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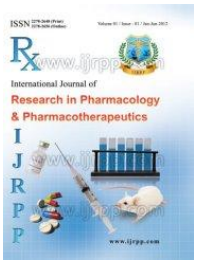

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Evaluation of gastroprotective efficacy of ethanolic extract of *Curcuma amada* rhizomes in rats

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ABSTRACT

The objective of this study was to investigate the gastroprotective efficacy of rhizomes of *Curcuma amada* (*mango ginger, Ambahaldi*), Zingiberaceae in rats. The antiulcer activity of ethanolic extract was evaluated in Indomethacin, ethanol induced gastric ulcer model & stress ulcer by cold water immersion method in wistar rats. The rhizome extracts were prepared by a successive solvent extraction process with petroleum ether, chloroform, ethanol & water. Phytochemical analysis & acute oral toxicity were carried out using standard methods. The preliminary phytochemical screening of *Curcuma amada* revealed the presence of resins, tannins & flavanoids etc. The Ethanolic extract at a concentration of 200mg/kg & 400 mg/kg exhibit a protective effect on ulcer induced models & was compared with standard drugs Ranitidine, Misoprostol, Omeprazole. Percentage ulcer inhibition of Ethanolic extract at 400 mg/kg for Indomethacin, ethanol & stress induced ulcer model were 39.31%, 40 % & 35 % respectively. Among the doses studied ethanolic extract 400 mg/kg body weight was found to be optimum for significant reduction in ulcer. The flavanoids present in the Ethanolic extract might be responsible for the gastroprotective action probably by maintaining the antioxidant level in the gastrointestinal tract thereby reducing mucosal damage. The extract exhibit ulcer protection activity in dose dependent manner.

Keywords: Gastroprotective Efficacy, Ulcer Index, Flavanoids, *Curcuma amada*

INTRODUCTION

Peptic ulcer is the most prevalent gastrointestinal disorder, resulting from *H. pylori* infection, up-regulation of proton potassium ATPase (PPA) activity, down-regulation of gastric mucosal defense etc.[1]. It affects approximately 5-10% of the people during their life. [2, 3]

Although a number of antiulcer drugs, such as H_2 receptor antagonists, Proton pump inhibitors & cytoprotectants are available, all these drugs have side-effect, limitations & may even alter biochemical mechanisms of the body on chronic usage, indicating a need of substitute medication for an alternative system of medicine. Indian medicinal plants & their derivatives have been an invaluable source of therapeutic agents with fewer side effects to manage various disorders including Peptic ulcer disease. Hence the use of herbal drugs for prevention & treatment of various diseases is constantly increasing throughout the world. In traditional & ayurvedic medicine system *Curcuma*

amada was found to be a classic herb for digestive system because of its stomachic & carminative properties, which in turn provide prevention against major gastric disorders such as hyperacidity, gastritis & ulcer[4]. Many recent literature reviews indicated that many flavanoids & antioxidant possess antiulcerogenic & wound healing activity. Because the phytochemical investigation of *Curcuma amada* also revealed the presence of flavanoids, phenols, saponins, tannins & alkaloids[5] an attempt has been made to investigate the antiulcer & the gastroprotective activity of ethanolic extract of *Curcuma amada* rhizomes in various experimental animal models for gastric ulcer.

MATERIALS & METHODS

Plant collection & identification

Mango ginger (C*urcuma amada*) rhizomes were purchased from the local market of Bhopal, India & used for studies. Authentication was done by Dr. Zia-UL Hasan, Professor & head of department botany, Saifia college of science, Bhopal (MP), India. (Voucher specimen no.293/Bot /Saifia /2011).The rhizomes were dried as quickly as possible in shade & stored in airtight glass jars until use.

Preparation of extract

The collected rhizomes of the *Curcuma amada* were shade dried & powdered coarsely & then passed through 40 mesh sieve.[6] Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of rhizomes of *Curcuma amada* was soxhlet extracted with the solvents with increasing order of polarity i.e. petroleum ether, chloroform, ethanol, distilled water. The completion of extraction was confirmed by evaporating a few drops of the extract on the watch glass & ensuring that no residue remained after evaporating the solvent. The solvent was removed at low temperature under reduced pressure or the extract was evaporated to dryness using a water bath (60-70^{0}c), rotary evaporator. The extract obtained was stored in labeled, airtight, amber colored bottle in the refrigerator until use for phytochemical analysis & pharmacological studies. The extract obtained with each solvent was weighed to a constant weight & the percentage yield w/w basis was calculated (Ethanol extract: 5 %).

