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Evaluation of anti-ulcer activity of extracts of Crateva religiosa and Waltheria indica using Acetic acid induced ulcer model

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ABSTRACT

Crateva religiosa (CR) and Waltheria indica (WI) a native plant from Africa and Asia, and the most widely cultivated species in Northwestern India, It comprises 10 species from tropical and subtropical climates, ranging in size from tiny herbs to massive trees. It can be used as food, medicine, cosmetic oil, or animal feed. It is produced for its nutrient-dense pods, edible leaves, and blooms. It is between 3 and 8 centimeters tall. Numerous studies have shown that it has positive impacts on people. has been identified as having a large amount of bioactive substances. The plant's leaves, which are abundant in vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins, are the most used components of the plant. The goal of the current study was to assess the anti-ulcer potential of many plant extracts.

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

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1. 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 alkali1-2. 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. 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, the present report aims to provide a general overview on peptic ulcers, namely considering their epidemiology, main symptoms and clinical features, pathogenesis, where a particular emphasis will be given to H. pylori infection, pharmacological agents used in an effective management and also pointing out the latest challenges and opportunities of using plant phytochemicals as upcoming antiulcerogenic agents. Lastly, 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

The cultivated plant has spread far beyond its native area, becoming naturalized in many countries in Southeast Asia, Central America, tropical Africa, the Caribbean, the Arabian Peninsula, and tropical South America. With a spreading, open crown of dangling, feeble branches, padding foliage of tripinnate leaves, and thick, corky, deeply fissured whitish

bark, the tree typically reaches a height of 10 or 12 meters. It is frequently used in conventional prescription throughout its local and regional areas and is primarily valued for its edible natural products, leaves, blossoms, roots, and seed oil. . In addition to Afghanistan, Bangladesh, Sri Lanka, Southeast Asia, West Asia, the Arabian Peninsula, East and West Africa, the West Indies, southern Florida, Central and South America from Mexico to Peru, Brazil, and Paraguay, 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.5 Since the reports about the antiulcer activity of the leaves of Crateva religiosa (CR) and Waltheria indica (WI) sparsely documented, it was considered worthwhile to investigate the antiulcer activity of the flower and seeds extacts of Crateva religiosa (CR) and Waltheria indica (WI) and substantiate its ethnopharmacological claim of providing relief in PUD.

2. METHODOLOGY

Collection of plant material and extraction procedure

The flowers and seeds of Crateva religiosa (CR) and Waltheria indica (WI) were collected from the botanical garden of RKDF university Bhopal between 21/06/2024 to 12/12/2024.. Flowers and seed 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 flowers and seeds

The flowers and seeds of Crateva religiosa (CR) and Waltheria indica (WI) 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.6

Phytochemical estimations of the extracts

The extracts of Crateva religiosa (CR) and Waltheria indica (WI) were subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids. 7

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 the Purpose of Control, and Supervision on Experiments on Animals (CPCSEA).

Acute toxicity study

The intense oral poisonous quality examination was performed by the OPPTS (Office of prevention, pesticide and toxic substance) Up and Down method (Health Effect Test Guideline 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.8

Acetic acid induced ulcer 9-11

The described procedure was used. Prior to the investigation, the critters were fasted for a whole day. The stomach was exposed after the midline entry point beneath the xiphoid technique opened the mid-region under light ether anesthesia. The barrel-shaped form, which was 6 mm wide and firmly placed on the stomach's front serosal surface, was filled with 0.05 ml of icy acidic corrosive and allowed to remain there for 60 seconds. To prevent damage to the surrounding tissues, the corrosive arrangement was removed by washing the shape twice or three times with regular saline. The stomach separator was closed and the stomach was carefully replaced. For ten days following the recruitment of ulcers, the animals were given daily with ranitidine (50 mg/kg, p.o.) and different concentrations of Crateva religiosa (CR) and Waltheria indica (WI) blooms and seeds (500 mg/kg, p.o.), whereas the control group received only vehicle. On the tenth day, rats were given up, their stomachs were thrown out, and they were sliced open in the more notable ebb and flow.The stomach tests were examined utilizing a PC scanner and the all out mucosal zone and all out ulcerated zone were estimated utilizing open area picture preparing and examination program created at National Institute of Health, USA. The PC adaptation of the program was downloaded free from Scion (http://www.scioncorp.com) The scale was set on 6.1 pixels/mm. The ulcer record was resolved utilizing the recipe

The ulcers were given scores based on their intensity as follows
0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis,
3 = perforated or penetrated ulcer.

