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ASSESSMENT OF THE ANTI-ULCERATIVE POTENTIAL OF ROOT EXTRACT FROM HARI CHAMPA (*ARTABOTRYS HEXAPETALUS*)

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Abstract

Peptic ulcers rank among the most prevalent afflictions in humans, impacting over 10% of the global population. Although synthetic medications exist for treating peptic ulcers, they often come with adverse effects and potential drug interactions. Medicinal plants, renowned for their minimal side effects, serve as promising sources of medications. Artabotrys hexapetalus, an underrecognized plant with diverse medicinal properties, is the focus of this study aimed at evaluating its anti-ulcer properties. The plant material was collected, extracted, and subjected to both in vitro and in vivo tests following standard procedures. Results disclosed a percentage yield of 2.5% in the pet ether extract and 7.6% in the ethanolic extract. Phytochemical screening of the hydroalcoholic extract of Artabotrys hexapetulus roots identified various bioactive compounds, including alkaloids, glycosides, flavonoids, proteins, carbohydrates, and saponins. The total flavonoid and alkaloid content were measured at 0.965 and 0.574 mg per 100 mg of dried extract, respectively. In the 100 mg/kg and 200 mg/kg extract-treated groups, the ulcer index was found to be 3.30±0.0 and 3.05±0.15, respectively, compared to the control group's ulcer index of 6.5±0.15. Both extract doses (100 mg/kg and 200 mg/kg) resulted in an increase in gastric pH (3.85±0.15 and 4.15±0.15, respectively) compared to the control group (2.65±0.10). Total acidity in the 100 mg/kg and 200 mg/kg extract-treated groups was 55.75±0.15 and 43.15±0.20 mEq/lt, respectively, while free acidity was 41.15±0.10 and 36.50±0.20 mEq/lt, respectively. Pepsin activity was 3.10±0.25 and 2.65±0.15 Per ml/h for the 100 mg/kg and 200 mg/kg extract-treated groups, respectively.

Keywords: *Peptic ulcer, Medicinal plants, Artabotrys hexapetalus, Herbal medicines, Phytochemicals, Ulcer index, free acidity, Total acidity, Pepsin activity*

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INTRODUCTION:

The liver stands as the largest organ within the human body, playing a pivotal role in regulating essential physiological processes such as glucose, fat, and protein metabolism. Additionally, the liver contributes to detoxification, secretes bile acids crucial for digestion, and serves as a storage site for vitamins. The maintenance of a healthy liver is paramount for overall well-being due to these multifaceted functions (Rowe, 2017; Cesaratto et al., 2004).

Hepatic diseases pose a significant threat to overall health, with peptic ulcer disease, encompassing stomach and duodenal ulcers, emerging as a leading cause of morbidity and mortality for over a century. Numerous researchers highlight peptic ulcers as a prevalent human ailment, affecting more than 10% of the global population. The condition results from an imbalance between the offensive effects of hydrochloric acid and pepsin and the defensive effects of mucus and bicarbonate, often triggered by factors such as stress, nonsteroidal anti-inflammatory drugs (NSAIDs), and Helicobacter pylori infection. While spicy foods and agitation were initially considered intensive factors, bacterial infection by H. pylori or reactions to various drugs, especially NSAIDs, were identified as the primary causes. Key etiological factors include H. pylori infection, NSAIDs usage, emotional stress, alcohol misuse, and smoking (Kavitt et al., 2019; Sverdén et al., 2019).
Several medications, such as proton pump inhibitors and H2 receptor antagonists, are available for treating peptic ulcers. However, clinical trials have indicated increased relapses, adverse effects, and drug interactions with these synthetic drugs. In contrast, the therapeutic use of plants is deemed safe, cost-effective, and widely accessible. Plants offer a rich source of medications, and researchers are increasingly exploring natural remedies to address health concerns without causing harm to the body (Svanes et al., 2000; Ranjan et al., 2017).
Artabotrys hexapetalus (L.f.) Bhandari, a plant belonging to the Annonaceae family, is found in India, Sri Lanka, and southern China. This plant boasts a range of bioactive compounds with diverse pharmacological actions, including antimicrobial, anthelmintic, anticancer, anti-inflammatory, antibacterial, mosquito repellent, antifertility, antioxidant, and anti-leishmanial activities. *Artabotrys hexapetalus* also contains uterine stimulants, muscle relaxants, and cardiac stimulants (Puri, 2020; Quang et al., 2022). Given the beneficial effects of Artabotrys hexapetalus, this study aims to evaluate the anti-ulcer properties of *Artabotrys hexapetalus* root extract.

MATERIALS & METHODS:

Collection of plant material

The roots of *Artabotrys hexapetulus* were collected from local area of Bhopal in the month of March, 2023. Drying of fresh plant parts was carried out in sun but under the shade. Dried roots of *Artabotrys hexapetulus* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction

45 gram shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Defatted powdered of *Artabotrys hexapetulus* has been extracted with hydroalcoholic solvent (ethanol: water; 75:25v/v) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield

The yield was calculated by dividing weight of extract by weight of powdered drug multiplied by 100. The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage.

