ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics

Available Online at www.jddt.in

CODEN: - JDDTBP (Source: - American Chemical Society)

Volume 11, Issue 1, January-February: 2023, 43-50

Research Article

Hepatoprotective Effect of Herbal Medicinal Plant Extract against Paracetamol Induced Liver Injury in Rat

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Article Info: Received: 08-12-2022 / Revised: 12-01-2023 / Accepted: 20-02-2023

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Conflict of interest: No conflict of interest.

Abstract:

The purpose of this study was to examine the hepatoprotective efficacy of ***Curcuma amada rhizome*** extracts against Paracetamol induced liver damage in rats. Wistar albino rats were orally fed 500 mg/kg of body weight of Methanolic and Hydroethanolic ***Curcuma amada rhizome*** extract, with Silymarin serving as the reference. By restoring functional parameters, physical parameters, biochemical parameters, and decreasing blood enzymes alkaline phosphatase (ALP) and total bilirubin (TBL) in the selected animal, the methanolic and hydroethanolic extracts exhibited a potent hepatoprotective effect. The plant's chemical composition includes, among others, alkaloids, flavonoids, glycosides, steroids, terpenoids, phenolics, and saponins. The overall experimental data suggest that bioactive phytoconstituents, such as flavonoids and alkaloids, present in the Methanolic and Hydroethanolic extracts of ***Curcuma amada rhizome*** may be responsible for the plant's significant hepatoprotective effect. The results thus support the use of ***Curcuma amada*** as a hepatoprotectant.

Keyword

Hepatoprotective activity, ***Curcuma amada rhizome***.

Introduction

Curcuma name was coined by Linnaeus in 1753 in his Species Plantarum. The word likely gets its reference from the Arabic word 'kurkum', which means yellow colour. *Curcuma amada* Roxb. (Family: *Zingiberaceae*) is enduring rhizomatic fragrant herb which is known as Mango ginger and is available from month November to April. Mango ginger (*Curcuma amada* Roxb.) is an antique spice which has

morphological resemblance with ginger but it imparts a raw mango flavour. The genus originated in the Indo-Malayan region, and is broadly distributed in the tropics of Asia to Africa and Australia. **[1]**. In India it is found in regions of West Bengal, Madhya Pradesh, Chattisgarh Orissa, and Uttar Pradesh. It prospers well in wet deciduous woodland regions. Rhizomes of another species *Curcuma amada* (Mango ginger) normally known as Amba Haldi, grown in west Bengal and on the slopes of West coast of India. Mango ginger is grown in regions of Gujarat and discovered wild in pieces of West Bengal, U.P, Karnataka and Tamil Nadu. **[2**,**3]**, ***Curcuma amada*** Roxb.is a rhizomatous fragrant spice with a leafy tuft and grows upto height of 60-90 cm. Leaves are long, petiolate, oval lanceolate, tightening at the two closures, glabrous and green on the two sides. **[4, 5]**. Rhizome is small in size, 3×1 cm, cone like brownish; roots many, some ending in root tubers. Flowers are longer than the bracts, 4.5-5.5 cm; bract 3-4 cm; light yellow. Calyx 1-1.2 cm, 3 lobed at peak, violet dabbed, thickly pubescent. Corolla tube light yellow, Seeds 3.5-5 mm long obviate; aril white **[6,7,8]**. Indian medication utilizes turmeric powder for the treatment of biliary issues, cold and flu, anorexia, hepatic problems, diabetic injuries, rheumatics and sinusitis. The rhizome is very pungent, healing, bitter, laxative, vulnerary anthelmintic, tonic and emollient. It is also used as a medicine in various kapha and vata blood diseases. It is used in the treatment of bronchitis, vertigo, dropsy, skin diseases, elephantiasis, burns, boils, sprains, fevers, swellings, chronic gonorrhea, bruises, chicken pox, small pox, snake bites, scabies, dyspepsia, ring worm, etc. **[9-14]**. Here, we evaluated the ability of methanol and hydroethanol ***Curcuma amada rhizome*** extracts to prevent or minimize the severity of Paracetamol induced liver damage in rats.

Procurement and Authentication of the Plant

The stems of ***Curcuma amada rhizome*** were collected from the area around Alwar, Rajasthan, and then submitted to the Department of Botany at Sunrise University, Alwar, Rajasthan, where they were authenticated.

Preparation of extracts of *Curcuma amada rhizome*

In order to increase polarity, 500g of powdered ***Curcuma amada rhizome*** were extracted using a Soxhlet device and a solvent. By means of evaporation, the materials were concentrated [15].

