

Anti Ulcerogenic and Anti-Oxidant Activity of *Alpinia Galanga* Rhizomes Aqueous Extract in Indomethacin Induced Gastric Mucosal Damage in Wistar Albino Rats

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Peptic ulcer disease is common diseases of the gastrointestinal tract that occur with long term use of NSAIDs. Currently available drugs for acid peptic disease shows side effects, relapses after discontinuation and drug interactions. Many Indian medicinal plants and their active compounds are used for prevention and treatment of Peptic ulcer. To evaluate the anti-ulcerogenic and anti-oxidant activity of *Alpinia galanga* rhizome in Wistar Albino rats. Animals were divided into 4 groups with 6 animals in each. Group 1 received only vehicle 1% CMC orally for 7 days. Group 2 received 1% Carboxyl methyl cellulose (gastric mucosal injury was induced by Indomethacin in this group) orally for 7 days. Group 3 received *Alpinia galanga* rhizomes extract (AGRE) 200 mg/kg orally for 7 days. Group 4 received Ranitidine 20 mg/kg orally. On 7th day, overnight fasted rats were given last dose of drugs and after 60 minutes of the last dose, gastric mucosal damage was produced by Indomethacin 40 mg/kg through gastric lavage, in all groups except group 1. Six hours later, calculation of ulcer score was done using Ulcer index and percentage inhibition method. The number and severity of ulcers were recorded with arbitrary scale. The antioxidant action of AGRE was also assessed. Rats pre-treated with extract of *Alpinia galanga* rhizomes showed significant decrease in ulcer index and percentage inhibition of ulcers was comparable with Ranitidine. Also, there was a significant increase in antioxidant levels in rats treated with *Alpinia galanga* rhizomes extract showing its antioxidant property. The extract shows significant antiulcer activity against Indomethacin induced gastric ulcers probably due to its antioxidant property.

Keywords: *Alpinia galanga* rhizomes; Acid peptic Disease; Indomethacin; Ranitidine.

Gastro intestinal diseases are human ailments causing maximum discomfort, morbidity and mortality¹. Peptic ulcer is one of the common diseases of the gastrointestinal tract where there is a break in the mucosal integrity of the stomach or duodenum.²

It possibly due to disturbance in balance between the aggressive factors like pepsin, acid and *H. pylori* and the defensive factors such as bicarbonate, gastric mucus, and prostaglandins.

Most common symptoms of the peptic ulcer disease are upper abdominal burning pain,

heart burn, belching, nausea, vomiting, and weight loss. Symptoms of Peptic ulcer disease is aggravated by a variety of factors such as alcohol consumption, stress, *Helicobacter pylori* infection and NSAIDs and.3 NSAIDs are one of the most commonly used drugs for longer duration against diseases like Rheumatoid arthritis and gout. On chronic use most harassing side effect of NSAIDs is gastric ulceration.

Main stay of management of peptic ulcer includes chronic dietary control and pharmacological treatment. Pharmacological treatment of peptic ulcer includes H₂ antagonists, PPIs, Antacids, ulcer protectives and anti *H. pylori* drugs. These drugs have side effects, incidence of relapses, and drug interactions. These drugs cause side effects such as dizziness, diarrhea, constipation, accelerated osteoporosis, kidney damage, gynecomastia, hematopoietic changes. Also, safety of most of the presently used drugs is not established during pregnancy. Hence, there is need for search of effective and safe antiulcer agents.

Many Indian medicinal plants and their active compounds are used for prevention and treatment of Peptic ulcer. *Alpinia galanga*, commonly known in Tamil as “Pera-rattai” is used in Ayurveda against peptic ulcer Disease. In Ayurveda *A. galanga* is considered as *Vatashamahna* drug and is used as carminative, digestive tonic, anti-emetic, anti-fungal, anti-tumor, Anthelmintic, anti-diuretic, anti-ulcerative, anti-dementia. Also, it is one of the constituents in ayurvedic preparations like *Rasnadhi Choornam*, *Rasnerand aedhi kashayam* and *Maharasnadhi Kashayam*.⁴

This study was planned to evaluate anti-ulcer and anti-oxidant action of aqueous extract of *Alpinia galanga* rhizomes in gastric mucosal damage induced by indomethacin in rats.

MATERIALS AND METHODS

Ethical Clearance

Institutional Animal Ethics Committee (IAEC) clearance was taken before commencement of the study. (Letter No: 01/IAEC/MG /2014)

Collection of Plant Material (Extract)

Aqueous extract of *Alpinia galanga* rhizome (AGRE) was purchased from Chemiloids Pvt. Ltd, Vijayawada, A.P, India. Extract was dark

brown colour dry powder. The extract was kept in airtight glass container inside the refrigerator at 40C.

