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Hepatoprotective Activity of Methanolic Extract from *Curcuma Longa* Leaves Against Paracetamol-Induced Liver Damage in Rats

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Abstract: -
Objective:
This study aims to explore the hepatoprotective effects of Curcuma longa leaves methanolic extract against paracetamol-induced liver damage.
Materials & Methods:
Thirty rats of either sex was divided into five groups (n=6 per group). Group 1 served as the control and received a basal diet, maintained as the positive control group. Group 2 received paracetamol at a dose of 3 g/kg b. wt. Group 3 received paracetamol along with the standard drug Silymarin at a dose of 100 mg/kg. Groups 4 and 5 were treated with paracetamol along with Curcuma longa plant extracts at doses of 250 mg/kg and 500 mg/kg, respectively. Hepatoprotective potential was evaluated by measuring the levels of serum glutamic oxaloacetic transaminase (SGOT)/aspartate aminotransferase (AST) and serum glutamic-pyruvic transaminase (SGPT), along with other liver biomarkers. Histopathological changes were assessed using haematoxylin and eosin staining (HE).
Result:
Activities of serum alanine aminotransferase (ALT), AST, alkaline phosphatase (ALP), triglycerides (TG), bilirubin, and total protein were measured. Paracetamol exposure led to significant increases in urea, sodium, potassium, and chloride levels in rats. Moreover, it caused damage to liver structures. Pre-treatment with methanolic extract of Curcuma longa prevented severe alterations in biochemical parameters and liver structure disruptions induced by paracetamol.
Conclusion:
This study clearly demonstrates that pre-treatment with methanolic extract of Curcuma longa significantly attenuated physiological and histopathological alterations induced by paracetamol.
Key words: Hepatotoxicity, Paracetamol, Silymarin, *Curcuma longa*, SGOT, SGPT.

INTRODUCTION:

The liver serves as a pivotal site for detoxification and metabolism of various drugs and xenobiotics. Misuse or overuse of medications can lead to liver dysfunction, characterized by hepatocyte death, making liver disease a significant global health concern. Causative factors range from diseases that disrupt liver function to chemical exposures such as ethanol, CCl4, thioacetamide, and D-galactosamine, as well as pharmaceuticals like paracetamol. Given the undesirable effects of synthetic agents, there is a growing interest in evaluating the therapeutic potential of medicinal plants through comprehensive research methodologies [1].

Curcuma longa L. (Zingiberaceae), commonly known as turmeric, is an herbaceous plant extensively cultivated across Asia, notably in India and China [2]. Traditionally valued for its medicinal properties, turmeric offers a spectrum of biological activities including energy enhancement, antioxidant, antibacterial, anti-inflammatory, anticancer, and wound healing effects [3,4]. These benefits are attributed to its primary constituents, curcuminoids, which encompass demethoxycurcumin, bisdemethoxycurcumin, and curcumin [5]. Additionally, turmeric leaves, commonly incorporated into various Southeast Asian cuisines, are believed to possess antioxidant properties [6]. Despite this, turmeric leaves are often discarded as byproducts, except for their occasional use in animal feed post-harvest [7]. Research indicates that turmeric leaves harbour bioactive compounds, such as curcumin, various phenolic compounds, and flavonoids [8], yet there remains a dearth of literature elucidating their functionalists.

Given that curcumin, alongside other phenolic compounds and flavonoids found in medicinal plants, are recognized for their hepatoprotective effects [3], this study endeavours to explore the potential of turmeric leaf extract (TLE) in mitigating Paracetamol-induced hepatotoxicity. By examining the composition of functional compounds within turmeric leaf extract and their impact on liver toxicity using an in vivo model, our aim is to elucidate the hepatoprotective properties of turmeric leaves.

MATERIAL AND METHOD

Plant Material

The plant specimens were sourced from the local vicinity of Indore, Madhya Pradesh, India. Authentication was conducted by Dr. S.N. Dwivedi from the Department of Botany, Janata PG College, A.P.S. University, Rewa, Madhya Pradesh, India.

Preparation Of Extract

Air-dried and coarsely powdered leaves (1 kg) underwent Soxhlet extraction with methanol for 72 hours. The resultant ethanolic extract was concentrated using a water bath and subsequently dried under reduced pressure to yield a dark brown mass (95 g; yield: 9.5%).

Phytochemical Analysis

The methanolic extract of Curcuma longa underwent conventional phytochemical analysis for alkaloids, carbohydrates, glycosides, flavonoids, saponins, tannins, proteins, amino acids, etc.