Methodology for the evaluation of gastric lesions by using scion image

Open the photo document from the report by snapping on the record catch in the scion picture menu. Choose the image of a heart from the device bar, then follow the image's outline. Click "break down catch" at that point, then select "set scale" and set the scale to 6.1 pixels/mm. The region of the chosen image on the info page is then displayed after you click the study button once more and then compute. To determine the total area of the mucosal surface and ulcerated area, the same process is performed. record the area of ulcerated area and total mucosal area and calculate the ulcer index as described above. The stomach samples from groups that showed reduction in ulcer index were subsequently processed for histological examination. Three lists to be specific recovered glandular epithelial width, narrow thickness and collagen content were chosen to mirror the rate and nature of ulcer recuperating Regenerated lining epithelial width was defined as the average distance from the origin of regenerated lining epithelium to the surface of the ulcer. 12-13

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 posttest was used.

The standards are articulated as mean + SEM and $p<0.05$ was considered significant.

3. RESULTS AND DISCUSSION

The current study examines the effects of various flower and seed extracts from Waltheria indica (WI) and Crateva religiosa (CR) on duodenal and stomach ulcers. The flower extracts' phytochemical analysis showed that the petroleum ether extract contained alkaloids and steroids, the acetone extract contained proteins, carbohydrates, alkaloids, tannins, flavonoids, and glycosides, and the methanol extract contained saponins in addition to all of the components found in the acetone extract.In the acute toxicity study, no mortality was observed after treatment with the highest tested dose (5 g /kg p.o) of all the extracts of flower and seeds. Hence, 1/10th of the tested dose (500 mg/kg p.o) was selected for evaluation of anti-ulcer activity. The anti-ulcer effect was evaluated using the acetic acid induced ulcer. The petroleum ether, acetone, and methanolic flower and seed extracts of Crateva religiosa (CR) and Waltheria indica (WI) and ranitidine showed a significant reduction in ulcer index when compared to control ($p<0.001$). When compared to control, the flower's acetone extract was the most effective, resulting in a 79% reduction in the ulcer index. There was no discernible difference in the ulcer index between the chloroform extract of the floral and the seed extracts of Crateva religiosa (CR) and Waltheria indica (WI). The ulcer score was not significantly impacted by any of the therapies.14-15

Sections of ulcerated area revealed that there was a significant increase in regenerated glandular epithelium width after treatment with acetone extract of the flowers ($p<0.05$) The collagen content in the ulcerated tissue was significantly increased by petroleum ether, acetone and methanol extracts of the flowers and ranitidine, with acetone extract of the flowers showing the maximum effect. No significant difference on capillary density in scar tissue was observed after treatment with different extracts of flowers or ranitidine

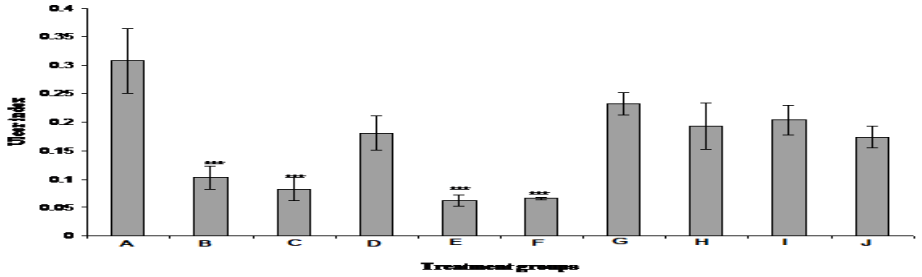


Figure 1: Effect of *Crateva religiosa (CR) and Waltheria indica (WI)* on ulcer index in acetic acid induced chronic gastric ulcer

A= Control; B= Standard (Ranitidine 50mg/kg p.o.); C= Flowers petroleum extract (500mg/kg p.o.); D= Flowers chloroform extract (500mg/kg p.o.); E= Flowers acetone extract (500mg/kg p.o.); F= Flowers methanol extract (500mg/kg p.o.); G= Seeds petroleum extract (5 00mg/kg p.o.); H= Seeds chloroform extract (500mg/kg p.o.); I= Seeds acetone extract (500mg/kg p.o.); J= Seeds methanol extract (500mg/kg p.o.). All values are mean ± SEM, n = 5-6. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to control group

