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Evaluation of Anti-ulcer Activity in Various Extracts of *Crateva religiosa* using Acetic acid induced Ulcer Model

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Abstract

Crateva religiosa is the most widely cultivated species in Northwestern India, is the sole genus in the family Capparacea.. It comprises 10 species from tropical and subtropical climates, ranging in size from tiny herbs to massive trees. The most widely cultivated species is *Crateva religiosa* (CR) The most used parts of the plant are rich in polyphenols, phenolic acids, flavonoids, alkaloids, tannins and saponins. The high number of bioactive compounds might explain the pharmacological properties. Many studies, *in-vitro* and *in-vivo*, have confirmed these pharmacological properties. The present investigation was carried out to evaluate anti-ulcer activity of various extract of the plant parts using acetic acid induced ulcer models.

Keywords: *Crateva religiosa* (CR) Anti-ulcer activity, acetic acid induced ulcer

Introduction

Peptic ulcers (PU) are lesions or sores in the gastrointestinal mucosa that spread across the muscularis mucosae. They are usually characterized by inflammation, increased oxidative stress, blood flow reduction, neutrophil infiltration, and various stages of necrosis. In addition to causing patients great distress by interfering with their daily routines and inflicting mental anguish, peptic ulcers are a non-fatal illness primarily characterized by recurrent episodes of epigastric pain that are frequently alleviated by food or alkali[1-2]. The condition is primarily classified according to its anatomical origins, including duodenal (found in the duodenal bulb, the area most exposed to gastric acid) and gastric (found along the stomach's lower curvature) ulcers. Studies have shown that peptic ulcer disease (PUD) occurs because of an imbalance between aggressive injurious (e.g., pepsin, HCl) and defensive mucosa-protective factors (e.g., prostaglandins, mucus and bicarbonate barrier and adequate blood flow). All ulcers of the upper gastrointestinal tract were originally thought to be caused by the aggressive action of pepsin and gastric acid on mucosa. However, the denomination "peptic ulcer" has lately pointed to Helicobacter pylori infection, where the chronic use of non- steroidal anti-inflammatory drugs (NSAIDs) and acetylsalicylic acid (ASA) are some of the disease-causing factors.

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Thus, based on the latest advances on this field and stress the fact that PUD is an important cause of morbidity and health care costs, a special emphasis was given on plant products safety and security, in order to trigger the interest in deepening skills on this matter and to ensure an effective managing competence for health-related systems.[3-4]

Crateva religiosa is frequently used in conventional prescription throughout local and regional areas and is primarily valued for its edible natural products, leaves, flower, seeds and roots. . It has been developed and naturalized in various parts of Pakistan, India, and Nepal. Swiftly growing deciduous or evergreen tree that typically reaches a height of 4 to 10 meters. It features thick, corky, whitish bark, fluffy foliage of tripinnate leaves, and an open, spreading crown of delicate, dangling branches. Since the reports about the antiulcer activity of the leaves of *Crateva religiosa* (CR) sparsely documented, it was considered worthwhile to investigate the antiulcer activity of *Crateva religiosa* (CR) substantiate its ethnopharmacological claim of providing relief in PUD.[5-6]

Material and Methods

Collection of plant material and extraction procedure

The flowers and seeds of *Crateva religiosa* (CR) were collected from the botanical garden of RKDF university Bhopal. different extracts of *Crateva religiosa* were authenticated by the Head of Department of botany Dr.Zia Ul Hasan Professor of Safia College of Science Bhopal. Plant authentication no. is 840 /Bot/Safia/2024.

Extraction of plant parts

The plant parts of *Crateva religiosa* (CR) were shade dried and reduced to coarse powder in a mechanical grinder and passed through sieve No. 40. The powdered material obtained was then subjected to successive extraction in batches using petroleum ether, chloroform, and acetone and methanol solvents in a Soxhelet extractor. The different extracts obtained were evaporated in rotary evaporator to get a semisolid mass.[7]

Phytochemical estimations of the extracts

The extracts of *Crateva religiosa* (CR) were subjected to qualitative analysis for the various phytoconstituents like alkaloids, glycosides, phytosterols, tannins, proteins, amino acids and flavonoids.[8]

Experimental animals

Male albino Wistar rats weighing between 200-250 gm were used. Institutional Animal Ethics Committee permitted the experimental procedure; animals were maintained under standard conditions in an animal house approved by Committee for Control, and Supervision of Experiments on Animals (CCSEA).[9]

