Available online on 15.01.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Qualitative Phytochemical Investigation and Acute Oral Toxicity Study on Ethanolic Extract of *Curcuma longa* Leaves

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Article Info:

Article History:

Received 21 Oct 2023
Reviewed 06 Dec 2023
Accepted 29 Dec 2023
Published 15 Jan 2024

Cite this article as:

Chourasiya P, Gour R, Patel AK, Qualitative Phytochemical Investigation and Acute Oral Toxicity Study on Ethanolic Extract of *Curcuma longa* Leaves, Journal of Drug Delivery and Therapeutics. 2024; 14(1):73-78

DOI: http://dx.doi.org/10.22270/jddt.v14i1.6244

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Abstract

The study focused on evaluating the acute toxicity and phytochemical composition of *Curcuma longa* leaves using Swiss albino mice. The investigation involved a two-phase acute toxicity test, with doses up to 5000 mg/kg administered orally. The results indicated no toxicity at lower doses, while signs of weakness, salivation, and reduced movement were observed at 1600 mg/kg. Lethal effects were noted at 5000 mg/kg, establishing the LD50 at 2154.06 mg/kg. Phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, tannins, and volatile oils in the ethanolic extract. The extract exhibited a 27.37% yield, characterized by a yellowish-orange color and a sticky consistency. This comprehensive investigation sheds light on both the acute toxicity profile and the diverse phytochemical components presents in *Curcuma longa* leaves.

Keywords: *Curcuma longa*, Phytoconstituent, Acute oral toxicity, Lokes method.

INTRODUCTION

Toxicology, the foundational study of poisons, is crucial for understanding the effects of substances on living organisms. The Organization for Economic and Development (OECD) defines acute toxicity as the rapid onset of adverse effects resulting from the oral administration of a single or multiple doses within 24 hours. This field, akin to both science and art, parallels medicine in its approach, involving the collection and utilization of observational data to predict outcomes of substance exposure in both humans and animals. Throughout history, humans have classified certain plants as either harmful or safe. Recent attention has shifted towards traditional herbal therapies, such as Ayurveda Medicines, presumed to have fewer side effects compared to allopathic drugs. Consequently, there is a significant focus on identifying plants devoid of toxicity for safe human consumption. [1]

At the core of turmeric's bioactive components lies the flavonoid group known as Curcuminoids, consisting of curcumin (diferuloylmethane), demethoxy curcumin, and bisdemethoxy curcumin. Among these, curcumin constitutes the major portion, accounting for roughly 90% of the curcuminoid content in turmeric. Turmeric also comprises sugars, proteins, and resins. The extensively researched component is curcumin, which constitutes 0.3-5.4% of raw turmeric.[2]Curcuminoids, encompassing curcumin (diferuloylmethane), demethoxy curcumin, and bisdemethoxy curcumin, constitute the essence of turmeric. Alongside these compounds, turmeric contains volatile elements such as tumerone, atlantone, and zingiberone, in addition to sugars, proteins, and resins. Curcumin, a lipophilic polyphenol, exhibits minimal solubility in water but displays a notable level of stability within the acidic pH environment of the stomach.[3]

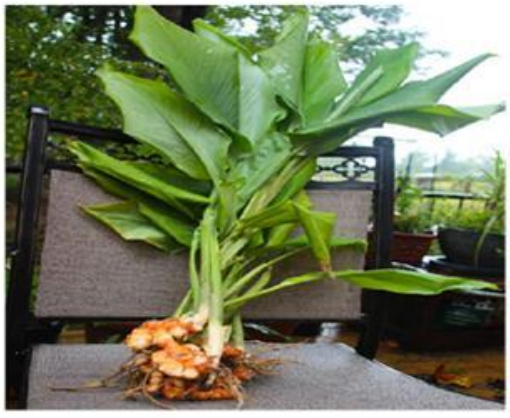

Figure 1: *Curcuma Longa* Plant.

MATERIALS AND METHODS

Selection and Collection of plant materials

Based on a review of literature and traditional assertions in different communities, the selection of *Curcuma Longa* L. was made for the present study. The plant material, specifically the leaves, was gathered from the medicinal garden located at Swami Vivekanand College of Pharmacy.