Phytochemical screening

The screening was carried out on the Ethanolic extracts of the Rhizomes of *Curcuma amada* to determine the active principles or secondary plant constituents. Tests were carried out for carbohydrates, reducing sugar, tannins, polyphenols, flavanoids, alkaloids, gum, saponins, amino acid, resins & steroids.

Animals

Healthy albino wistar rats of either sex weighing 150-200 g were used for the evaluation of the acute oral toxicity test and anti ulcer activity. The animals were used after an acclimatization period of 10 days to the laboratory conditions. Animal was obtained from animal house of VNS institute of pharmacy, Bhopal. Ethical clearance for the handling of animals and procedures used in the study was obtained from institutional animal ethical committee before the start of the study. (Ref. no: VNSIP/IAEC/2012/6871). All animals were stored in standard cages and maintained at 25^{0}c, under 12 hrs dark/ light cycle. The animals were fed with standard rat feed and water was given prior to experiment animal were kept for 12 hrs fasting.

Acute toxicity study [7]

Young, healthy female Wistar rats weighing 200-250 g selected by random sampling technique were used in the study as per OECD 423 guidelines. The animal were fasted prior to dosing overnight, provided only water after which extract were administered to the groups orally at the single dose 5 mg/kg body weight by gavage using a stomach tube or a suitable intubation canula and the group were observed for 14 days. If mortality was observed in 2-3 animals out of 6, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300, 2000 mg/kg body weight. The animals were observed for toxic symptom such as behavioral changes, locomotion, convulsion, sleep, comma and mortality for 24 hours (in every 30 min interval for first 4 hours after dosing).

Gastroprotective efficacy

Three models (Indomethacin, ethanol & stress) with effective induction of ulcer experimentally in

rats were employed to evaluate the gastroprotective efficacy of Ethanolic extract of *Curcuma amada*.

Indomethacin Induced Ulcers

Five groups of 6 Wistar rats weighing 150-200 g are used. The standard (Misoprostol- 0.012 mg/kg P.O) and test extract (ethanolic-200mg/kg & 400 mg/kg) were administered orally in 0.5% carboxymethyl cellulose (CMC) solution 10 min prior to oral Indomethacin administration (40 mg/kg). Six hours later, the rats were sacrificed[8] and their stomachs removed. Formal-saline (2% v/v) is then injected into totally ligated stomachs for overnight. The next day, the stomachs were opened along the greater curvature, then washed in warm water, and examined to determine the ulcer scores.

Ulcer index was determined using the formula

Where, $\mathbf{U_N}$ = Average of number of ulcers per animal, $\mathbf{U_S}$ = Average of severity scores**,**

$$\textbf{Ulcer index} = U_N + U_S + U_P \times 10^{-1}$$

$\mathbf{U_P}$ =Percentage of animals with ulcers

Based on the intensity, the ulcers were given **scores** as follows: **0**= no ulcer, **1** = superficial mucosal erosion, **2** = deep ulcer or transmural necrosis, **3** = perforated or penetrated ulcer.

$$\textbf{Percentage inhibition} = \frac{UIC - UIT}{UIC} \times 100$$

Where UIC=Ulcer index of control group, UIT= Ulcer index of test group.[6]

Ethanol Induced Ulcers

Five groups of 6 wistar rats weighing between 150-200 g were fasted before the administration of ethanol. The standard drug (Ranitidine- 50mg/kg P.O) or extract (ethanolic-200 mg/kg & 400 mg/kg,P.0) was administered 1 hour before ethanol administration. Ethanol (90%) was administered to all the animals at a dose of 0.5ml/100g & after 1 hour, all the animals were sacrificed & ulcer index was determined.[9]

Stress Ulcer by Cold Water Immersion

Five Groups of 6 wistar rats weighing 150-200g were used. After oral administration of standard (Omeprazole-10 mg/kg) and test extracts (ethanolic-200 mg/kg & 400 mg/kg,P.O), the rats were placed vertically in individual restraint cages in water at 22 ^{0}c for 1 hour. Then, they were removed, dried and injected intravenously via the tail vein with 30 mg/kg Evans blue. Ten min later, they are sacrificed by cervical dislocation and their stomachs removed. Formal-saline (2% v/v) is then injected into totally ligated stomachs for overnight. The next day, the stomachs were opened along greater curvature, washed in warm water, and examine to determine ulcer scores.[10]

STATISTICAL ANALYSIS

Ulcer indices were shown as the mean ± SEM & level of ulcer protection presented as percentage inhibition Statistical comparison were performed by one way ANOVA followed by Dunnett's "t" test. The results were considered statistically significant if p-values were less than 0.05.