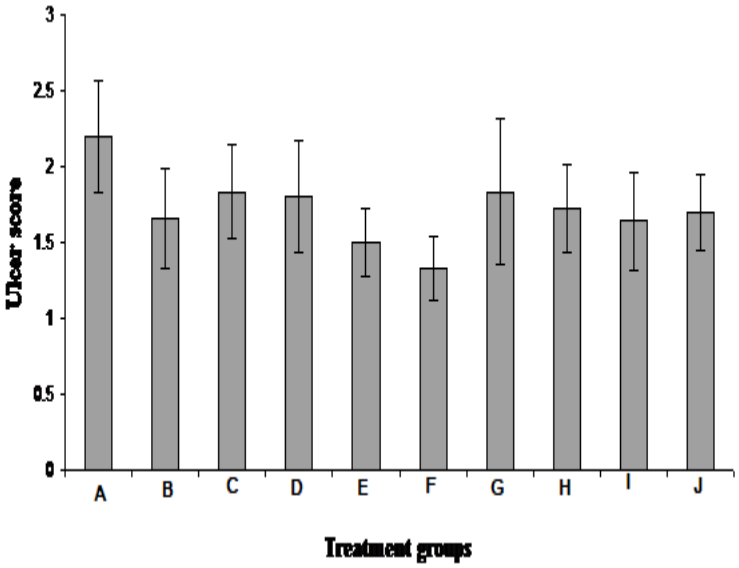


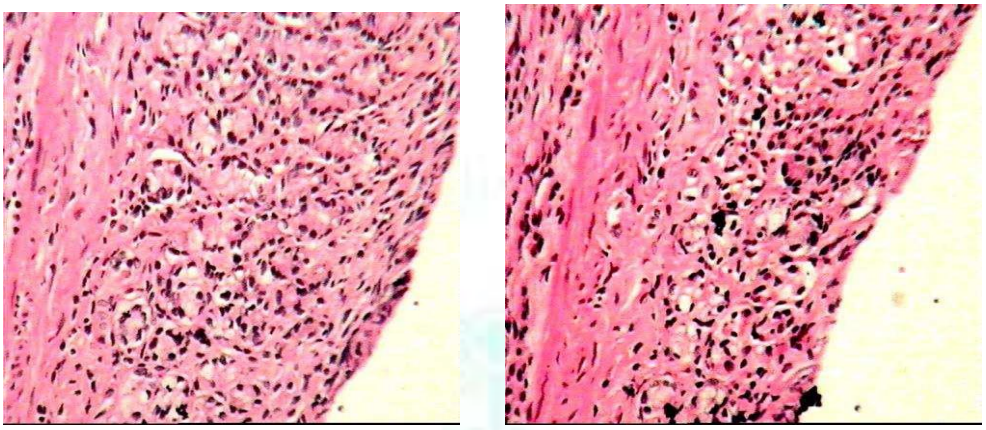
Figure 2: Effect of *Crateva religiosa (CR) and Waltheria indica (WI)* on ulcer score in acetic acid induced chronic gastric ulcer

A= Control; B= Standard (Ranitidine 50mg/kg p.o.); C= Flowers petroleum extract (500mg/kg p.o.); D= Flowers chloroform extrac t (500mg/kg p.o.); E= Flowers acetone extract (500mg/kg p.o.); F= Flowers methanol extract (500mg/kg p.o.); G= Seeds petroleum extract (500mg/kg p.o.); H= Seeds chloroform extract (500mg/kg p.o.); I= Seeds acetone extract (500mg/kg p.o.); J= Seeds methanol extract (500mg/kg p. o.). All values are mean ± SEM, n = 5-6. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to control group