Collection and authentication of plant material

The leaves of *Curcuma Longa* L. (Zingiberaceae) were obtained from the medicinal garden at Swami Vivekanand College of Pharmacy in Indore, Madhya Pradesh, India. Taxonomic identification was conducted at the Department of Botany, Janata PG College, A.P.S. University, Rewa (486001), M.P., India, under the supervision of Dr. S.N. Dwivedi. The reference voucher specimen number is J/Bot./2023-0118, dated 20/09/2023.

Preparation of Extract

The Maceration method was employed for the extraction process. Fresh leaves were collected, thoroughly washed to remove soil and impurities, and then air-dried for 20 days. Subsequently, 60.52 grams of the air-dried leaf powder of *Curcuma Longa* was soaked in 96% ethanol solvent for 24 hours, filtered to obtain the first macerate, and then immersed again in 96% ethanol for another macerate. The two macerates were combined and poured into a porcelain evaporating dish. The resulting extract was concentrated on a boiling water bath until a semi-solid mass was achieved. The residue obtained was dried, weighed (15.67 gm), stored in a cool, dry place, and used for further experiments. The percentage yield was calculated using the following formula.[4]

The ethanolic extract was prepared by Maceration extraction technique and the % yield was calculated, its physical characteristics are tabulated as below.

Table1: Percentage extractive value and physical characteristics of extract

Extract	% yield	Colour	Odour	Consistency
Ethanolic extract	27.37%	Yellowish-orange colour	Characteristics	Sticky (semi-solid)

Figure 2: Dried Leaves

Figure 3: Extract concentrated on boiling water bath

Qualitative phytochemical analysis [5, 6]

The phytochemical screening of aqueous extract was done to identify the secondary metabolites present in the aqueous extract of *Curcuma Longa*L.using different chemical reagents.Tests were performed for the presence determination of Alkaloids, Flavonoids, Saponins, Carbohydrates, Tannins, Proteins and Terpenoids etc. Phytochemical screening was performed using standard procedure.

I. Detection of Alkaloids: -

a) Dragendroff's Test: 2 ml of ethanolic extract was taken and few drops of Dragendroff's reagent was added to it. Formation of orange brown precipitate indicated the presence of alkaloids.

b) Mayer's Test: 2 ml of ethanolic extract was taken and few drops of Mayer's reagent (Potassium mercuric iodide) was added to it. Formation of precipitate indicated the presence of alkaloids.

c) Wagner's Test: 2 ml of ethanolic extract was taken and few drops of Wagner's reagent was added to it. Formation of reddish-brown precipitate indicated the presence of alkaloids.

d) Hager's Test: 2 ml of ethanolic extract was taken and few drops of Hager's reagent was added to it. Formation of yellow precipitate indicated the presence of alkaloids.

II.Detection of Flavonoids: -

a) Lead acetate Test: - Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

b) Sulphuric acid Test: Extracts were treated with few drops of sulphuric acid. Formation of brownish red colour precipitate indicates the presence of flavonoids.

c) Zinc Test: Extracts were treated with zinc in the presence of heat. Formation of red colour indicated the presence of flavonoids.

III. Detection of Tannins: -

a) Nitric acid test: Extract were treated with dilute nitric acid. Appearance of deep red colour indicated the presence of Tannins.

b) Ferric chloride Test: Extract were treated with Ferric chloride solution. Appearance of deep blue colour indicated the presence of Tannins.

IV. Test for Saponins: -

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. There is no formation of foam which indicates the absence of saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. There is no formation of foam which indicates the absence of saponins.

V. Test for Terpenoids

a) Salkowski Test: 5 ml of ethanolic extract were taken and 2 ml of Chloroform was added to it. Further, 3 ml of concentrated sulphuric acid was added to it. Formation of reddish-brown coloured layer at the interface was formed which indicated the presence of terpenoids.

VI. Test for Glycosides

a) Legal's Test: 2 ml of ethanolic extract was taken and 1 ml of pyridine and 1 ml of sodium nitroprusside was added to the extract. Yellow colour indicated the presence of glycosides.

b) Baljet's Test: 2 ml of ethanolic extract was taken and 1 ml of sodium picrate solution was added to it. Formation of yellowish orange colour indicated the presence of glycosides.

