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EXTRACTION, PHYTOCHEMICAL AND ANTIULCER EFFECT OF HYDROALCOHOLIC EXTRACT OF ALPINIA GALANGA

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Abstract:
Peptic disorders like Gastroesophageal reflux disease, gastritis, peptic ulcer, duodenal ulcer, etc., are the common in today's life style. This may be due to stressful life style or improper balance diet. The pathology behind these disorders may be discrepancy between offensive and defensive mechanisms either by excess secretion of acid and pepsin or diminished ability of the gastro-duodenal mucosal barrier to protect against stomach acid-pepsin secretion. Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of the most commonly used medicines and proven to be effective for certain disorders. Some people use NSAIDs on daily basis for preventive purpose. But a variety of severe side effects can be induced by NSAIDs. Studies have shown that edible natural ingredients exhibit preventive benefit of gastric ulcer. Therefore present study was designed to evaluate antiulcer activity of hydroalcoholic extract of Alpinia galanga (A. galanga) rhizomes in rats. Qualitative analysis of various phytochemical constituents and quantitative analysis of total flavonoids and alkaloid were determined by the well-known test protocol available in the literature. The in vivo anti-ulcer activity of hydroalcoholic extract was assessed against ethanol-induced gastric ulcer in rats. Depending on the model, outcome measures were volume and pH of gastric fluid, free acidity, total acidity, pepsin activity and ulcer index as well as percent inhibition of ulcer index. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, and saponins. The total flavonoids content of A. galanga extract was (0.942mg/100mg), followed by alkaloids (0.855mg/100mg) respectively. Further hydroalcoholic extract of 100 and 200mg/kg/p.o, the volume of gastric content, total/free acidity and pepsin activity was significantly decreased and pH of the gastric juice was significantly increase. The findings of this study confirmed that A. galanga extract has anti-ulcer pharmacologic activity due to one or more of the secondary metabolites present in it. Therefore, this study validates its anti-ulcer use in Indian folk medicine. Further investigations on isolation of specific phytochemicals and elucidating mechanisms of action are needed.

Keywords: *Alpinia galanga, Phytochemical constituents, Antiulcer, Non-steroidal anti-inflammatory drugs, Ethanol-induced gastric ulcer.*

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INTRODUCTION:

Peptic ulcer is one of the most prevalent GIT disorders with increased morbidity which affects approximately 5-10% of people during their life [1]. Peptic ulcer disease is a disease of multiple etiologies, till date there is continuous research to elucidate the exact pathogenesis of peptic ulcer, although scientist and researcher proposed a common ground to understand the possible pathogenesis of peptic ulcer. Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and Helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors in the stomach [2]. Such factors could range from natural causes, infections and lifestyle [3-5]. Various treatment options (proton pump inhibitors, histamine receptor antagonists prostaglandins analogs, and cyto protective agents) are available for the management of peptic ulcer. But majority of these drugs generate several undesirable adverse reactions (headache, abdominal pain, bowl upset, dizziness, constipation and diarrhoea) and also may alter normal biochemical homeostasis of the body on chronic use (elevated serum aluminium levels due to antacids and sucralfate, reduced calcium absorption by proton pump inhibitors) [6]. In recent years, a lot of work has been carried out on natural drugs to elucidate their potential effectiveness in gastric ulcer prevention. Herbal medication is promising as an alternative treatment to available synthetic drugs for the treatment of ulcer probably due to availability, affordability, lesser adverse effects and proved effectiveness [7, 8]. Many natural herbs have been pharmacologically reported possessing potent anti-ulcer activity [9-12]. *Alpinia galanga* (L.) Willd. (Zingiberaceae) known as Galangal, a member of the ginger family and native to Southern China and Tailand, is primarily used as a favoring especially in the preparation of fresh Tai curry paste and Tai soup [13]. It is widely cultivated in Southeast Asian countries, e.g., China, Indonesia, Tailand, India, and Philippines [14]. Galangal exhibited diferent pharmacological activities such as antimicrobial, anti-inflammatory, carminative, antipyretic, aphrodisiac, and emmenagogue and traditionally has been used for the treatment of various diseases such as kidney disorders, diabetes, cough, tuberculosis, bronchitis, rheumatism, asthma, and heart diseases [15]. Hence, the objective of the present investigation is to evaluate the anti-ulcer activity of *A. galanga* against ethanol-induced gastric ulcer in Wister albino rat model.

