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Role of *Abrus precatorius* Linn. in Indomethacin Induced Gastric Ulcer in Rats

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ABSTRACT

Role of *Abrus precatorius* Linn. in indomethacin induced gastric ulcers was studied in rats. *Abrus precatorius* Linn. leaves significantly reduced ulcer index induced by indomethacin. The plant leaves produced gastric anti secretory effect by decreasing gastric volume and acidity it further increased gastric mucin, which showed gastric cytoprotective effect. The plant prevented the increased lipid peroxidation during ulceration by indomethacin. Activities of the anti oxidant enzymes were enhanced during ulceration by this plant leaves. Results were comparable to that of ranitidine, a standard anti ulcer drug. Anti ulcerogenic activity of *Abrus precatorius* Linn. leaves was, thus, mediated through anti oxidant defense mechanism.

Keywords: *Abrus precatorius Linn, indomethacin, ranitidine, gastric ulcer, SOD, CAT, GSH.*

Introduction

Abrus precatorius Linn. has been used in Hindu medicines from very early times, as well as in china and other ancient cultures [1]. The plant was considered beneficial for the hair and the seeds extract is used in the treatment of ulcer and skin affection [2]. Seeds of the plant are very much attractive, used in ornaments, but are highly poisonous. Seeds are reported to have anti diabetic property [3], may induce abortion [4], have anti oxidative property [5] as well as anti– inflammatory analgesic activity [6]. Saganuwan and Gulumbe [7,8] reported antimicrobial activity of the aqueous extract of *Abrus precatorius* Linn. against various bacteria. Karamoko *et al.* also showed antibacterial activities of the aqueous extract of the plant [9]. Other uses of the plant are observed in cancer [10] and in malaria [11]. Phytochemical components of the plant are abricin, abrin, abrisin, abrine, abraline, abrasine, abrusgenic acid-methylester, abruslectone, abrussic acid, anthocyanins etc. [12,13].

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Recently in screening program we observed anti peptic ulcer activity of the leaves of *Abrus precatorius* Linn. in albino rats [14]. In present communication, effect of *Abrus precatorius* Linn. leaves on indomethacin induced gastric ulcer in albino rats and the possible mechanism involved therein are being reported.

Materials and Methods

Plant Material

Abrus precatorius **Linn.**

Leaves of *Abrus precatorius* Linn.were collected from the medicinal plants garden of the University of North Bengal during July -August, 2013 and authenticated by the taxonomist of the department of Botany of the said University. A voucher specimen (Authentication no. VS/13/07/0029) was kept in the department for future reference. Leaves were shade dried and powdered. The powder was used as the test drug.

Preparation of the Test Drug

Leaves of *Abrus precatorius* Linn. were properly washed, shade dried and powdered. The powder was used as the test drug.

Experimental animals

Wistar strain albino rats (150 - 180 g) of either sex were used

Figure 1: *Abrus precatorius* **Linn.**

for the study. Rats were housed in colony cages (5 rats / cage) and were kept for at least a week in the experimental wing of the animal house (room temperature 25 – 28 degree centigrade and humidity 60 – 65% with 12 h light and dark cycle) before experimentation. Animals were fed on laboratory diet with water *ad libitum*. 8 rats were used for each set of experiment. The animal experiment was approved by the ethics committee of the Institute (No. PCM/ 08-09/05/1173 dt. 05/07/08).

Chemicals and Drugs

All chemicals used in this experiment were procured from Ranbaxy and SD Fine Chemicals, New Delhi, India. Indomethacin (Torrent Research Centre, Gandhinagar), ranitidine (Cipla pharmaceuticals) were used in the study.

Acute toxicity study

Acute toxicity study of *Abrus precatorius* Linn. was done by the method of Ghosh [15]. Rats were starved overnight (water is supplied *ad libitum*) and were divided into five groups of ten each.

Powdered leaves of *Abrus precatorius* Linn. (4.5% w/v) suspended in water was given to rats orally through feeding tube in increasing dose levels of 0.1, 0.5, 1, 3 and 5g/kg body weight. The animals were observed continuously for 2 hrs for the following :

a) Behavioral profile : alertness, restlessness, irritability and fearfulness.
b) Neurological profile: spontaneous activity, reactivity, touches response, pain response and gait.
c) Autonomic profile : defecation and urination.

The number of deaths, if any, was recorded after 24 and 72 hours.

Production of gastric ulcer

Indomethacin induced gastric ulcer was produced by the method of Parmar and Desai [16] with slight modification. Rats were fasted for 18 h when no food but water was supplied *ad libitum*. Indomethacin at a dose of 10 mg/kg (20mg/ml suspension in 1% carboxy methylcellulose in water was given to rats orally through a feeding tube in two doses at an interval of 15 hour. 1h after administration of last dose, pylorus part of the animals was ligated. Four hours after pyloric ligation the animals were sacrificed by cervical dislocation. Stomachs were taken out, gastric juice collected and the stomachs were then incised along the greater curvature to examine the ulcers.