VII. Test for Volatile oils

a) Odour test: The *Curcuma Longa* ethanolic extract possess characteristics volatile oils.

b) Filter paper test: The ethanolic extract has permanently removed the stains on filter paper, indicating the presence of volatile oils.

Table 2: Qualitative phytochemical analysis of ethanolic *Curcuma Longa* extract.

Phytochemicals	Name of test	Inference	Result
Alkaloids	Dragendroff's test	Orange brown precipitate	Positive
	Mayer's Test	Precipitate formation	Positive
	Wagner's Test	Reddish-brown precipitate	Positive
	Hager's Test	Yellow colouration	Positive
Flavonoids	Lead acetate test	Yellow precipitate	Positive
	Zinc test	Red colouration	Positive
	Sulphuric acid test	Brownish-red precipitate	Positive
Glycoside	Legal's test	Pink colouration	Positive
	Baljet's test	Yellowish-orange colour	Positive
Tannin and phenolic content	DiluteHNO_3test	Reddish-yellow colour	Positive
	Ferric chloride test	Deep blue colouration	Positive
Saponin	Foam test	Persistent foam not form	Negative
	Froth test	Formation of 1cm layer of foam	Negative
Terpenoids	Salkowski test	Layer formation at interface doesn't form	Negative
Volatile oil	Solubility test	Soluble in 90% alcohol	Positive

Selection of animals:

In acute toxicity studies per the literature survey Swiss albino mice are used as experimental model because they are physiologically very much related to human.

Experimental Animals

The experimental protocol was approved by the Animal Ethical Committee of Pinnacle Biomedical Research Institutional, (Approval No: 1824/PO/ERe/S/15/CPCSEA) and

were strictly in accordance with the norms of CPCSEA. New Delhi (CPCSEA stands for Committee for the Purpose of Control & Supervision of Experiments on Animals, India).

Healthy Swiss albino miceweighing 25-30g of either sex was obtained from the animal house of Pinnacle Biomedical Research Institutional, Bhopal for the evaluation of analgesics and anti-inflammatory activity. The animals were housed in well-ventilated standard polypropylene cages at controlled temperature (22ºC ± 3ºC) and relative humidity ranging between 50-70%. The animals had 12 hrs light: 12 hrs dark cycle. The animals were kept individually in the large spacious hygenic cages during the course of experimental period. The animals were allowed free access to standard laboratory pellets and drinking water ad libitum. The animals were allowed to acclimatize for seven days before being used for the studies.

Chemicals and reagents used: Ethanol& for screening of the phytochemicals, the reagents and the chemicals used was of laboratory were of analytical grade

Preparation of doses:

The ethanolic extract of *Curcuma Longa* and the standard drug was administered in a constant volume over range of doses to be tested by varying the concentration of dosing preparation. The test animal's size determined the maximum amount of liquid that could be given at once. The volume in rats was typically not more than 1 millilitre per 100g of body weight. The dilutions of the ethanolic extract and standard drug was prepared in normal saline acting as a vehicle. The freshly prepared doses were administered to the rats to avoid stability issues.[7]

Acute toxicity test (LD50) the Lokes method [8]

Laboratory animals used in the research: The animal models involved in this study was Swiss albino mice. The parameters evaluated during this were assayed including body weight of the mice, survival time, paw licking, salivation, stretching/writhing, erect fur, calmness, reduced movement, weakness, coma, convulsion, sleep and death. Albino mice were coded, weighed and randomized into three groups/dose with 3 mice per group/dose in two phases. In phase I, nine mice were randomized into three groups of three mice each and were given (5 mL) each of 30, 300 and 500 mg/ kg body weight (b. wt) of the extract orally. The mice were observed for paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first four (4) hours andsubsequently daily for seven (7) days. In phase II, another fresh set of nine mice were randomized into three groups of three mice each and were given 1600, 2900 and 5000 mg kg-1 body weight of the extract orally on the basis of the result obtained during the first phase. They were observed for signs of toxicity and mortality for the first four critical hours and thereafter, daily for 7 days.