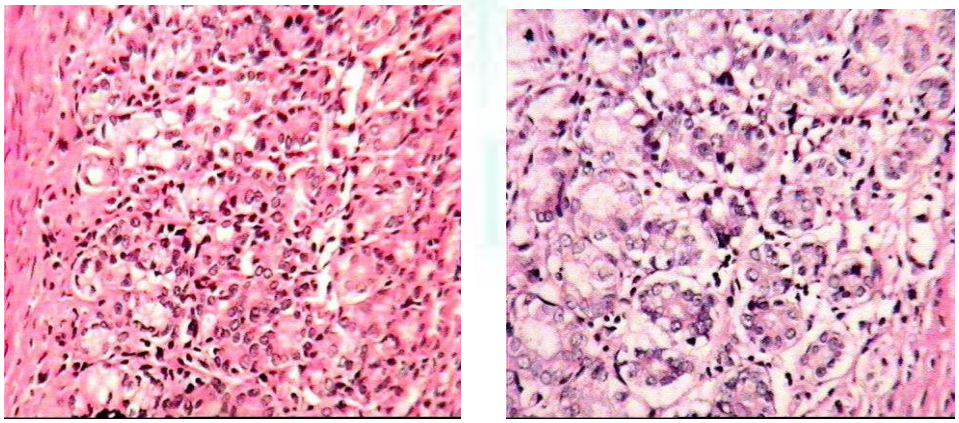
Table 1: Effect of *Crateva religiosa (CR) and Waltheria indica (WI)* on regenerated glandular epithelium width, capillary density and volume of collagen content

Treatment	Regenerated glandular epithelium width (μm)in 19600 μm^2	Capillary density (No)	Vol. of collagen content
Control	506±51.27	5.4±0.34	0.143±0.015
Ranitidine	647±24.42	5.3±0.25	0.355±0.017**
Petroleum Ether flower extract	546±46.34	6.6±0.62	0.224±0.001**
Acetone flower extract	702±23.74*	4.4±1.65	0.152±0.007***
Methanol flower extract	566±24.67	5.63±24.64	0.224±0.013**
Chloroform flower extract	522±20.22	5.6±0.32	0.151±0.002*
Petroleum ether seeds extract	601±21.64	4.3±1.75	0.241±0.006
Acetone seeds extract	765±22.56	6.56±32.76	0.177±0.011***
Methanol seeds extract	525±31.21	5.5±0.26	0.212±0.0043
Chloroform seeds extract	541±21.21	5.±0.31	0.214±0.003**

All values are mean ± SEM, n = 5-6. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to control group

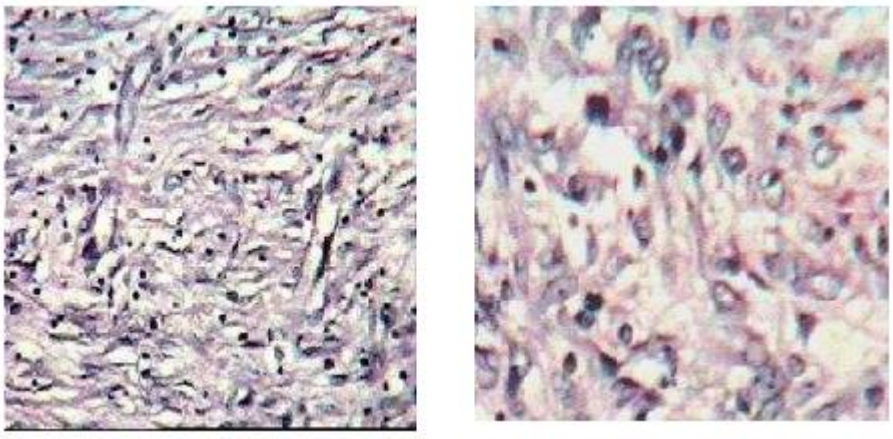


Normal (X100) **Ulcerated Control (X100)**

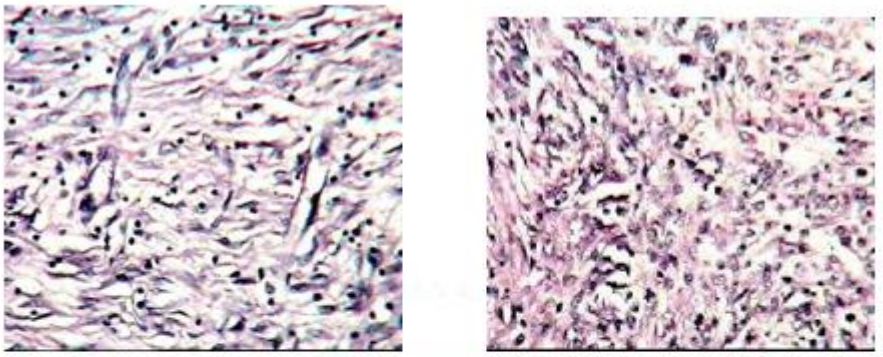


Ranitidine treated (50mg/kg.p.o.) ***Acetone flower extract treated (500mg/kg.p.o) (X100)***