Experimental Animals

Hepatoprotective activity was assessed in rats of either sex (110-145 g) obtained from the animal house. Animals were housed in polyacrylic cages with six animals per cage, maintained at a temperature of 24 ± 2 ºC and relative humidity of 30-70%. A 12-hour light/dark cycle was maintained at 25 ± 2°C. Rats had free access to standard pellet diet and water ad libitum, with a one-week acclimatization period before experimentation. All procedures were approved by the Institutional Animal Ethics Committee.

Drugs And Dosing Schedule

Animals were divided into six groups: control, PCM-treated, PCM + silymarin-treated, and two PCM + extract-treated groups. PCM was administered orally in a single dose of 3g/kg body weight per day for 7 days. Additionally, animals in PCM + silymarin and PCM + extract groups received silymarin suspension (100 mg/kg body weight, IP) and methanolic extract at doses of 100 mg/kg and 200 mg/kg body weight, IP, respectively, for 7 days. Control group received distilled water.

Serum Analysis

On day 5 of treatment, animals were anaesthetized and sacrificed. Blood was collected from the heart, and serum was separated by centrifugation for analysis of biochemical parameters including serum transaminases (SGOT, SGPT), total protein, total albumin, alkaline phosphatase, and total bilirubin content.

Histopathological Examination

Liver tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned (4 µm thickness), and stained with haematoxylin and eosin for light microscopic examinations.

Statistical Analysis

Data were expressed as mean ± S.E.M and analyzed using ANOVA followed by Dunnett test, with $p < 0.05$ considered significant.

RESULTS

Percent Yield of the 70% Methanol Crude Extract and Solvent Fractions

The leaves of Curcuma longa were extracted with 70% methanol using a Soxhlet apparatus, yielding 23.4% of crude extract.

Acute Oral Toxicity Test

The acute oral toxicity test indicated that the 70% methanol leaf extract of Curcuma longa did not cause gross behavioural changes or mortality within 24 hours or 14 days, suggesting a median lethal oral dose greater than 2000 mg/kg in rats.

HEPATOPROTECTIVE ACTIVITY

Effect on Body Weight, Change in Body Weight and Absolute and Relative Liver Weight 70% Methanol Extract

The impact of the 70% methanol extract on body weight, alterations in body weight, and the absolute and relative weights of the liver in rats are depicted in Table 1. While Group I (Control) displayed no significant change in body weight, Groups II, III, IV, and V exhibited a noticeable increase in body weight ($p<0.05$). Notably, the absolute and relative weights of the livers in rats treated with paracetamol were notably elevated compared to the control group.

Table 1 Effect of 70% Methanol Extract of the Leaves of *Curcuma longa* on Body Weight, Change in Body Weight and Absolute and Relative Liver Weight of Rats Administered with PCM.

Groups	Change in body weight (g)		Liver Weight (g)	Relative liver weight
	Initial	Final		
Control	185.07 ± 4.06	187.07 ± 6.6	5.11 ± 3.20	4.11 ± 2.02
PCM treated	175.16 ± 6.16	178.23 ± 3.04	6.01 ± 3.20	4.57 ± 1.18
PCM + Silymarin	180.02 ± 5.45	183.49 ± 6.75	4.24 ± 3.20	3.42 ± 2.10
PCM + Extract (100g)	182.39 ± 3.48	184.39 ± 6.19	5.37 ± 3.20	4.05 ± 2.06
PCM + Extract (200g)	184.27 ± 2.35	186.53 ± 4.25	4.18 ± 3.20	3.03 ± 1.20

Impact on Serum Biochemical Markers of Liver Injury 70% Methanol Extract

The hepatotoxic agent, PCM, induced significant liver damage, evident from elevated levels of liver chemistry biomarkers such as AST, ALT, and ALP, alongside a decline in liver function biomarkers including total protein, albumin, and bilirubin (refer to Table 2). PCM administration resulted in a marked increase in AST, ALT, and ALP levels. However, rats pretreated with crude 70% methanol extract of Curcuma longa leaves at doses of 100 mg/kg and 200 mg/kg exhibited a significant reduction in AST, ALT, and ALP levels ($p<0.05$) compared to the PCM-administered group. Notably, the higher dose (200 mg/kg) demonstrated superior hepatoprotective activity compared to the lower dose.