Animals

The Central Drug Research Institute in Lucknow, India, supplied 150-200 g Wistar albino rats. The animals were given a normal pellet diet (Hindustan lever Ltd., Bangalore) and had access to drink at will. Each animal was acclimated for one week prior to usage. After examination, the Institutional Animal Ethics Committee authorized the experimental procedures. The medication was administered to the animals using an oral gavage tube. All animals were cared for ethically in accordance with CPCSEA norms, and rats were subjected to frequent inspections. The laboratory settings are supervised by a licensed veterinarian [16].

Chemicals

All chemical and solvent was of analytical quality. Micro Lbs in Goa, India, provided a complimentary sample of silymarin. India's Span Diagnostics Ltd. supplied standard kits for SGOT, SGPT, ALP, and others.

Preliminary phytochemical analysis

An initial phytochemical analysis was conducted to establish which phytoconstituents were present in each extract.

Toxicity studies

According to the OECD 423 guidelines [16], all of the extracts were submitted to an acute toxicity test. Albino female rats were used for research into acute toxicity. Before giving the extract orally at doses of 100, 200, and 500mg/kg bw, the animals were fasted for 24 hours with just water and then observed for up to 72 hours for toxic signs. All extracts had a therapeutic oral dose of 500 mg/kg body weight.

Carbon tetrachloride induced hepatotoxicity

The rats, of both sexes, were split up into 6 groups of six each. (n = 6) **[16, 17]**

Group I (Control): administered water (5 mililitre/kilogram, p.o.) *o.d.* for nine days.
Group II (-ve control): administered water (5 mililitre/kilogram, p.o.) *o.d.* for nine days, meanwhile on the seventh day, paracetamol (1 g/kg p.o.) was administered.
Group III (+ve control): administered the normal medicine silymarin (25 mg/kg, p.o.) *o.d.* for nine days, meanwhile on the seventh day, paracetamol (1 g/kg p.o.) was administered.
Group IV and V (Test Sample) administered The M**ethanolic (*MECA*) & Hydroethanolic (*HEECA*)** (500 mg/kg) extract of ***Curcuma amada rhizome*** *o.d.* for nine days, meanwhile on the seventh day, paracetamol (1 g/kg p.o.) was administered.

Blood was collected from animals on the last day by puncturing the retro orbital plexus. Blood samples were allowed to clot at room temperature for 45 minutes. SGOT & SGPT [18], ALP [19], serum bilirubin [20], and serum protein [21] were measured after serum was separated by centrifugation at 2500 rpm at 30°C for 15 minutes and used for the assessment of different biochemical parameters. After the collection of blood samples, the animals were sacrificed while deeply sedated with ether. In evaluating the drug's preventive impact, morphological data such as animal weight and liver weight have also been considered. The hepatoprotective chemical decreases the liver weight per 100 g of rat body weight [22, 23].

Histopathology studies

In each group of animals, a small piece of liver tissue was removed and washed in normal saline. The liver tissues were paraffin embedded after being fixed in 10% buffered neutral formalin for 48 hours and then in bovine solution for 6 hours. Staining with hematoxylin and eosin was performed on slices that were 5 mm thick and cut using a microtome. These slices were looked at using a light microscope at a magnification of 100X [24].

Statistical Significance

The results of the study were expressed as mean ± SEM, n=6. ANOVA **[25]** was used to analyze and compare the data, followed by Dunnet's **[26]** test for multiple comparisons.

Results

The phytoconstituents in the various extracts were detected using chemical analysis. Results show that the highest concentrations of pharmacologically active chemicals, including glycosides, sponins, phytosterols, and flavonoids, are found in the Methanolic (***MECA***) and Hydroethanolic (***HEECA***) extract of ***Curcuma amada rhizome***. In light of this, the pharmacological studies choose to focus on these extracts. Table 1 displays the results. At a dosage of 5000 mg/kg, there was no toxicity or behavioural alterations seen in the animal groups that were subjected to the graduated dose. This result indicates that 500 mg/kg, p.o. of Methanolic (***MECA***) & Hydroethanolic (***HEECA***) extracts of ***Curcuma amada rhizome*** were safe or non-toxic to rats. After receiving a thiopentone sodium (40 mg/kg) intramuscular injection, all test groups

