effects on the liver have drawn attention since a number of flavonoidal and phenolic compounds were isolated from it. The aim of this study was to assess the potential of a flavonoid-rich extract of the entire *Curcuma caesia* plant to mitigate the hepatotoxic effects of paracetamol on mice.

2. MATERIAL AND METHODS

Paracetamol tablets and silymarin capsules (Limarin-140) was purchased from Rajshree medical store, Bhopal. All other chemicals used for this study were of analytical grade.

Preparation of the plant material

In the month of December, the *Curcuma caesia* plant was gathered from the Bhopal, Madhya Pradesh, area and verified by a botanist at RB Science, Bhopal, Madhya Pradesh, with voucher number RBS/Botany/Herb/005. After being dried in the shade, the entire plant was ground into a powder using a blender, sieved with a #40 sieve, and sealed in an airtight container.

Extraction

200g of plant material was uniformly packed in the Soxhlet apparatus and extracted using a hot continuous extraction method using different solvents of increasing polarity (methanol, petroleum ether, and chloroform). Following the aforementioned extractions, the aqueous extraction was completed using the cold maceration technique. Rotating vacuum evaporation was used to concentrate the extracts after they had been hot-filtered. The extracts were gathered and put in desiccators to eliminate the surplus moisture after final evaporation was accomplished using a water bath (Singh & Singh, 2017).

Phytochemical screening

To determine if common plant secondary metabolites were present or absent, the extracts from both plants were subjected to qualitative phytochemical analysis. Triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids were all evaluated. The analytical response to these tests was either colour intensity or precipitate development (Khandelwal, 1997).

Total Phenolic content

The Folin-Ciocalteu technique was used to determine the total phenolic content in the extracts of both plants (Tiwair et al., 2017). Methanol was used to macerate the extract, which was then diluted to create a 1 mg/mL solution. Three millilitres of water, 0.5 millilitres of Folin-Ciocalteu reagent, and 200 microliters of the extract solution were combined. After three minutes, two millilitres of a 20% w/v sodium carbonate aqueous solution were added. The mixture was then left in the dark for an hour, and a UV-Vis spectrophotometer was used to detect the absorbance at 750 nm. To create a calibration curve, standard solutions of gallic acid (10–100 ppm) were made in a similar manner. The control solution was made and incubated in the same manner as the other samples, and it comprised 200 µL of methanol and the appropriate reagents. Milligrams of gallic acid equivalent (GAE) per 100 grams of the dry sample were used to express the results.

Hepatotoxicity induced by paracetamol

Four-month-old Wistar albino rats of both sexes weighing 120–180 g were used in the experiment. In a cross-ventilated animal housing with a temperature of 25±2°C, a relative humidity of 44–56%, and light and dark cycles of 12:12 hours, the animals were acclimated to conventional laboratory settings. Throughout the experiment, they were fed a regular pallet diet and given unlimited access to water. The experiment was authorized in accordance with CPCSEA rules and by the institutional ethics committee.

Preparation of Paracetamol and Silymarin

For oral delivery, paracetamol was made in a 0.5% sodium CMC solution. In the current investigation, a toxicant dose of 2g/kg of paracetamol was chosen. Normal saline was used to dissolve silymarin, and a conventional dosage of 100 mg/kg p.o. was used.

Experimental Design

The rats were divided into 5 groups comprising of 6 animals in each group as follows:

Group I: Normal control rats received 1ml/100gm of 0.5% sodium CMC using an intragastric tube for 7days; **Group II:** Negative control rats received paracetamol 2 g/kg, p.o. for inducing hepatotoxicity; **Group III:** Rats received Silymarin (100 mg/kg, p.o.) for 7 days and paracetamol 2g/kg, p.o. on 6th day; **Group IV** Rats received aqueous extract of *Curcuma caesia* 100 mg/kg once daily for 7 days and paracetamol 2g/kg, p.o. on 6th day; **Group V** Rats received aqueous extract of *Curcuma caesia* 200 mg/kg once daily for 7 days and paracetamol 2g/kg, p.o. on 6th day.

Sample collection

The experiment ended on the seventh day, and the rats were killed by cervical dislocation. An orbital puncture was used to draw blood, which was then left to clot at room temperature for half an hour. Centrifugation was used to separate the serum for 15 minutes at 30°C and 3000 rpm (Pimple et al., 2007).

Biochemical estimation

The serum samples were subjected to biochemical parameters examination like ALT, SGOT, SGPT, Bilirubin, Triglyceride and Cholestrol levels were estimated by using standard kits.

Histopathological studies

The livers were promptly removed, preserved in 10% formalin, and stained with hemotoxylin and eosin before being examined under a microscope for signs of necrosis, fatty alterations, degeneration, and any indications of hepatotoxicity.

