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EVALUATION OF ANTI- ULCER PROPERTIES OF *ARTABOTRYS HEXAPETALUS* ROOT EXTRACT

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ABSTRACT

Peptic ulcers are one of the most common illnesses in humans, affecting over 10% of the global population. A number of synthetic medications, are available to treat peptic ulcers, however shoes adverse effects and drug interactions. Medicinal plants are a great source of medications with zero side effects. *Artabotrys hexapetalus* is one such unrecognized plant with various medicinal properties. Thus, this work aim at evaluation of anti- ulcer properties of *Artabotrys hexapetalus* root extract. The plant material was collected, extracted & subjected to in vitro & in vivo tests as per standard procedures. The results revealed that percentage yield in pet ether & ethanolic extract was estimated to be 2.5% & 7.6% respectively. The phytochemical screening of the hydroalcoholic extract of *Artabotrys hexapetulus* roots reveals the presence of several bioactive compounds, including alkaloids, glycosides, flavonoids, proteins, carbohydrates, and saponins. Total flavonoid & alkaloid content was found to be 0.965& 0.574 mg/ 100 mg of dried extract. In100 mg/kg and 200 mg/kg extract treated group the ulcer index was found to be 3.30±0.0 & 3.05±0.15 respectively while in control group the ulcer index was seen to be 6.5 ±0.15. Both doses of the extract (100 mg/kg and 200 mg/kg) show an increase in gastric pH of 3.85±0.15 & 4.15±0.15 respectively compared to the control group which is 2.65±0.10. The total acidity in 100 mg/kg & 200 mg/kg extract treated group was observed to be 55.75±0.15 & 43.15±0.20 mEq/lt respectively. While the free acidity in 100 mg/kg & 200 mg/kg extract treated group was estimated to be 41.15±0.10 & 36.50±0.20 mEq/lt respectively. The pepsin activity was found to be 3.10±0.25 & 2.65±0.15 Per ml/h for 100 mg/kg & 200 mg/kg extract treated group respectively. Thus results of all parameters indicate towards fact that the *Artabotrys hexapetalus root* extract have influential antiulcer activity.

Keywords: Peptic ulcer, Medicinal plants, *Artabotrys hexapetalus*, Phytochemicals, Ulcer index, free acidity, Total acidity.

INTRODUCTION

The liver is the biggest organ in the human body. The primary function of the liver is to regulate several physiological processes such as glucose, fat, and protein metabolism. It also aids in detoxification, bile acid secretion, which is necessary for digesting, and vitamin storage. In this regard, maintaining a healthy liver is critical for a healthy individual. As a result of these functions, hepatic diseases pose the greatest threat to overall health (Rowe, 2017; Cesaratto *et al*., 2004).

Peptic ulcer disease, which encompasses both stomach and duodenal ulcers, has been a leading cause of morbidity and mortality for more than a century. According to numerous

researchers, peptic ulcers are one of the most common illnesses in humans, affecting over 10% of the global population. Peptic ulcer is defined as an imbalance between the offensive effects of hydrochloric acid and pepsin and the defensive effects of mucus and bicarbonate caused by stress and the use of nonsteroidal anti-inflammatory medicines (NSAIDs) and Helicobacter pylori. Peptic ulcers develop as a result of eating spicy foods and becoming agitated. These two components were discovered to be merely intensive factors. However, the true causes were shown to be a bacterial infection caused by the H. pylori bacteria or a reaction to many types of drugs, most notably NSAIDs. In general, the key etiological factors of peptic ulcer include H. pylori bacteria, NSAIDs, emotional stress, alcohol misuse, and smoking (Kavitt *et al*., 2019; Sverden *et al*., 2019).

A number of medications, including proton pump inhibitors and H2 receptor antagonists, are available to treat peptic ulcers, however clinical trials have revealed an increase in relapses, adverse effects, and drug interactions. However, therapeutic uses of plants are safe, cost-effective, and widely available. Various plants are a great source of medications. Increasing health concerns have prompted researchers to revitalise natural goods and cure ailments without hurting the body (Svanes *et al*., 2000; Ranjan *et al*., 2017).

Artabotrys hexapetalus (L.f.) Bhandari is an Annonaceae plant. There have been reports of this species in India, Sri Lanka, and southern China. *Artabotrys hexapetalus* contains a number of bioactive chemicals with pharmacological action. Antimicrobial, anthelmintic, anticancer, anti-inflammatory, antibacterial, mosquito repellent, antifertility, antioxidant, and anti-leishmanial activities are all possessed by Artabotrys hexapetalus. Artabotrys hexapetalus contains uterine stimulants, muscle relaxants, and cardiac stimulants (Puri, 2020; Quang *et al.,* 2022). Considering the beneficial effect of *Artabotrys hexapetalus* this work aim at evaluation of 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 AND DISCUSSION

The percentage yield in pet ether & ethanolic extract was estiated to be 2.5% & 7.6% respectively. The phytochemical screening of the hydroalcoholic extract of *Artabotrys hexapetulus* roots reveals 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.

Total flavonoid & alkaloid content was found to be 0.965& 0.574 mg/ 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 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. As in100 mg/kg and 200 mg/kg extract treated group the ulcer index was found to be 3.30±0.0 & 3.05±0.15 respectively while in 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 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 & 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 100 mg/kg & 200 mg/kg extract treated group was observed to be 55.75±0.15 & 43.15±0.20 mEq/lt respectively. While the free acidity in 100 mg/kg & 200 mg/kg extract treated group was estimated to be 41.15±0.10 & 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. The pepsin activity was found to be 3.10±0.25 & 2.65±0.15 Per ml/h for 100 mg/kg & 200 mg/kg extract treated group 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**	
	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**	
	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 *A. 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* has anti-ulcer effect. In conclusion, our findings indicated that the anti-ulcer activity of the hydroalcoholic extract of *Artabotrys hexapetulus* was due to the active phytoconstituents' anti-secretory, cytoprotective, and antioxidant characteristics. These findings point to the possibility of using *Artabotrys hexapetulus* as an adjuvant in the treatment of stomach ulcers. The decrease in pepsin activity, along with the reductions in free acidity and ulcer index observed in the previous data, collectively suggests that the hydroalcoholic extract of *Artabotrys hexapetulus* may have a comprehensive protective effect on the gastric mucosa and could potentially be used in the management of gastric ulcers.

Further studies are necessary to elucidate the underlying mechanisms through which the extract influences pepsin activity and explore its potential as an anti-ulcer agent. The findings contribute to our understanding of the effects of *Artabotrys hexapetulus* extract on gastric parameters and its potential role in ulcer management. More research is needed to isolate the active ingredients responsible for the anti-ulcer activity and to determine the specific mechanism of action in gastric ulcer healing.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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