exhibited sleepiness. Rats given **Paracetamol** slept for longer periods overall and had considerably later sleep onset times (measured in minutes). Methanolic (***MECA***) and hydroethanolic (***HEECA***) ***Curcuma amada rhizome*** extracts (500 mg/kg, p.o.) and silymarin significantly enhanced sleep start but significantly decreased sleep duration in rats compared to a **Paracetamol** treatment group. The results are shown in Table 2. Both the weight and volume of the livers of those who were given, **Paracetamol** showed growth, suggesting that the livers of those people had enlarged. ***Curcuma amada rhizome*** extracts *ie.,* (***MECA***) and hydroethanolic extract (***HEECA***) (500 mg/kg, p.o.) substantially recovered liver weight compared to the control group. The results are shown in Table 3. Pretreatment with ***Methanolic (MECA) & Hydroethanolic (HEECA) extract of Curcuma amada rhizome*** (500 mg/kg, p.o.) and silymarin (25 mg/kg, p.o.) showed a capacity to prevent the hepatotoxicity by reducing serum marker enzymes. Findings are shown in Table 4. An elevation in total bilurubin and a decrease in total protein content were observed in **Paracetamol**-treated groups. Total bilirubin was significantly decreased and total protein was significantly increased after pretreatment with a Methanolic (***MECA***) and Hydroethanolic (***HEECA***) extract of ***Curcuma amada rhizome*** (500 mg/kg, p.o. Outcomes are shown in Table 4.

Table 1: Preliminary Phytochemical studies of Extracts of *Curcuma amada rhizome* Stem

Constituents	*Methanolic Extract (MECA)*	*Hydroethanolic Extract (MECA)*
Carbohydrate	+	+
Glycosides	-	-
Oil and fats	-	+
Proteins	+	+
Saponins	-	-
Phenolic comp. and tannins	+	+
Phytosterols	+	+
Alkaloids	+	+
Gums and mucilage	+	-
Flavonoids	+	+

Table 2: Effect of *Methanolic (MECA) & Hydroethanolic (HEECA) extract of Curcuma amada rhizome* stem on functional parameters in Paracetamol induced hepatotoxic rats.

Treatment/ Dose	Onset of sleep(Sec.)	Duration of sleep (Min.)
Normal	169.2 ± 2.13	105.02 ± 1.43
Paracetamol	92.25± 5.19*	224.2 ± 6.94*
Silymarin	187.5 ± 5.22***	154.6 ± 3.29***
MECA (500 mg/kg)	178.8 ± 3.74**	252.2 ± 3.16**
HEECA (500 mg/kg)	188.6 ± 4.54***	189.44 ± 4.72***

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with **Paracetamol** induced group and * P<0.05, when compared with normal group.

Table 3: Effect of *Methanolic (MECA) & Hydroethanolic (HEECA) extract of Curcuma amada rhizome* ste\m on Physical Parameters in Paracetamol induced hepatotoxic rats.

Treatment/ Dose	Liver Weight	Liver Volume
Normal	7.29 ± 0.04	7.49 ± 0.06
Paracetamol	8.98 ± 0.56*	8.54 ± 0.48*
Silymarin	7.98 ± 0.42***	7.69 ± 0.57***
MECA (500 mg/kg)	8.22 ± 0.44***	8.12 ± 1.44**
HEECA (500 mg/kg)	7.86 ±0.65***	7.69 ± 0.98***

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with **Paracetamol** induced group and * P<0.05, when compared with normal group.

Table 4: Effect of *Methanolic (MECA) & Hydroethanolic (HEECA) extract of Curcuma amada rhizome* stem on serum enzyme parameter in Paracetamol induced hepatotoxic rats.

Treatment/ Dose	SGPT U/L	SGOT U/L	ALP U/L	Total Bilirubin (mg/dl)	Total Protein (gm/dl)
Normal	75.02 ± 4.63	189.06± 3.52	187.02 ± 7.02	0.55 ± 0.11	8.68 ± 0.35
Paracetamol	198.23 ± 6.43*	378.78 ± 7.32*	368.34 ± 6.45*	6.68 ± 7.04*	6.23 ± 0.12*
Silymarin	92.43 ± 4.32***	163.63 ± 3.54***	246.32 ± 7.24***	0.64 ± 2.43***	8.84 ± 4.68***
MECA (500 mg/kg)	168.04 ± 4.54***	276.14 ± 5.53***	262.21 ± 5.35***	0.84 ± 3.29***	6.82 ± 5.12**
HEECA (500 mg/kg)	142.31 ± 5.66***	189.12 ± 7.55***	254.19 ± 5.52***	0.82 ± 0.65***	7.24 ± 2.54***

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with **Paracetamol** induced group and * P<0.05, when compared with normal group.