Phytochemical Screening

The phytochemical screening was performed according to standard protocol.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Chang *et al.*, 2002). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Estimation of total alkaloids content

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered (Margraf *et al.*, 2015). This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the

reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

***In vivo* anti-ulcer activity**

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC).

Toxicity study

Healthy adult male albino rats were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the hydroalcoholic extract of *Artabotrys hexapetulus* were administered to each group of rats (Each group carries 6 rats) and they were observed continuously for 1 hour and then at half-hourly intervals for 4 hour, for any gross behavioural changes and further up to 72 hour, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 425 (OECD, 2008). The hydroalcoholic extract of *Artabotrys hexapetulus* was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for antiulcer evaluation was 100 and 200 mg/kg respectively.

Ulcer induced by absolute ethanol

The rats were divided into four groups of six each.

Group I (Toxicant control) received absolute ethanol (1 ml/animal)

Group II was treated with ranitidine (50 mg/kg)

Groups III was treated with hydroalcoholic extract of *Artabotrys hexapetulus* 100 mg/kg/p.o.

Groups IV was treated with hydroalcoholic extract of *Artabotrys hexapetulus* 200 mg/kg/p.o.

The animals were treated with ranitidine (100 mg/kg), dose of hydroalcoholic extract of *Artabotrys hexapetulus* 100 and 200 mg/kg (once daily) for 5 days after the induction of ulcer, while the control group received only the vehicle. The rats were fasted for 24 h and they received 1 ml of absolute ethanol orally. The animals were sacrificed after 1 h of ulcerogen administration, and their stomachs were excised and the gastric contents were aspirated. The contents were subjected to centrifugation at 1000 rpm for 10 min and then analyzed for pH (digital pH meter), pepsin activity, total and free acidity (Mousa *et al.*, 2019).

Antiulcer screening

The ulcer index was determined using the formula

Ulcer index = 10/X

Where X = Total mucosal area/Total ulcerated area.

Based on their 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.

RESULTS & DISCUSSION:

The percentage yield in pet ether and ethanolic extracts was estimated to be 2.5% and 7.6%, respectively. Phytochemical screening of the hydroalcoholic extract of *Artabotrys hexapetulus* roots revealed the presence of several bioactive compounds, including alkaloids, glycosides, flavonoids, proteins, carbohydrates, and saponins. These compounds have the potential to contribute to the plant's medicinal properties and may be responsible for various therapeutic effects.

The total flavonoid and alkaloid content was found to be 0.965 and 0.574 mg per 100 mg of dried extract. The relatively low values of total flavonoids and alkaloid content in the extract suggest that the roots of *Artabotrys hexapetulus* may contain lower concentrations of these compounds compared to some other plant species. However, it's important to note that the pharmacological effects of a plant extract are not solely determined by the concentration of individual compounds. Synergistic interactions between different bioactive compounds and other factors, such as the presence of trace elements and other phytochemicals, can also influence the overall therapeutic potential of the extract.

The data from the ulcer index indicates that the hydroalcoholic extract of *Artabotrys hexapetulus* has a potential anti-ulcer effect in the ethanol-induced ulcer model in rats. Both doses of the extract (100 mg/kg and 200 mg/kg) show a trend toward reducing ulcer severity compared to the control group. In the 100 mg/kg and 200 mg/kg extract-treated groups, the ulcer index was found to be 3.30±0.0 and 3.05±0.15, respectively, while in the control group, the ulcer index was seen to be 6.5±0.15.

These results suggest that the hydroalcoholic extract of *Artabotrys hexapetulus* may possess anti-ulcer properties, possibly through mechanisms that contribute to the reduction of gastric injury caused by ethanol. The significant reduction in the ulcer index

observed with the higher dose indicates the potential of the extract to mitigate ulcer development.

The data suggests that the hydroalcoholic extract of *Artabotrys hexapetulus* has a potential effect on gastric pH in the ethanol-induced ulcer model in rats. Both doses of the extract (100 mg/kg and 200 mg/kg) show an increase in gastric pH of 3.85±0.15 and 4.15±0.15, respectively, compared to the control group, which is 2.65±0.10. The increase in pH indicates a reduction in gastric acidity, which could be beneficial in mitigating the damaging effects of ulceration. The total acidity in the 100 mg/kg and 200 mg/kg extract-treated groups was observed to be 55.75±0.15 and 43.15±0.20 mEq/lt, respectively, while the free acidity in the 100 mg/kg and 200 mg/kg extract-treated groups was estimated to be 41.15±0.10 and 36.50±0.20 mEq/lt, respectively.

The reduction in free acidity is an important observation, as excessive gastric acid secretion can contribute to ulcer formation and exacerbate gastric injury. The extract's ability to modulate acid secretion could contribute to its potential gastroprotective effects and ulcer-healing properties. Pepsin activity was found to be 3.10±0.25 and 2.65±0.15 Per ml/h for the 100 mg/kg and 200 mg/kg extract-treated groups, respectively.