The LD50 was then calculated as the square root of the product of the lowest lethal dose and highest nonlethal dose i.e., the geometric mean of the consecutive doses for which 0% and 100% survival rates were recorded in the second phase, the oral median lethal dose were calculated using the formula:

Oral median lethal (LD50) dose=√Minimum toxic dose × maximum toxic dose

Table 3: Effect of *Curcuma Longa* on body weight

	DOSE with grouping according to phase I & II		Weight of Mice		
			Day 1	Day 4	Day 7
Phase I	30 mg/Kg (3 mg/mL)	Mice 1	25.34	26.45	28.40
		Mice 2	26.23	26.54	27.10
		Mice 3	27.50	27.40	29.00
	300 mg/Kg (30 mg/mL)	Mice 1	24.40	25.00	25.40
		Mice 2	24.70	25.00	27.50
		Mice 3	27.40	27.50	27.10
	500 mg/kg (50 mg/mL)	Mice 1	27.90	26.45	26.10
		Mice 2	26.23	26.54	25.54
		Mice 3	27.50	27.40	28.90
Phase II	1600 mg/Kg (160 mg/mL)	Mice 1	25.34	26.45	28.40
		Mice 2	28.40	27.80	27.00
		Mice 3	27.50	27.40	27.00
	2900 mg/Kg (290 mg/mL)	Mice 1	28.60	27.70	27.10
		Mice 2	25.09	Death	Death
		Mice 3	27.50	26.97	26.00
	5000 mg/kg (500 mg/mL)	Mice 1	28.90	Death	Death
		Mice 2	28.80	Death	Death
		Mice 3	25.00	Death	Death

RESULTS

Acute toxicity test (LD50) the Lokes method

The acute toxicity tests were conducted in two phases using the ethanolic extract of *Curcuma longa* leaves. In the first phase, doses of 30 mg/kg (3 mg/mL), 300 mg/kg (30 mg/mL), and 500 mg/kg (50 mg/mL) were administered to three mice each. No signs of toxicity were observed, and all mice survived in each dose group.

In the second phase, higher doses of 1600 mg/kg (160 mg/mL), 2900 mg/kg (290 mg/mL), and 5000 mg/kg (500 mg/mL) were administered. Mice in the 1600 mg/kg group showed signs of weakness, Salivation, and reduced movement, but all survived. In the 2900 mg/kg group, one mouse experienced convulsions, another died, and the third exhibited sleep-like symptoms. In the 5000 mg/kg group, all three mice died. These results indicate that the ethanolic extract demonstrated toxicity at higher doses in the second phase, with lethal effects observed at 5000 mg/kg.

Table 4: Phase I Acute Toxicity Test of Ethanolic Extract of leaf of *Curcuma Longa*

DOSE		Sign of toxicity	Survival
30 mg/Kg (3 mg/mL)	Mice 1	NO	Yes
	Mice 2	NO	Yes
	Mice 3	NO	Yes
300 mg/Kg (30 mg/mL)	Mice 1	NO	Yes
	Mice 2	NO	Yes
	Mice 3	NO	Yes
500 mg/kg (50 mg/mL)	Mice 1	NO	Yes
	Mice 2	NO	Yes
	Mice 3	NO	Yes

Table 5: Phase II Acute Toxicity Test of Ethanolic Extract of leaf of *Curcuma Longa*

DOSE		Sign of toxicity	Survival
1600 mg/Kg (160 mg/mL)	Mice 1	Weakness	Yes
	Mice 2	Salivation	Yes
	Mice 3	reduced movement	Yes
2900 mg/Kg (290 mg/mL)	Mice 1	convulsion	Yes
	Mice 2	Death	No
	Mice 3	Sleep	Yes
5000 mg/kg (500 mg/mL)	Mice 1	Death	No
	Mice 2	Death	No
	Mice 3	Death	No

Oral median lethal (LD50) dose=√Minimum toxic dose × maximum toxic dose

Minimum toxic dose: 1600 mg/Kg (160 mg/mL)

Maximum toxic dose: 2900 mg/Kg (290 mg/mL)

(LD50) dose=√1600×2900

= 2,154.06 mg/kg

Low Dose: 200 mg/Kg (20 mg/mL)

High Dose: 400 mg/kg (40 mg/mL)

DISCUSSION

The acute toxicity evaluation of *Curcuma longa* leaves revealed noteworthy insights into its safety profile. The OECD definition of acute toxicity emphasizes the rapid onset of adverse effects following substance administration within a short duration. In our study, the ethanolic extract exhibited no signs of toxicity at lower doses (30 mg/kg, 300 mg/kg, and 500 mg/kg) during the first phase, suggesting a favorable safety profile within this dosage range.