MATERIALS AND METHODS:

Plant material:

The rhizomes of *A. galanga* were collected from Bhimbetka Bhojpur, Raisen (Madhya Pradesh) in the month of November, 2020.

Chemicals and reagents:

All the drugs, solvents and chemicals used in the study were of analytical grade. Ranitidine was obtained as a gift sample from Scan Research Lab, Bhopal, MP, India. All other chemicals e.g. Methanol, ether, formalin, sodium hydroxide, citric acid monohydrate, trichloroacetic acid, sodium nitrate, sodium potassium tartrate, ethylene diamine tetra acetic acid disodium salt were purchased from S. D. Fine Chemicals, Mumbai, India. Tris buffer, Topfer's reagent, Folin's Reagent and Phenolphthalein were purchased from Hi-Media Pvt. Ltd., Mumbai, India.

Extraction by maceration process:

Rhizomes of *A. galanga* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether. The extraction was continued till the defatting of the material has been taken place. 50 gm of dried powdered rhizomes of *A. galanga* has been extracted with hydroalcoholic solvent (ethanol: water: 80:20) using maceration process for 48 hrs. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Phytochemical screening:

Hydroalcoholic extract of *A. galanga* rhizomes was subjected to qualitative phytochemical investigation for the identification of the different phytoconstituents using standard tests and procedures [16, 17].

Total flavonoids content estimation:

Determination of total flavonoids content was based on aluminium chloride method [18]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Total alkaloid determination:

The plant extract (20mg) was dissolved in 1ml of 2 N HCl and filtered. 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 120µg/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 [19].

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 rhizome of *A. galanga* 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[(97)]. The hydroalcoholic extract of rhizome of *Alpinia galanga* 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.

Experimental designs:

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 rhizome *A. galanga* 100 mg/kg/p.o.

Groups IV was treated with hydroalcoholic extract of rhizome of *A. galanga* 200 mg/kg/p.o.

The animals were treated with ranitidine (100 mg/kg), dose of hydroalcoholic extract of rhizome of *A. galanga* 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.

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 DISCUSSIONS:

The crude extracts so obtained after the maceration process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The yield of *A. galanga* extracts was 2.6 %w/w. The results of preliminary phytochemical screening of hydroalcoholic extract of *A. galanga* rhizome are shown in Table 1. The extract showed the presence of flavonoids, alkaloids and saponins. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **$Y=0.032X + 0.018$, $R^2=0.998$**, where X is the quercetin equivalent (QE) and Y is the absorbance. Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: **$Y=0.007X+ 0.024$, $R^2=0.995$**, where X is the Atropine equivalent (AE) and Y is the absorbance. Results were shown in Table 2. The acute oral toxicity study was done according to the OECD 425 guidelines. No adverse changes and mortality were observed in animals, which orally received hydroalcoholic extract (2000 mg/kg) of *A. galanga* rhizomes. This indicates that 2000 mg/kg is maximum safe dose. So $1/10^{th}$ and $1/20^{th}$ *i.e.* 200 and 100 mg/kg of body weight, of the maximum safe dose were selected for studying *in vivo* anti-ulcer effects. The hydroalcoholic extract of *A. galanga* and ranitidine significantly decreased the volume of gastric content, total/free acidity and pepsin activity and significantly enhance the pH; this suggests that it having an anti secretory effect. (Table 3-7). The cause of gastric ulcer is due to stress induced increase in gastric acid (HCl) secretion and these acid secretions promote ulceration due to exposure of the unprotected lumen of the stomach to the accumulating acid [20-22]. Ethanol is responsible for disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. The generation of free radicals was produced by continuous release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol [23]. Ethanol induced gastric ulceration may be occurred due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic tissue injuries. Alcohol has ability to penetrate the gastric mucosa and causing the cellular damage which increases the permeability to sodium and water. In other hand, the accumulation of intracellular calcium

causes the pathogenesis of gastric injury that leads to cell death and exfoliation of surface epithelium. The present study observed that the *A. galanga* significantly reduced ethanol induced ulcer by cytoprotective action via antioxidant effect. The *A. galanga* extract showed cytoprotection against the ethanol induced ulceration by reducing the gastric acid secretion. The results of this study found that *A. galanga* established a cytoprotective action against ethanol induced cellular damage in the gastric mucosa of rats. Cytoprotection of anti-ulcer drugs has been recognised due to the generation of prostaglandins [24]. The modern approach towards a potent antiulcer agent involves a delicate balance of controlling the synthesis, secretion and metabolism of proteins, glycoproteins and lipids, so as to strengthen the mucosal integrity [25]. Several scientific studies revealed that the phytoconstituents like flavonoids, tannins, terpenoids and saponin were responsible for gastro protective agents [26]. Tannins possess as an antiulcer agent by its astringency property and vasoconstriction effects. Due to precipitation of micro proteins on the ulcer site, a protective layer was formed which hinders gut secretions and protects the mucosa from toxins and other irritants. Previous studies have recommended that these above active compounds had ability to stimulate mucus, bicarbonate and prostaglandin secretion and neutralize with the deteriorating effects of reactive oxidants in gastrointestinal lumen [27]. Therefore, *A. galanga* possess antiulcer activity, may be due to presence of flavonoids.