The reduction in pepsin activity is an important observation, as excessive pepsin activity can contribute to the degradation of the gastric mucosa and exacerbate gastric injury. The extract's ability to modulate pepsin activity could contribute to its potential gastroprotective effects and ulcer-healing properties.

Table 1: % Yield of hydroalcoholic extract of *Artabotrys hexapetulus*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	2.5%
2.	Ethanolic	7.6%

Table 2: Phytochemical screening of roots extract of *Artabotrys hexapetulus*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	-ve
	Hager's Test	+ve
2.	**Glycosides**	
	Legal's Test	+ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	-ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	+ve
7.	**Saponins**	
	Froth Test	+ve
8.	**Diterpenes**	
	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	-ve

Table 3: Estimation of total flavonoids and alkaloid content of roots extract of *Artabotrys hexapetulus*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.965	0.574

Table 4: Effect of hydroalcoholic extract of *Artabotrys hexapetulus* on ulcer index by ethanol induced ulcers in rats

Treatment and dose	Ulcer Index
Control	6.5 ±0.15
Ranitidine (50 mg/kg, p.o.)	2.45±0.15***
Hydroalcoholic extract of *Artabotrys hexapetulus* (100 mg/kg, p.o.)	3.30±0.0**
Hydroalcoholic extract of *Artabotrys hexapetulus* (200 mg/kg, p.o.)	3.05±0.15***

Table 5: Effect of hydroalcoholic extract of *Artabotrys hexapetulus* on gastric parameters i.e. pH by ethanol-induced ulceration in rats

Treatment and dose	pH
Control	2.65±0.10
Ranitidine (50 mg/kg, p.o.)	4.50±0.20***
hydroalcoholic extract of *Artabotrys hexapetulus* (100 mg/kg, p.o.)	3.85±0.15**
hydroalcoholic extract of *Artabotrys hexapetulus* (200 mg/kg, p.o.)	4.15±0.10***

Table 6: Effect of hydroalcoholic extract of *Artabotrys hexapetulus* on gastric parameters i.e. total acidity ethanol- induced ulceration in rats

Treatment and dose	Total acidity (mEq/lt)
Control	77.85±0.15
Ranitidine (50 mg/kg, p.o.)	36.12±0.30 ***
hydroalcoholic extract of *Artabotrys hexapetulus* (100 mg/kg, p.o.)	55.75±0.15*
hydroalcoholic extract of *Artabotrys hexapetulus* (200 mg/kg, p.o.)	43.15±0.20 ***

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant

Table 7: Effect of hydroalcoholic extract of *Artabotrys hexapetulus* on gastric parameters i.e. free acidity by ethanol-induced ulceration in rats

Treatment and dose	Free acidity (mEq/lt)
Control	56.32±0.40
Ranitidine (50 mg/kg, p.o.)	24.58±0.20 ***
hydroalcoholic extract of *Artabotrys hexapetulus* (100 mg/kg, p.o.)	41.15±0.10**
hydroalcoholic extract of *Artabotrys hexapetulus* (200 mg/kg, p.o.)	36.50±0.20 ***

Table 8: Effect of hydroalcoholic extract of *Artabotrys hexapetulus* on gastric parameters i.e. pepsin activity by ethanol-induced ulceration in rats

Treatment and dose	Pepsin activity (Per ml/h)
Control	3.54±0.15
Ranitidine (50 mg/kg, p.o.)	2.35±0.15 ***
hydroalcoholic extract of *Artabotrys hexapetulus* (100 mg/kg, p.o.)	3.10±0.25**
hydroalcoholic extract of *Artabotrys hexapetulus* (200 mg/kg, p.o.)	2.65±0.15***

CONCLUSION

The hydroalcoholic extract of *Artabotrys hexapetulus* demonstrates an anti-ulcer effect. In summary, our results suggest that the anti-ulcer activity of the hydroalcoholic extract of *Artabotrys hexapetulus* can be attributed to the anti-secretory, cytoprotective, and antioxidant characteristics of its active phytoconstituents. These findings open up the possibility of considering *Artabotrys hexapetulus* as a potential adjuvant in the treatment of stomach ulcers. The reduction in pepsin activity, coupled with the decreases in free acidity and ulcer index as observed in the earlier data, collectively indicates that the hydroalcoholic extract of *Artabotrys hexapetulus* may exert a comprehensive protective effect on the gastric mucosa, suggesting its potential application in the management of gastric ulcers.

However, further studies are essential to elucidate the underlying mechanisms by which the extract influences pepsin activity and to explore its potential as an anti-ulcer agent. These findings contribute to our understanding of the impact of *Artabotrys hexapetulus* extract on gastric parameters and its potential role in ulcer management. Additional research is warranted to isolate the active ingredients responsible for the anti-ulcer activity and to delineate the specific mechanism of action in gastric ulcer healing.

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