Acute toxicity study

The acute oral toxicity study was performed according to the OECD guidelines 2004. The different extracts were suspended using 0.5% sodium carboxy methylcellulose and were administered orally. The concentration was adjusted in such a way that it did not exceed 1ml/kg b/w of the animal

Acetic acid induced ulcer

The method described by Okabe et al was followed. The animals were fasted for 24 hours prior to the experiment. Under light ether anesthesia the abdomen was opened by midline incision below the xiphoid process and the stomach was exposed. Glacial acetic acid (0.05ml) was added to the cylindrical mould of 6mm diameter placed tightly over the anterior serosal surface of the stomach and this was allowed to remain over there for 60 seconds. The acid solution was removed by rinsing the mould with normal saline twice or thrice to avoid damage to the surrounding tissues. The stomach was placed back carefully and the abdominal wall was closed. The animals were treated with ranitidine (50 mg/kg, p.o) and with different extracts of *Crateva religiosa* (200 mg/kg p.o) once daily for 10 days after the induction of ulcer while the control group received vehicle only. Rats were sacrificed on the 10th day, stomachs were removed and they were cut open along the greater curvature.[10-12]

Statistical analysis

The arithmetical implication was assessed using one-way analysis of variation (ANOVA) followed by Dunnet comparison test. For comparing nonparametric ulcer scores, ANOVA followed by non-parametric Dunn post-test was used.

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Evaluation Parameters for antiulcer activity

Macroscopic evaluation

The stomachs were opened along the wider curvature, rinsed with saline to eated animals extract stomach contents and blood clots, and inspected for ulcer formation by lens magnifier. The ulcer index was determined using the formula [13]

Percentage inhibition of ulceration

$$\% \text{ Inhibition of Ulcer} = \frac{\text{Ulcer index of Control group} - \text{Ulcer index of Test group}}{\text{Ulcer index of Control group}} \times 100$$

Table 1: Effect of different extracts of *Crateva religiosa* on Ulcer Index and present inhibition in acetic acid induced ulcer model

Treatments	Ulcer Index	% Inhibition of ulcer
Control (Saline 5 ml/kg)	87.32±2.10	-
Ranitidine (50 mg/kg)	12.23±0.80*	73.31*
Petroleum ether flowers extract (200 mg/kg)	27.22±1.61	56.14
Petroleum ether Fruits extract (200 mg/kg)	25.23±1.78	58.42
Petroleum ether Seeds extract (200 mg/kg)	27.45±2.07	55.88
Petroleum ether Leaves extract (200 mg/kg)	24.21±2.60	59.59
Chloroform flowers extract (200 mg/kg)	27.57±2.48	55.74
Chloroform Fruits extract (200 mg/kg)	23.21±1.28	60.73
Chloroform Seeds extract (200 mg/kg)	24.01±1.18	59.82
Chloroform Leaves extract (200 mg/kg)	22.95±1.27	61.03
Acetone flowers extract (200 mg/kg)	23.57±2.13	60.32
Acetone Fruits extract (200 mg/kg)	24.31±3.21	59.47
Acetone Seeds extract (200 mg/kg)	22.24±2.61	61.85
Acetone Leaves extract (200 mg/kg)	21.11±1.88*	63.14*
Methanol flowers extract (200 mg/kg)	27.71±1.72	55.58
Methanol Fruits extract (200 mg/kg)	25.32±1.25	58.32
Methanol Seeds extract (200 mg/kg)	27.35±2.03	55.99
Methanol Leaves extract (200 mg/kg)	26.51±2.71	56.96

Values are expressed as Mean ± S.D. *p< 0.05

Methods for biochemical estimations like gastric content, total acidity and pH

Collection of gastric juice

Gastric juice was collected from pylorus-ligated rats as mentioned earlier. The gastric juice collected was centrifuged for 1000 rpm for 10 minutes and the volume of gastric juice was measured. This gastric juice was used for biochemical estimations as follows.[14-15]

Measurement of gastric content in peptic ulcer

The stomachs were removed and the volume of the gastric juice was collected and measured.