Statistical analysis

All the values are expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and posthoc Dunnet's *t*-test by employing statistical software, GraphPad Prism 5. Differences between groups were considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

Extraction yield and phytochemical screening

1.5% w/v petroleum ether extract was obtained as a result of the extraction process, indicating that the plant material had been defatted. 5.5%w/v chloroform extract, 12.36%w/v methanol extract, and 7.97%w/v aqueous extract were produced from the defatted plant material. The aqueous extract was found to contain alkaloids, glycosides, phenolics, flavonoids, carbohydrates, saponins, sterol, and terpenes, according to the phytochemical screen. The methanol extract did not include glycosides (Table 1).

Table 1 Phytochemical screening results

Test for	Extract			
	Petroleum ether	Chloroform	Methanol	Aqueous
Alkaloids (Dragendorf's test)	-	-	+	+
Carbohydrates (Molish test)	-	-	+	+
Glycosides (Keller Killiani test)	-	-	-	+
Flavonoids (Ammonia test)	-	-	+	+
Terpenoids (Burchard-Lieberman test)	-	-	+	+
Tanins (Ferric chloride test)	-	-	+	+
Saponins (Foam test)	-	+	+	+
Phytosterols (Burchard-Lieberman test)	+	+	+	+

+ indicates a positive observation, - indicates negative observation

The total phenolic content in the methanolic and aqueous extracts was found to be 63.17 and 78.54 GAE mg/g of dry extract.

Acute toxicity study

According to OECD guidelines, the aqueous extract with the highest phenoic content was selected for an acute toxicity assessment in order to calculate the LD50 value. The results showed that the LD50 was 500 mg/kg. Therefore, we decided to use 50 and 100 mg/kg for the hepatoprotective investigation.

Hepatoprotective study

ALP leakage in the blood, which is frequently linked to hepatonecrosis, can be found in animal models of liver injury caused by paracetamol from transaminases. Several biochemical parameters were used in this investigation to assess the preventive impact of aqueous extracts of the entire *Curcuma caesia* plant against hepatotoxicity caused by paracetamol (Table 2).

Effect of extract on SGOT and SGPT levels

Rats treated with paracetamol had higher levels of SGOT and SGPT, measuring 177.37 and 157.48, respectively, compared to the normal control group's 86.05 and 64.24 levels. When compared to the hazardous group, the aqueous extract reduced the levels of SGOT and SGPT.

Effect of extracts on alkaline phosphatase (ALP) level

Rats treated with paracetamol had an increased ALP level (136.52), while the normal control group's level was 68.42. When compared to the harmful group, the aqueous extracts of the entire *Curcuma caesia* plant reduced the amount of ALP.

Effect of extracts on total bilirubin (TB) level

Rats treated with paracetamol had a TB level of 4.08, while the normal control group had a 1.3 level. Compared to the hazardous group, the *Curcuma caesia* extract dramatically reduced the amount of TB. The aqueous extract's TB values at 50 and 100 mg/kg were 3.44 and 2.87, respectively.

Effect of extracts on total cholesterol (TC) level

Rats treated with paracetamol had an elevated level of TC (86.37), while the normal control group had a level of 75.83. In contrast to the hazardous group, the aqueous extract reduced the levels of TC.

Effect of extracts on triglyceride level

When compared to the normal rat group, which had a triglyceride level of 127.92, the rats treated with paracetamol had a greater amount (173.2). When compared to rats in the normal control group, the triglyceride level was dramatically reduced by the *Curcuma caesia* aqueous extract. Aqueous extracts containing 50 and 100 mg/kg had TP levels of 157.9 and 161.9, respectively.

Table 2 Effect of aqueous extract of *Curcuma caesia* on serum biochemical parameters in PCM induced hepatic injury in rats.

GROUP	ALP (IU/L)	SGOT/AST (IU/L)	SGPT/ALT (IU/L)	TOTAL BILURUBIN (mg/dl)	TRIGLTCERIDE (mg/dl)	CHOLESTROL (mg/dl)
Group I (Normal Control)	68.42 ± 0.62	86.05± 3.57	64.25 ± 2.84	1.3 ± 0.32	127.92±5.36	75.83 ± 2.34
Group II (-ve Control PCM)	136.52 ± 0.81	177.37 ± 7.81	157.48 ± 1.25	4.08 ± 0.68	173.2±9.94	86.37 ± 3.15
Group III (Std. Silymarin)	79.65 ± 0.37**	83.71 ± 4.79**	87.39 ± 4.97**	2.06 ± 0.76	156.0±2.63	75.53 ± 0.49
Group IV (AECC, 50mg/kg)	116.35 ± 0.89*	112.17 ± 5.35**	118.12 ± 5.97*	3.44 ± 0.46	157.9±5.68	71.56 ± 0.68
Group V (AECC, 100mg/kg)	103.63 ± 0.52**	106.18 ± 2.45**	110.62 ± 4.96**	2.87 ± 0.32	161.9±6.83	78.40 ± 2.69