Phytochemical Screening

Alpinia galanga rhizomes extract was screened for presence or absence of various secondary metabolites such as alkaloids, glycosides, steroids, flavonoids, tannins and polyphenols by using standard procedures.⁵

Drugs and Chemicals

Indomethacin obtained from Sigma Chemicals, Bangalore, India. Ranitidine of Koprani Laboratories was procured from local medical store. All other solvents and chemicals used were obtained from SD-fine Ltd, Mumbai, Maharashtra, India.

Animals

Wistar male albino rats weighing 180 ± 20 g of age 10 to 12 weeks from birth were obtained from King's institute, Chennai. They were procured before 2 weeks and kept in the animal house of the Mahatma Gandhi Medical College & Research Institute to familiarise them to new environment. The environment of the room was maintained on a 12-hour light/dark cycle. They were fed with standard laboratory diet and access to water ad libitum. The care of animals in departmental animal house was taken according to the CPCSEA Guidelines.⁶

Acute Toxicity Study and Dose Selection

Acute toxicity of *Alpinia galanga* rhizomes extract (AGRE) was done using revised OECD/OCED guidelines. Overnight fasted, Wistar rats of either sex was used for testing. The dose was calculated according to the body weight so that equal volume of the drug is administered to them.

Limit Test at 2000 mg/kg

As the one animal survived after 2000 mg/kg BW of AGRE, 4 more rats were given the same dose and observed individually for first 4 hours continuously and then occasionally for further 4 hours till 24 hours after the oral administration. After a period of 24 and 72 h they will be observed for any lethality or death. As no animal died, LD₅₀ of test drug can be taken as greater than 2000 mg/kg. Accordingly, the dose selected was 1/10th of acute toxicity study.

Indomethacin Induced Gastric Mucosal Damage

Wistar male albino rats weighing $180 \pm$

20 g were divided into 4 groups with 6 animals in each. Group 1 (control) received only vehicle 1% Carboxyl methyl cellulose (CMC) orally for 7 days. Group 2 (positive control) received 1% CMC (gastric mucosal injury was induced by Indomethacin in this group) orally for 7 days. Group 3 (Test group) received *Alpinia galanga* rhizomes extract (AGRE) 200 mg/kg (in 1% CMC) orally for 7 days. Group 4 (Standard Control) received Ranitidine 20 mg/kg (in 1% CMC) orally for 7 days.

On 7th day, overnight fasted rats were given last dose of drugs and after 60 minutes of the last dose, gastric mucosal damage was produced by Indomethacin 40 mg/kg (in 1% CMC) through gastric lavage, in all groups except group 1

After six hours of Indomethacin administration, the animals were sacrificed using ether. Their stomachs were removed and fixed on a cork plate. The gastric mucosa was inspected using a magnifying lens for any visible mucosal damage. Haemorrhagic spots greater than 2 mm diameter are taken as ulcers. The number of ulcers and their severity were recorded by arbitrary scale

Severity Score 0 was used for normal stomach, 0.5, 1 and 1.5 was used if there is red coloration, spot ulcers and haemorrhagic streaks respectively while 2 and 3 was used if Ulcers = 3 but = 5 and = 5 respectively.

Ulcer index was calculated as using formula $UI (\text{Ulcer index}) = UN (\text{Average of number of ulcers}) + US (\text{average of severity score}) + UP (\text{Percentage of animals with ulcer}) \times 10^{-1}$

Percentage inhibition was calculated by formula $\% \text{ Inhibition} = (UI \text{ positive control group} - UI \text{ treated group}) / UI \text{ positive control group}$

Antioxidant Levels Estimation

After the examination of gastric mucosa Stomach weighed and kept in cold condition. Then it was cross chopped with surgical scalpel into fine slices. The tissue was homogenized in normal saline. The prolonged homogenization under hypotonic condition was designed to disrupt cells as far as possible so as to release soluble protein and leave only membrane and non-vascular matter in a sedimentable form. It was then centrifuged at 5000 rpm for 20 min and clear supernatant was kept frozen at -20°C until analysis.