Determination of total acidity in peptic ulcer

The stomachs were removed and the content was measured before drained into a centrifuge tube and subjected to centrifugation at 2000 rpm for 10 min. pH of the gastric secretion was recorded with a pH meter. The total acidity of the gastric secretion was determined by titration with 0.01N NaOH and phenolphthalein as indicator.[16]

The total acidity is expressed as mEquiv/l using the following formula: n×0.01×40×1000

where, n is volume of NaOH quantified, 40 is the molecular weight of NaOH, 0.01 is normality of NaOH and 1000 is the factor represented in litre.[17]

Determination of pH in peptic ulcer

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using pH meter.1

Table 2: Effect of different extracts of *Crateva religiosa* on various ulcer parameters in acetic acid induced ulcer model

Treatments	Gastric volume (ml)	pH	Total acidity (mEq/l)
Control (Saline 5 ml/kg)	6.18±0.05	2.3±0.07	625.15±26.15
Ranitidine (50 mg/kg)	3.41±0.07*	4.3±0.04*	327.12±22.31*
Petroleum ether flowers extract (200 mg/kg)	5.72±0.06	2.7±0.12	578.22±24.13
Petroleum ether Fruits extract (200 mg/kg)	5.15±0.04	3.2±0.24	543.31±25.12
Petroleum ether Seeds extract (200 mg/kg)	5.13±0.14	3.4±0.12	544.24±23.12
Petroleum ether Leaves extract (200 mg/kg)	5.12±0.02	3.2±0.14	517.21±12.34
Chloroform flowers extract (200 mg/kg)	5.18±0.24	2.7±0.43	548.42±20.18
Chloroform Fruits extract (200 mg/kg)	5.98±0.16	2.9±0.16	535.42±36.25
Chloroform Seeds extract (200 mg/kg)	5.28±0.06	3.1±0.06	535.42±25.10
Chloroform Leaves extract (200 mg/kg)	5.82±0.23	3.0±0.02	538.24±27.31
Acetone flowers extract (200 mg/kg)	5.16±0.05	2.9±0.17	541.15±27.43
Acetone Fruits extract (200 mg/kg)	5.15±0.06	3.2±0.04	546.37±26.37
Acetone Seeds extract (200 mg/kg)	5.17±0.07	3.4±0.12	523.14±25.12
Acetone Leaves extract (200 mg/kg)	4.23±0.02*	3.9±0.04*	485.24±17.32*
Methanol flowers extract (200 mg/kg)	5.58±0.16	3.6±0.05	527.36±19.38
Methanol Fruits extract (200 mg/kg)	5.48±0.06	3.4±0.06	538.45±32.17
Methanol Seeds extract (200 mg/kg)	5.34±0.03	3.1±0.02	541.14±28.32
Methanol Leaves extract (200 mg/kg)	5.16±0.05	3.4±0.07	564.25±27.43

Values are expressed as Mean ± S.D. *p< 0.05

Results and Discussion

Acute toxicity study

The different parts extracts of *Crateva religiosa* extracts was found to be safe in the dose used and there was no mortality up to a dose of 4000 mg/kg body weight. There is zero mortality (0/3).[18]

Determination of ulcer index and percentage inhibition of ulcer in acetic acid induced ulcer model

Acetic acid administration resulted the gastric mucosal damage. The ulcer index of animals in acetic acid induce ulcer model, in case of control group of animals was 87.32±2.10. Acetone leaves extract of *Crateva religiosa* 200 mg/kg treated animals were showed 21.11±1.88 and percentage inhibition of ulcer was found 63.14 that is significantly reduced the ulcer index ($p<0.05$) as compared to control group Table 1 .The reduction in ulcer index and percent inhibition of ulcer by other plant part extracts was not found significantly.

pH of gastric contents in acetic acid induced ulcer model

In control animals, pH was found 2.3, Acetone leaves extract of *Crateva religiosa* 200 mg/kg was showed significant rise in pH 4.23, as compared to control group .The increase in pH shown by standard drug was 3.41, which is statistically significant ($p<0.05$).

Total acidity and gastric contents in acetic acid induced ulcer model

The control group was showed gastric total acidity 625.15 mEq/litre. Acetone leaves extract of *Crateva religiosa* 200 mg/kg was showed significant decrease in total acidity 485.24±17.32 mEq/litre,. Other extracts i.e. petroleum ether, chloroform, methanol does not showed significant reduction in the total acidity and compared to control group of animals. Gastric contents in acetic acid induced ulcer model, control group was fount 6.18 ml, standard group treatment with ranitidine 50mg/kg show reduction i.e. 3.41ml, Acetone leaves extract of *Crateva religiosa* 200 mg/kg treated was showed significant reduction in gastric contents.

Conclusion

The petroleum ether, chloroform ,acetone, and methanolic extracts of various parts of *Crateva religiosa (CR)* were found to be effective in healing the ulcer, induced by acetic acid. The acetone leaves extracts of *Crateva religiosa (CR)* showed significant effect in various ulcer parameters as standard animal groups and better antisecretory activity as evidenced by reduction in the mean volume of gastric secretion, rise in pH and reduction in total acidity.

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