Table 2 Effect of 70% Methanol Extract of the Leaves of *Curcuma longa* on Liver Function and Liver Chemistry of Rat Administered with PCM

S. No.	Groups	AST (mg/dl)	ALT (mg/dl)	ALP (mg/dl)	Albumin (mg/dl)	Total Protein (mg/dl)	T.B. (mg/dl)
1.	Control	78.12 ± 3.02	98 ± 4.32	158.35 ± 2.37	2.95 ± 0.37	7.05 ± 0.37	3.95 ± 0.89
2.	PCM treated	218.45 ± 15.12	235.07 ± 7.06	347.94 ± 3.98	3.85 ± 9.15	6.10 ± 7.15	7.23 ± 5.49
3.	PCM + Silymarin	89.73 ± 6.84	92.45 ± 4.56	152.94 ± 3.56	4.05 ± 0.39	5.01± 0.21	3.11 ± 0.37
4.	PCM + Extract (100g)	115.70 ± 5.62	127.30 ± 4.07	195.26 ± 3.22	4.10 ± 0.54	5.95 ± 0.46	3.09 ± 016
5.	PCM + Extract (200g)	85.25 ± 1.37	120.57 ± 5.78	175.65 ± 3.54	4.15 ± 0.43	7.01 ± 0.85	3.15 ± 0.93

Histopathological results

Microscopic examination of the liver in the normal group revealed typical features including polygonal nuclei with nucleoli, abundant cytoplasm, and bilobed nuclei, with no discernible changes or disarrangement in hepatic cells (refer to Figure 1). Conversely, the toxic control group treated with paracetamol exhibited severe histopathological alterations. Prolonged administration of paracetamol for seven days resulted in profound impairment of liver structure, characterized by disorganized hepatic strands, histological signs of necrosis, enlarged sinusoids, vacuole formations in hepatocytes, leukocytic infiltrations, and dilation and congestion of blood vessels with haemorrhage (refer to Figure 2).

Administration of the methanolic extract of Curcuma longa partially restored the cellular arrangement around the central vein and reduced necrosis (refer to Figure 5). Additionally, it facilitated the normalization of blood vessel morphology (refer to Figure 4). In comparison, the standard group treated with Silymarin alongside paracetamol exhibited less disarrangement and degeneration of hepatocytes, indicative of marked regenerative activity (refer to Figure 3).

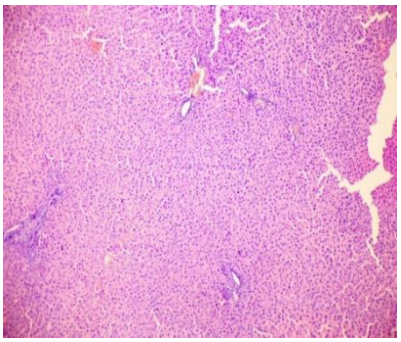

Figure 1

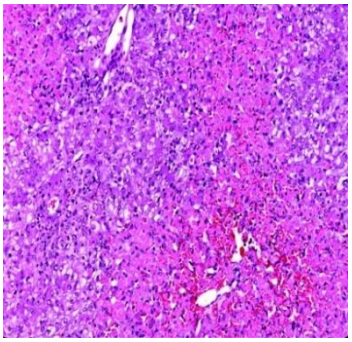

Figure 2

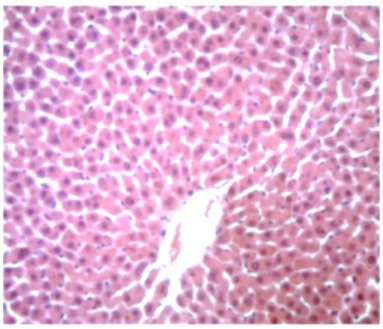

Figure 3

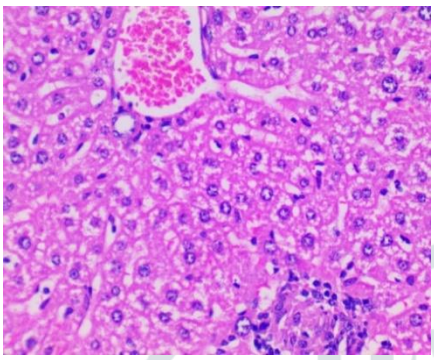

Figure 4

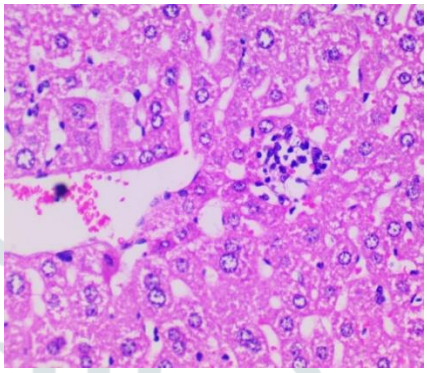

Figure 5

Figure 1-5: Histological monograph of extract and standard 1. Normal; 2. Paracetamol (3g/kg); 3. Silymarin (100mg/kg) 4. Paracetamol + Methanolic extract (100 mg/kg); 5. Paracetamol + Methanolic extract (200mg/kg).