However, the second phase, involving higher doses (1600 mg/kg, 2900 mg/kg, and 5000 mg/kg), uncovered dose-dependent toxicological effects. At 1600 mg/kg, mice displayed weakness, salivation, and reduced movement, yet all survived. The 2900 mg/kg group witnessed convulsions, one fatality, and sleep-like symptoms, while all three mice succumbed to the 5000 mg/kg dose. These outcomes underscore a dose-related toxicity pattern, reinforcing the importance of dosage considerations in the utilization of *Curcuma longa* extract.

The LD50, a crucial metric in toxicity assessment, was determined as 2154.06 mg/kg. This value serves as a reference point for estimating the median lethal dose, providing valuable information for future applications and regulatory considerations. The LD50 establishes a threshold beyond which adverse effects become more pronounced, guiding safe dosage determination.

CONCLUSION

In conclusion, the acute toxicity evaluation of *Curcuma longa* leaves illuminated its safety profile, with no observed toxicity at lower doses. However, caution is warranted at higher doses, as evidenced by dose-dependent toxic effects. The LD50 of 2154.06 mg/kg provides a pivotal reference for establishing safe dosage parameters. These findings contribute valuable insights into the safety considerations of *Curcuma longa* extract, paving the way for informed applications in therapeutic and medicinal contexts. Further studies, including chronic toxicity assessments, will enhance our understanding of the long-term safety implications and facilitate the responsible utilization of this natural remedy.

Acknowledgments

I am thankful to Head of Pinnacle Biomedical Research Institutional, Bhopal, for providing me the facilities to carry out my research work

Funding source: NO

Competing interests/Conflicts of interest: NO

REFERENCES

1. Vijaya Chavan Lobo, Acute toxicity studies of Some Indian Medicinal plants, Journal of Pharmacognosy and Phytochemistry, 2010; 2. https://doi.org/10.1016/S0975-3575(10)80094-X
2. Heath DD, Khwaja F, Rock CL. Curcumin content of turmeric and curry powders. FASEB J. 2004; 18:A 125.
3. Wang YJ, Pan MH, Cheng AL, Lin LI, Ho YS, Hsieh CY et al. Stability of curcumin in buffer solutions and characterization of its degradation products. J Pharm Biomed Anal, 1997; 15:1867-76. https://doi.org/10.1016/S0731-7085(96)02024-9 PMid:9278892
4. Gostry Aldica Dohude, Inhibitory test of turmeric leaves extract (*Curcuma Longa*. L) against the growth of Streptococcus mutans bacterial growth in vitro, Journal of Syiah Kuala Dentistry Society, 2022; 7(2):73-77. https://doi.org/10.24815/jds.v7i2.30229
5. C.K. Kokate, A.P. Purohit, S.B. Gokhale. Pharmacognosy. Nirali Prakashan, 53rd edition, 2017; 7.1-7.46.
6. Dr. K.R. Khandelwal, Dr. Vrunda Sethi. Practical pharmacognosy techniques and experiments. Nirali Prakashan, 24th edition, 2014, 23.13-23.15, 25.1-25.9.
7. Erhirhie Earnest Oghenesuvwe, Ekene, Nwoke E. and Ajaghaku Daniel Lotanna, Guidelines on dosage calculation and stock solution preparation in experimental animals' studies, 2014, 4(18):100-106.
8. Zakari Abdu, Acute Toxicity (LD50) Studies Using Swiss Albino Mice and Brine Shrimp Lethality (LC50 and LC90) Determination of the Ethanol Extract of Stem Bark of Echinaceae angustifolia DC, Natural Products Chemistry & Research, 2016; 4(6). https://doi.org/10.4172/2329-6836.1000240