Figure 3: Sections stained with hematoxylin and eosin (H&E) displaying the regenerated glandular epithelium width in stomachs of rats treated with ranitidine and acetone flower extract of *Crateva religiosa (CR) and Waltheria indica (WI)* Lam in acetic acid induced ulcer model.

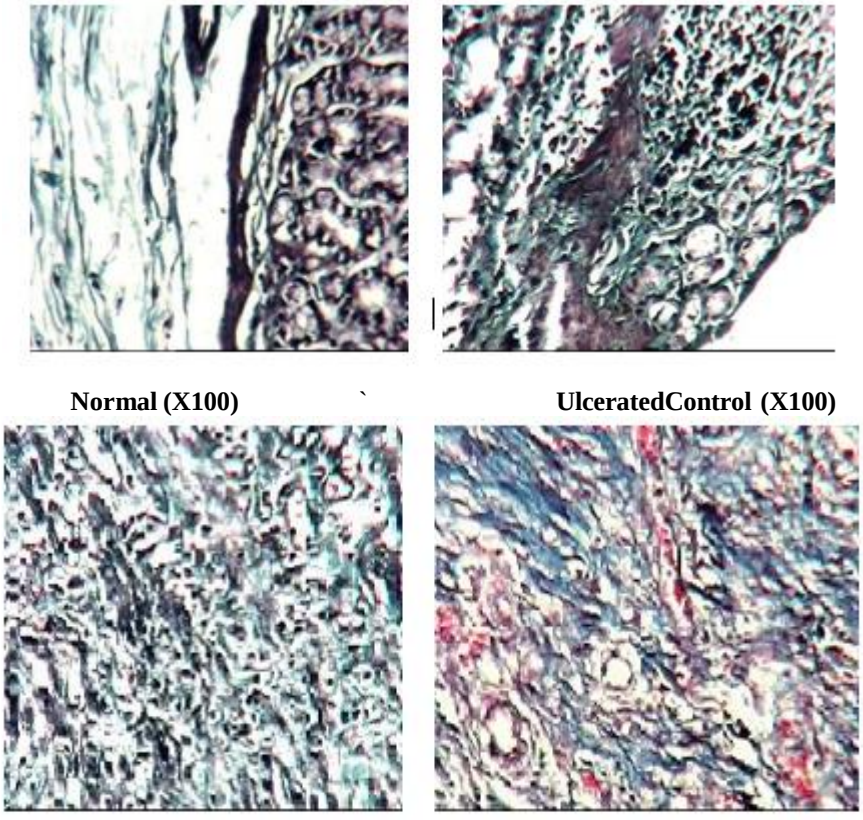


Normal (X100) ***Ulcerated Control (X100)***



Ranitidine treated (50mg/kg.p.o.) **Acetone flower extract treated (500mg/kg.p.o) (X100)**

Figure 4: Sections stained with periodic acid schiff's stain displaying capillary density in stomachs of rats treated with ranitidine and acetone flower extract of *Crateva religiosa (CR) and Waltheria indica (WI)* in acetic acid induced ulcer model



Normal (X100) **UlceratedControl (X100)**

Ranitidine treated (50mg/kg.p.o.) (X100) **Acetone flower extract treated (500mg/kg.p.o)**

Figure 5: Sections stained with masson's trichome displaying collagen in stomachs of rats treated with ranitidine and acetone flower extract of *Crateva religiosa (CR) and Waltheria indica (WI)* in acetic acid induced ulcer model.

4. CONCLUSION

The petroleum ether, acetone, and methanolic flower extracts of *Crateva religiosa (CR) and Waltheria indica (WI)* were found to be effective in healing the ulcer induced by acetic acid. The petroleum ether, acetone and methanolic extracts of *Crateva religiosa (CR) and Waltheria indica (WI)* were also found to increase the collagen content in the ulcerated tissues. The acetone flower extract significantly increased the regenerated glandular epithelium width.

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