RESULTS

Phytochemical screening showed that the Ethanolic extract contain tannins, flavanoids & resins. Acute toxicity results showed that the LD_{50} was greater than 2000mg/kg.

Indomethacin Induced Ulcers

In Table 1, ulcer inhibition was evident in all treatment groups of the extract of *C .amada* compared to the negative control. However statistically significant ulcer inhibition (38.62% and 39.31%, *p< 0.01) could be seen at doses of 400 mg/kg of the Ethanolic extract.

Ethanol Induced Ulcers

The Ethanolic extract of *C. amada* at all the doses provided protection from ulcer & the protection was dose dependent. The Ethanolic extract at doses of 200 mg/kg & 400 mg/kg provides statistically significant protection (20% & 40 %, *p<0.01) when compared with negative control (Table 2).

Stress Ulcer by Cold Water Immersion

The Ethanolic extract at all the doses provided protection from ulcer & the protection was dose dependent. The Ethanolic extract at doses of 200 mg/kg & 400 mg/kg provides statistically significant protection (20% & 35 %, *p<0.01) when compared with negative control (Table 3).

DISCUSSION & CONCLUSION

The gastroprotective efficacy of the Ethanolic extract of *C.amada* against Indomethacin, ethanol induced ulcers & stress ulcer by cold water immersion was established. Our finding showed that the Ethanolic extracts at doses of 200 mg/kg & 400 mg/kg body weight had gastroprotective effects on acute experimental gastric ulcer in rats. The present finding concluded that Ethanolic extract had potential gastroprotective efficacy, which was not superior to the respective effect observed with standard drug (Ranitidine, Omeprazole & Misoprostol).

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Table 1: Effect of Ethanolic extract on Indomethacin induced ulcer in rats (n = 6)

S.No	Treatment groups	Doses	Ulcer Index	%Inhibition
Groups I	Vehicle control	-	-	-
Group II	Negative control(CMC + Indomethacin)	40 mg/kg	11.6±0.23	-
Group III	Positive control(Misoprostol)	0.012 mg/kg	6.9±0.12*	40.51
Group 1V	Ethanolic extract	200 mg/kg	7.1±0.14*	38.62
Group V	Ethanolic extract	400 mg/kg	7.0±0.13*	39.31

Data were expressed as mean±SEM. Significant at *p<0.01(ANOVA followed by Dunnett's test) when compared to negative control, n=6.

Table 2: Effect of Ethanolic extract on Ethanol induced ulcer in rats (n = 6)

S.No	Treatment groups	Doses	Ulcer Index	%Inhibition
Groups I	Vehicle control	-	-	-
Group II	Negative control(CMC + Ethanol)	5 ml/kg	9.0±0.25	-
Group III	Positive control(Ranitidine)	50 mg/kg	5.2±0.16*	41.77
Group 1V	Ethanolic extract	200 mg/kg	7.2±0.15*	20.0
Group V	Ethanolic extract	400 mg/kg	5.4±0.14*	40.0

Data were expressed as mean±SEM. Significant at *p<0.01(ANOVA followed by Dunnett's test) when compared to negative control, n=6.

Table 3: Effect of Ethanolic extract on cold water immersion induced ulcer in rats (n = 6)

S.No	Treatment groups	Doses	Ulcer Index	%Inhibition
Groups I	Vehicle control	-	-	-
Group II	Negative Control (cold water)	-	8.0±0.22	-
Group III	Positive control(Omeprazole)	10 mg/kg	3.1±0.13*	61.25
Group IV	Ethanolic extract	200 mg/kg	6.4±0.14*	20.0
Group V	Ethanolic extract	400 mg/kg	5.2±0.12*	35.0

Data were expressed as mean±SEM. Significant at *p<0.01(ANOVA followed by Dunnett's test) when compared to negative control, n=6.

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