DISCUSSION

Paracetamol, a widely used analgesic and antipyretic drug, has been associated with hepatocellular damage or necrosis at higher doses in both experimental animals and humans [14]. The induction of paracetamol-induced hepatotoxicity has been established as a reliable method for screening hepatoprotective agents. Metabolized primarily in the liver and eliminated through conjugation with sulphate and glucuronide, paracetamol metabolism can lead to the generation of toxic metabolites, notably N-acetyl-p-benzoquinone imine (NAPQI), through hepatic cytochrome P-450 activation [15]. These reactive metabolites can cause initial cell stress by depleting glutathione (GSH) or binding to various cellular structures, ultimately resulting in liver damage [17].

Serum markers such as AST, ALT, ALP, and bilirubin are commonly used to assess liver injury [18, 19]. Paracetamol administration significantly increased the levels of these enzymes and bilirubin, indicative of hepatotoxicity [20]. However, co-administration of the tested plant extracts mitigated the elevation of these markers, suggesting hepatoprotective effects. This aligns with the notion that normalization of AST, ALT, and ALP levels reflects the restoration of hepatic parenchyma and hepatocyte regeneration [21].

Paracetamol-induced hepatotoxicity is also associated with reduced serum albumin levels, likely due to the formation of protein adducts by toxic metabolites such as NAPQI [22]. Curcuma longa demonstrated excellent hepatoprotective properties by preventing the elevation of serum biochemical parameters associated with paracetamol toxicity. Additionally, it enhanced the activity of catalase, an enzyme involved in detoxifying hydrogen peroxide, thus reducing oxidative stress in the liver [23].

Increased levels of TBARS in the liver indicate enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defence mechanisms. GSH depletion is considered a key mechanism in paracetamol-induced hepatotoxicity, as it is involved in detoxifying free radicals and maintaining cellular integrity [16]. Curcuma longa showed superior ability to reduce oxidative stress by increasing GSH levels and preventing lipid peroxidation compared to other tested plants.

Phytochemical analysis revealed that Curcuma longa contains compounds such as triterpenes, flavonoids, and alkaloids, which likely contribute to its hepatoprotective activity [24]. Overall, Curcuma longa extract demonstrated significant protection against paracetamol-induced liver damage, making it a promising natural remedy for hepatoprotection.

CONCLUSION

This study investigated the hepatoprotective activity of aqueous extract of Curcuma longa. The extract, particularly at a dose of 200 mg/kg, exhibited hepatoprotective activity comparable to silymarin, as evidenced by significant decreases in transaminase enzyme levels and preservation of hepatocellular membrane integrity ($P<0.05$). Identifying the natural compounds present in plants can pave the way for developing new therapeutic agents, and the results of this study highlight Curcuma longa as a promising source for hepatoprotective activity. Its cost-effectiveness and availability make it a viable option for natural treatment. Further clinical trials are warranted to develop formulations suitable for widespread use, especially considering the rising costs of conventional treatments and the need for more accessible remedies for chronic diseases.