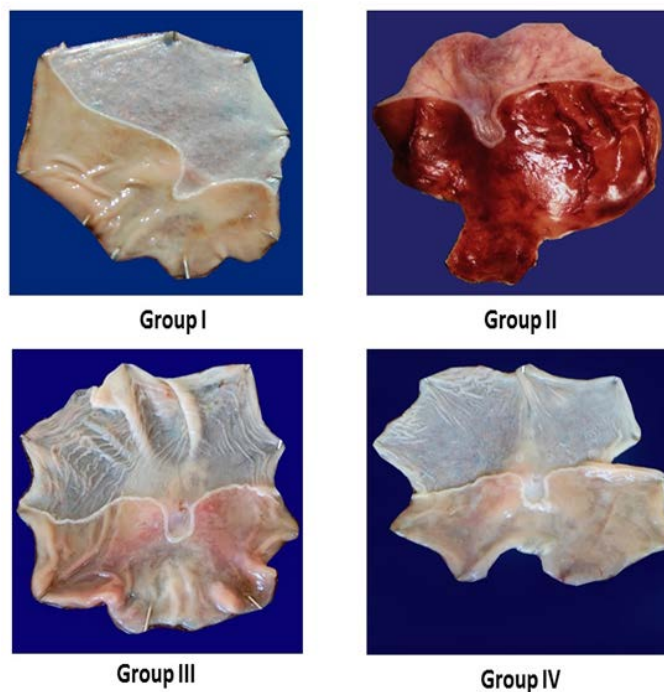


Fig. 1. Showing Stomach of control rat (Group 1); Indomethacin induced gastric ulcer (Group 2), Effect of *AGRE* on gastric ulcer induced by Indomethacin (Group 3), Effect of Ranitidine on gastric ulcer induced by Indomethacin (Group 4)

The antioxidant action of AGRE was assessed by measuring TBARS levels, Glutathione (GSH) activity, Superoxide dismutase (SOD) activity and Catalase activity in tissues by the methods of Ohkawa, Kakkar and Sinha *et. al.*, respectively.

Statistical Analysis

Results were expressed as Mean \pm SD. Statistical analysis was done using analysis of variance followed by Post hoc test Bonferroni. *p* value <0.05 was considered as statistically significant.

RESULTS

Phytochemical Analysis of *Alpinia Galanga* Rhizomes Extract

The preliminary tests of *Alpinia galanga* rhizomes extract showed the presence of alkaloids, steroids, terpenoids, flavonoids, polyphenols and tannins.

Acute toxicity study and dose selection

Alpinia galanga rhizomes extract up to a dose of 2 g/kg BW orally did not showed any

alteration in the behaviour of the animals. All the animals were active and there was no mortality. This showed that the oral median lethal dose of AGRE is more than 2 g/kg BW. So, dose 200 mg/kg, *p.o.*, which was 1/10th of 2000 mg/kg, was chosen for further study.

Effect of *Alpinia Galanga* Rhizomes Extract on Gastric Mucosa

In positive control group (group 2), there was visible gastric mucosal damage after 6 h of Indomethacin administration. In *Alpinia galanga* rhizomes extract (Group 3) and standard drug Ranitidine pre-treated group (Group 4), there was decrease in intensity of gastric mucosal damage but Ranitidine was found better than the AGRE. (Figure 1)

Effect of *Alpinia Galanga* Rhizomes Extract on Ulcer Index and Percentage Protection

The group pre-treated with standard drug Ranitidine (Group 4) and the group pre-treated with extract of *Alpinia galanga* rhizomes (Group 3) showed significant decrease ($p < 0.001$) in ulcer index compared to the positive control (Group 2). Percentage inhibition of ulcers *Alpinia galanga*

Table 1. Effect of pre-treatment of *Alpinia galanga* rhizomes extract on ulcer index and percentage protection on gastric ulcers induced by Indomethacin

Groups	Ulcer Index	Percentage protection (%)
Group I (CMC)	0.83 \pm 0.25	–
Group II (CMC+IND)	7.0 \pm 1.6	–
Group III (AGRE + IND)	2.33 \pm 0.21*	66.71
Group IV (RAN + IND)	1.66 \pm 0.11*	76.29

Significance at **p* value <0.05 compared to positive control (Group 2). CMC: 1% CMC, CMC + IND: 1% CMC + Indomethacin 40 mg/kg, AGRE + IND: *Alpinia galanga* rhizomes extract 200 mg/kg + Indomethacin 40 mg/kg, RAN + IND: Ranitidine 20 mg/kg + Indomethacin 40 mg/kg

Table 2. Effect of *Alpinialangala rhizomes* Extract on levels of TBARS, SOD and Catalase in indomethacin induced gastric ulcer model