The data obtained were analyzed by one-way ANOVA followed posthoc Dunnet's *t*-test. Each value represents the mean ± S.E.M., n= 6. *P < 0.05, ** P < 0.01, compared with negative control group

Histopathology

To directly demonstrate the hepatotoxicity of PCM and the impact of *Curcuma caesia* aqueous extracts, a histopathological investigation was conducted. In the liver tissue of rats given PCM, there were noticeable alterations in the hepatocyte structure. The biochemical changes in serum and liver were supported by the histological picture of the liver tissues of rats treated with *Curcuma caesia* aqueous extracts, which showed minor centrilobular fatty changes, necrosis, and bile duct

proliferation (Figure 1).

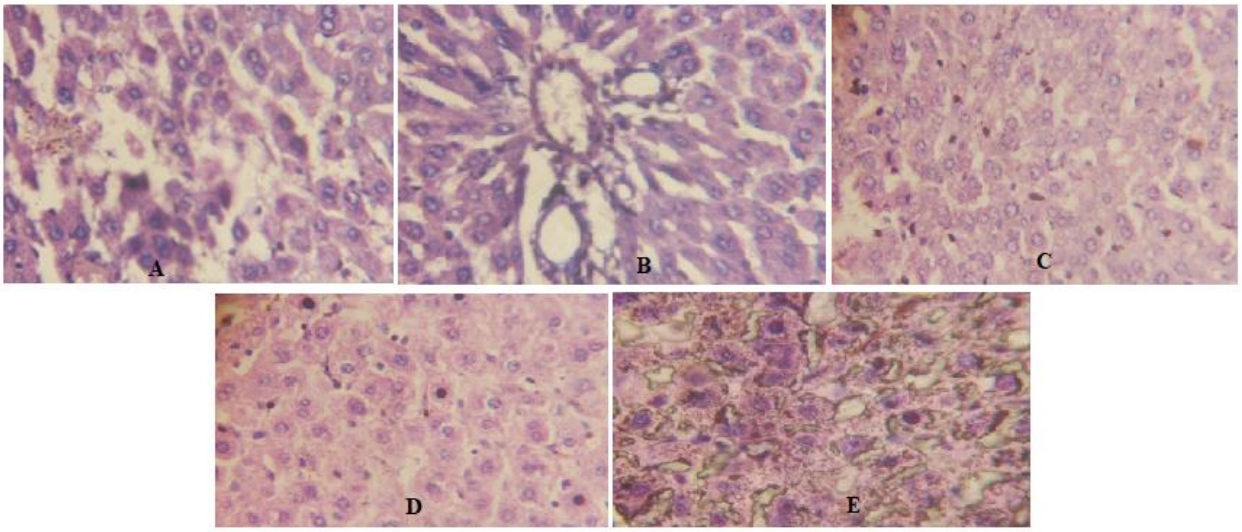

Figure 1. Histopathology of liver section (A) normal (B) paracetamol treated (C) Silymarin treated (D, E) Extract treated

4. CONCLUSION

Serum levels of the enzymes ALT, AST, and ALP were markedly elevated after PCM therapy, suggesting chemically induced hepatocellular damage. These enzymes' serum levels are highly sensitive indicators used to diagnose liver disorders. The enzymes that are typically found in the cytosol are released into the bloodstream when the hepatocellular plasma membrane is disrupted. To determine the kind and degree of liver damage, this can be measured. The injection of PCM was also seen to increase the levels of the serum marker enzymes ALT, SGOT, SGPT, serum bilirubin, serum triglycerides, and cholesterol in the current investigation. All blood values were lower in the group treated with an aqueous extract of *Curcuma caesia* and silymarin than in the group treated with PCM. An obvious sign of improved liver cell activity is the stabilization of serum ALT, SGOT, SGPT, bilirubin, triglyceride, and cholesterol levels by *Curcuma caesia* aqueous extract.

In contrast to the group that received PCM, the biochemical analysis unequivocally shows that the hepatic cells in the group treated with an aqueous extract of *Curcuma caesia* (50 and 100 mg/kg, p.o.) are normal. Because it may restore the liver function that has been damaged by PCM, the aqueous extract of *Curcuma caesia* can be regarded as an effective hepatoprotective in nature. Therefore, it was determined that *Curcuma caesia* extract has hepatoprotective properties

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