Discussion

Many variables, such as chemicals and medicines, may cause liver damage or injury. Because it is clinically relevant, **Paracetamol** was employed to produce hepatotoxicity in this investigation. Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are indicators of hepatocellular damage [27].

Paracetamol is a common antipyretic and analgesic. Several research in animals and people have shown that greater dosages of acetaminophen cause liver damage. Paracetamol-induced hepatotoxicity has been utilized as a reliable approach for screening Hepatoprotective drugs. Paracetamol is largely metabolized in the liver and excreted by the kidney after conjugation with sulphate and glucuronide. Furthermore, paracetamol hepatotoxicity has been linked to the creation of hazardous metabolites when a portion of the drug is converted by hepatic cytochrome P-450 to the highly reactive metabolite N acetyl-p-benzoquinoneimine (NAPQI). Toxic metabolites (N-acetyl-p-benzoquineimine)

have the ability to alkylate and oxidize intracellular GSH, resulting in liver damage. GSH depletion causes enhanced lipid peroxidation by extracting hydrogen from a polyunsaturated fatty acid and, eventually, liver injury owing to larger paracetamol dosages. Reactive metabolites may cause early cell stress by a variety of methods, including glutathione (GSH) depletion or binding to enzymes, lipids, nucleic acids, and other cell components. AST and ALP are normally seen in significant concentrations in the liver. These enzymes are released from the cells as a result of hepatocyte necrosis or aberrant membrane permeability, and their levels in the blood rise. ALT is a sensitive marker of acute liver injury, and its rise in non-hepatic illnesses is rare. ALT is a more specific hepatic paranchymal enzyme than AST. **[28]**.

The administration of paracetamol to experimental animals resulted in a statistically significant increase in the levels of enzymes such as SGOT, SGPT, ACP, ALP, and others, suggesting chemical-induced liver injury. The inhibitory impact of ***Curcuma amada rhizome*** Stem Methanolic (***MECA***) and Hydroethanolic (***HEECA***) extracts (500 mg/kg, p.o.) on hepatotoxicity was compared to that of the positive control group. Pretreatment of the animals with ***Methanolic (MECA) & Hydroethanolic (HEECA) extract of Curcuma amada rhizome*** Stem (500 mg/kg, p.o.) provided considerable protection in biochemical parameters such as SGOT, SGPT, ACP, and ALP against **Paracetamol**-induced increases. Furthermore, there was an increase in the weight of the liver treated with **Paracetamol** when compared to the control. The ***Methanolic (MECA) & Hydroethanolic (HEECA) extract of Curcuma amada rhizome*** Stem (500 mg/kg, p.o.) therapy keeps the liver weight close to normal. A control rat's liver segment reveals a normal hepatic architectural wall caused by the central vein. The liver samples of **Paracetamol**-treated rats showed gross necrosis of the centrilobular hepatocytes characterized by gross necrosis, degeneration, karyolysis, and eosinophilic infiltration, which was significantly prevented by treatment with the ***Methanolic (MECA) & Hydroethanolic (HEECA) extract of Curcuma amada rhizome*** Stem (500 mg/kg, p.o.) that demonstrated hepatoprotective activity A number of scientific studies have shown that some flavonoids, triterpenoids, and steroids have antioxidant characteristics that protect the liver. Administration of ***Methanolic (MECA) & Hydroethanolic (HEECA) extract of Curcuma amada rhizome*** Stem (500 mg/kg, p.o.) that demonstrated significant hepatoprotective activity; while qualitative phytochemical investigations on the ***Methanolic (MECA) & Hydroethanolic (HEECA) extract of Curcuma amada rhizome*** Stem (500 mg/kg, p.o.) that demonstrated positive flavonoids by chemical tests. Furthermore, it has been observed that the plant's flavonoid contents have antioxidant qualities and have been proven to be effective in the treatment of liver damage. [**29]**

Treatment with hepatoprotective medications may help the liver's hepatocytes endure **Paracetamol**'s damaging effects. The findings showed that ***Curcuma amada rhizome*** stem extract (500 mg/kg, p.o.) exhibits considerable hepatoprotective action when tested in methanolic (***MECA***) and hydroethanolic (***HEECA***) solvents. The data showed that the hepatotoxic effects of Paracetamol were significantly reduced.

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