Table 1: Phytochemical screening of extract *of Alpinia galanga*

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

Table 2: Estimation of total flavonoids and alkaloid content of *Alpinia galanga*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	total alkaloid content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.942	0.855

Table 3: Effect of hydroalcoholic extract of rhizome of *A. galanga* on ulcer index by ethanol induced ulcers in rats

Treatment and dose	Ulcer Index
Control	7.00 ±0.25
Ranitidine (50 mg/kg, p.o.)	2.50±0.20***
Hydroalcoholic extract of rhizome of *A. galanga* (100 mg/kg, p.o.)	3.50±0.18**
Hydroalcoholic extract of rhizome of *A. galanga* (200 mg/kg, p.o.)	3.02±0.18***

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test).

Table 4: Effect of hydroalcoholic extract of rhizome of *Alpinia galanga* on gastric parameters i.e. pH by ethanol-induced ulceration in rats

Treatment and dose	pH
Control	2.70±0.14
Ranitidine (50 mg/kg, p.o.)	4.60±0.18***
Hydroalcoholic extract of rhizome of *A. galanga (*100 mg/kg, p.o.)	3.98±0.18**
Hydroalcoholic extract of rhizome of *A. galanga* (200 mg/kg, p.o.)	4.44±0.18***

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test).

Table 5: Effect of hydroalcoholic extract of rhizome of *Alpinia galanga* on gastric parameters i.e. total acidity ethanol- induced ulceration in rats

Treatment and dose	Total acidity (mEq/lt)
Control	78.10±0.50
Ranitidine (50 mg/kg, p.o.)	35.01±0.30 ***
Hydroalcoholic extract of rhizome of *A. galanga* (100 mg/kg, p.o.)	58.50±0.30*
Hydroalcoholic extract of rhizome of *A. galanga* (200 mg/kg, p.o.)	45.40±0.30 ***

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test).

Table 6: Effect of hydroalcoholic extract of rhizome of *Alpinia galanga* on gastric parameters i.e. free acidity by ethanol-induced ulceration in rats

Treatment and dose	Free acidity (mEq/lt)
Control	55.16±0.40
Ranitidine (50 mg/kg, p.o.)	25.3±0.30 ***
Hydroalcoholic extract of rhizome of *A. galanga* (100 mg/kg, p.o.)	40.5±0.40**
Hydroalcoholic extract of rhizome of *A. galanga* (200 mg/kg, p.o.)	37.40±0.40 ***

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test).

Table 7: Effect of hydroalcoholic extract of rhizome of *Alpinia galanga* on gastric parameters i.e. pepsin activity by ethanol-induced ulceration in rats

Treatment and dose	Pepsin activity (Per ml/h)
Control	3.50±0.20
Ranitidine (50 mg/kg, p.o.)	2.50±0.20 ***
Hydroalcoholic extract of rhizome of *A. galanga* (100 mg/kg, p.o.)	3.30±0.31**
Hydroalcoholic extract of rhizome of *A. galanga* (200 mg/kg, p.o.)	2.70±0.32***

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test).

CONCLUSION:

The preliminary phytochemical investigation of hydroalcoholic extract of *A. galanga* rhizomes showed the presence of flavonoids, saponins, alkaloids. Hydroalcoholic extract was screened for acute oral toxicity and was found to be non toxic. Hydroalcoholic extract of *A. galanga* rhizomes possesses significant anti-ulcer activity. In conclusion, our results showed that the anti-ulcer activity of the extract was a result of the probable gastric ulcer healing mechanism (anti-secretory, and cytoprotective properties) of its active phytoconstituents. These findings suggest the potential for use of *A. galanga* as an adjuvant in the treatment of gastric ulcer. Further, studies are needed for the isolation of active constituents responsible for the anti-ulcer activity and to elucidate the exact mechanism of action in gastric ulcer healing.

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