REFERENCE

1. J.C Reed "Effects of curcumin on ethanol-induced hepatocyte necrosis and apoptosis: implication of lipid peroxidation and cytochrome c" Vol.379, pg-27-60, (2009)
2. Verma R.V., Kumari P., Maurya R.K., Kumar V., Verma R.B., Singh R.K. Medicinal properties of turmeric (*Curcuma longa* L.): A review. *Int. J. Chem. Stud.* 2018;6:1354–1357. doi: 10.13040/IJPSR.0975-8232.4.03
3. Uchio R., Higashi Y., Kohama Y., Kawasaki K., Hirao T., Muroyama K., Murosaki S. A hot water extract of turmeric (*Curcuma longa*) suppresses acute ethanol-induced liver injury in mice by inhibiting hepatic oxidative stress and inflammatory cytokine production. *J. Nutr. Sci.* 2017;6 doi: 10.1017/jns.2016.43.
4. Bomdyal R.S., Shah U.M., Doshi S.Y., Shah A.V., Khirade P.S. Antibacterial activity of curcumin (turmeric) against periopathogens—An in vitro evaluation. *J. Adv. Clin. Res. Insights.* 2017;4:175–180. doi: 10.15713/ins.jcri.188.
5. Na L.-X., Li Y., Pan H.-Z., Zhou X.-L., Sun D.-J., Meng M., Li X.-X., Sun C.-H. Curcuminoids exert glucose-lowering effect in type 2 diabetes by decreasing serum free fatty acids: A double-blind, placebo-controlled trial. *Mol. Nutr. Food Res.* 2013;57:1569–1577. doi: 10.1002/mnfr.201200131.
6. Rubya A.J., Kuttan G., Babub K.D., Rajasekharanb K.N., Kutta R. Anti-tumour and antioxidant activity of natural curcuminoids. *Cancer Lett.* 1995;94:79–83. doi: 10.1016/0304-3835(95)03827-J.
7. Liu Y., Nair M.G. Curcuma longa and Curcuma mangga leaves exhibit functional food property. *Food Chem.* 2012;135:634–640. doi: 10.1016/j.foodchem.2012.04.129.
8. Choi W.Y., Lee H.Y. Enhancement of Antioxidant Activities of *Curcuma longa* Leaves by Ultra High Pressure Extraction. *Korean J. Med. Crop Sci.* 2014;22:121–126. doi: 10.7783/KJMCS.2014.22.2.121.
9. Braga M.C., Vieira E.C.S., de Oliveira T.F. *Curcuma longa* L. leaves: Characterization (bioactive and antinutritional compounds) for use in human food in Brazil. *Food Chem.* 2018;265:308–315. doi: 10.1016/j.foodchem.2018.05.096.
10. Rietman S and Frankel S. A colorimetric determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases.Am. J. Clin. Pathol. (1957) 28: 56-63.
11. Gornall A, Bardawil J and David MM. Determination I S U Q \ E X U P G P HP (1949) 177: 751.
12. Kind PRN and King EJ. Determination of serum alkaline phosphatase. J. Clin. Path. (1954) 7: 132-136.
13. Malloy HT and Evelyn KA. The determination of bilirubin with the photometric colorimeter. J. Biol. Chem. (1937) 119: 481-490.
14. Vermeulen, N.P.E., Bessems, J.G.M. and Van de straat, R., "Molecular aspects of paracetamol induced hepatotoxicity and its mechanism -based prevention" Drug Metabolism Reviews., 24: 367-407 (1992)
15. Savides, M.C., Oehme, F., Nash S.L., and Leipold H.W., "The toxicity and biotransformation of single doses of acetaminophen in dog and cats" Toxicology and Applied Pharmacology., 3: 95-111 (1983).
16. Mitchell, J.R., Jollow, D.J, Potter, W.Z., Gillette, J.R. and Brodie, B.B. "Acetaminophen induced hepatic necrosis. I. Role of covalent binding in vivo" Journal of Pharmacology and Experimental Therapeutics 187: 211- 217(1973).
17. Pauli-Magnus, C., Stieger, B., Meier, Y. Kullak-Ublick, G.A. and Meier, P.J. "Enterohepatic transport of bile salts and genetics of cholestasis" Journal of Hepatolology.,43(2): 342-357 (2005).
18. Kozer, E., Evans, S., Barr, J., Greenberg, R., Soriano I, Bulkowstein, M, Petrov, I., Chen-Levi, Z., Barzilay, B. and Berkovitch, M., "Glitathione- dependent enzymes and antioxidant status in erythrocytes from children treated with high-dose paracetamol" British Journal of Clinical Pharmacology, 55(3): 234- 40 (2003).
19. Girish, C., Koner, B.C., Jayanthi, S., Rao, K.R., Rajesh, B. and Pradhan, S.C. "Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice" Indian Journal of Medical Research, 129(5): 569- 578 (2009).
20. Sallie, R., Tredger, J.M. and William, R. "Hepatoprotective, DNA damage prevention of Spirulina Platensis on CCL4 induced hepatotoxicity in mice" Biopharmaceutics Drug Disposal., 12: 251-259 (1991)
21. Thabrew, M. and Joice, P., "A comparative study of the efficacy of Pavette indica and Osbeckia octanda in the treatment of liver dysfunction" Planta Medical., 53: 239–241 (1987).
22. Zaher, H., Buters, J.T, Ward, J.M., Bruno, M.K., Lucas, A.M., Stern, S, T, Cohen, S.D., and Gonzalez, F.J., "Protection against acetaminophen toxicity in CYP1A2 and CYP2E1double null mice" Toxicology Applied Pharmacology, 152(1): 193-9 (1998).
23. Parmar, S.R., Dave, G.S., Patel, H.V. and Kalia K., "Hepatoprotective value of some plants extract against CCL4 toxicity in male rats" Journal of Cell and Tissue Research., 9(1): 1737- 1743 (2009).
24. Gupta, A.K. and Misra, N. "Hepatoprotective activity of aqueous ethanolic extract of chamomile capitula in paracetamol intoxicated albino rats" American Journal of Pharmacology and Toxicology, 1: 17-20 (2006).