Treatment	TBARS (nmol/g tissue)	GSH (μ g/mg tissue)	SOD (IU/g tissue)	Catalase (IU/g tissue)
Group 1(CMC)	0.12 \pm 0.03	2.6 \pm 0.5	68.42 \pm 10.48	127 \pm 17.9
Group 2(CMC+IND)	0.69 \pm 0.16	0.9 \pm 0.2	21.51 \pm 3.6	56.59 \pm 10.4
Group 3(AGRE + IND)	0.24 \pm 0.07*	2.1 \pm 0.34*	93.18 \pm 17.26*	119.5 \pm 14.9*
Group 4(RAN + IND)	0.27 \pm 0.03*	2.2 \pm 0.35*	74.14 \pm 13.01*	120.4 \pm 16.76*

Significance at **p* value <0.05 as compared to positive control. CMC: 1% CMC, CMC + IND: 1% CMC + Indomethacin 40 mg/kg, AGRE + IND: *Alpinia galangarhizomes* extract at 200 mg/kg + Indomethacin 40 mg/kg, RAN + IND: Ranitidine at 20 mg/kg + Indomethacin 40 mg/kg

rhizomes extract pre-treated group was comparable with standard group. (Table 1)

Effect of *Alpinia Galanga* Rhizomes Extract on TBARS and Antioxidant Levels

In positive control group a marked increase in the TBARS level and a concomitant decrease in the antioxidant levels were noted. However, Pre-treatment with *Alpinia galanga* rhizomes extract and standard drug Ranitidine significantly reduced the TBARS level. The activities of SOD, CAT and glutathione were significantly lower in positive control group compared to normal control. Pre-treatment with *Alpinia galanga* rhizomes extract showed a significant increase in SOD, CAT and glutathione in the positive control group but was less than standard drug Ranitidine. (Table 2)

DISCUSSION

The peptic ulcer is common terminology used for both gastric ulcers and duodenal ulcers. It possibly due to disturbance in balance between the aggressive factors and the defensive factors

Therapy for peptic ulcer is well established with many effective antiulcer agents but there are adverse effects with these drugs. Due to high morbidity associated, there remains continuous need for safe anti-ulcer medicines, which made researchers to look for safer alternative herbal therapies.⁷

In the present study, antiulcer and antioxidant activities of hydroalcoholic extract of *Alpinia galanga* rhizomes were investigated by experimental animal models. An acute toxicity study was done to find out the dose that can be used safely for experiments rather than to provide complete toxicity data on the extract.

After acute administration of Indomethacin, there was visible gastric lesions and increase in ulcer index. In the groups pre-treated with AGRE, there was significant decrease in intensity of gastric lesions and ulcer index. These findings suggested that *Alpinia galanga* rhizomes have a cytoprotective effect. Our study findings were same as previous study done by Matsuda H *et. al.*, in 2003 on gastro-protective effect of *Alpinia galanga* rhizomes, but study results were contradicting to study done by Al-Yahya *et. al.*, in 1990, who showed *Alpinia galanga* rhizomes did

not protect gastric mucosa against Indomethacin.^{8,9}

Mucus production is stimulated by prostaglandins, which also directly inhibit gastric acid secretion by parietal cells and protects stomach from acid damage. NSAID's like Indomethacin causes damage to gastric and duodenal cells by inhibition of prostaglandin synthesis, thus decreasing prostaglandin levels.¹⁰ *Alpinia galanga* rhizomes extract has been proved to inhibit Indomethacin induced mucosal damage; there is a likelihood of its intervention in prostaglandin synthesis.¹¹

In positive control group TBARS levels increased and reduced activities of enzymes GSH, SOD and Catalase in stomach have been observed in this study. The increase in level of TBARS and decrease in the enzymatic antioxidant defense mechanism is indicative of lipid peroxidation.¹²

Production of oxygen free radicals significantly increased may be due to gastric mucosal injury is associated with increased lipid peroxidation.

The over expression of antioxidant enzymes plays an important role in protecting cells from oxidative damage. Pretreatment with *Alpinia galanga* rhizomes extract for 7 days significantly decreased TBARS levels and increased the levels of SOD and Catalase suggestive of its antioxidant potential.^{12,13}

The phytochemical analysis of *Alpinia galanga* rhizomes extract revealed presence of Flavonoids followed by alkaloids, steroids, terpenoids.

Many studies have reported anti-ulcer and gastric protection activity a of flavonoids.^{14,15} So, the antiulcer and anti-oxidant effect of *Alpinia galanga* rhizomes extract might be due to the presence of Flavonoids.

CONCLUSION

Alpinia galanga rhizomes extract showed a significant antiulcer activity against indomethacin induced gastric ulcers. *Alpinia galanga* rhizomes extract offered the protection, probably due to its antioxidant property. Further studies are warranted to explain exact mechanism of antiulcer effect of *Alpinia galanga* rhizomes extract.

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