Antiulcer Study

Rats were divided into 4 groups.
1. Control: Rats took normal diet and water as well as vehicle of drug.
2. Indomethacin treated : Rats were treated with indomethacin as mentioned earlier.
3. Indomethacin + Powdered leaves of *Abrus precatorius* Linn. was given to the rats orally through feeding tube 30 minutes prior to each dose of indomethacin as discussed before.

4. Indomethacin + Ranitidine : Ranitidine was given in the dose of 50 mg/kg p.o. 30 minutes before each dose of indomethacin. Dose of ranitidine was selected based on the report of Khare *et al.* [17].

Evaluation of Ulcer Index

This was done by the method of Szelenyi and Thiemer, [18].

Gastric lesions were counted and the mean ulcerative index was calculated as follows :
I – Presence of edema, hyperemia and single sub mucosal punctiform hemorrhage.
II – Presence of sub mucosal hemorrhagic lesions with small erosions.
III – Presence of deep ulcer with erosions and invasive lesions.
Ulcer index = (number of lesion I) x1 + (number of lesion II) x2 + (number of lesion III) x 3.

Biochemical estimations

Collected gastric juice from the rat's stomach was centrifuged and its volume and pH were measured. Gastric juice was further used for the estimation of free and total acidity as described by Hawk *et al* [19], pepsin content by the method of Anson [20], Musin by our methos [21] and total protein by the method of Lowry *et al* [22].

The mucosal tissue from rat's stomach was scrapped and then homogenized (5%) in ice cold 0.99% saline with a Potter – Elvehjem glass homogenizer for 30 sec. The homogenate was used for the estimations of DNA [23], nitric oxide [24] , lipid peroxides [25], superoxide dismutase [26], catalase [27], glutathione [28], and glutathione peroxidase [29] .

Statistical Analysis

The values were expressed as mean ± SEM and were analyzed

using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20th versions. Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p< 0.05$.

Results and Discussion

Acute toxicity study

Result of acute oral administration of powdered leaves of *Abrus precatorius* Linn. in various doses of 0.1, 0.5, 1, 3 and 5g/kg indicated no mortality up to 72 hrs after treatment. All rats were healthy and active during the experimental period.

Anti gastric ulcer activity of *Abrus precatorius* Linn.

Results relating to effects of *Abrus precatorius* Linn. and ranitidine against indomethacin induced gastric ulcer in rats were shown in Table -1.

Indomethacin produced massive ulcers in glandular part of rat's stomach. Incidence of ulceration was 100% . Acute dilatation and hemorrhage were seen in stomach. In one rat perforation of the stomach was noted. Ulcer index came 30.4 ± 1.52. Pretreatment of rats with *Abrus precatorius* Linn. leaves gave significant protection (27.30%, 66.48% and 66.78% by the doses 0.5 g/kg, 1.0 g/kg and 1.5 g/kg respectively) to the animals from forming ulcers by indomethacin. Ranitidine (50 mg/kg), however, gave 71.05% protection.

Anti secretory effect

Results were shown in Table – 2. In control rats volume and pH of gastric juice were 1.34 ± 0.04 and 2.91 ± 0.08 respectively. Indomethacin increased volume of gastric juice (3.61 ± 0.07) and decreased its pH (1.61 ± 0.07) in rats. Changes were statistically significant ($p<0.001$). Pretreatment of rats with *Abrus precatorius* Linn. leaves, however, could decrease volume of gastric juice (1.39 ± 0.08) and increase its pH (2.71 ± 0.06). Effects were comparable to that of ranitidine as in this group gastric juice volume and pH were 1.36 ± 0.07 and 2.85 ± 0.05 respectively.

Effect of *Abrus precatorius* Linn. leaves on gastric acidity

Table – 3 showed effects of *Abrus precatorius* Linn. leaves and ranitidine on free and total acidity of gastric juice during indomethacin induced gastric ulcer in rats. Indomethacin significantly increased both free and total gastric acidity. Free and total gastric acidity of control rats were 11.31 ± 0.49 and 31.28 ± 0.52 respectively. For indomethacin group values came 23.69 ± 1.11 (free acidity) and 77.34 ± 2.93 (total acidity). *Abrus precatorius* Linn. leaves could decrease free and total gastric acidity (12.12 ± 0.46 and 49.71 ± 1.53 respectively). Ranitidine also decreased raised free and total gastric acidity during indomethacin induced gastric ulcers.

Cytoprotective effect

Effects of *Abrus precatorius* Linn. and ranitidine on gastric pepsin and mucin during indomethacin induced gastric ulcer in rats were shown in Table - 4. It appears from the table that indomethacin elevated activity of gastric pepsin (46.22 ± 1.35, control - 31.62 ± 1.15) and lowered mucin content of gastric juice (1.23 ± 0.15, control - 5.68 ± 0.31). Changes were statistically significant ($p<0.001$). *Abrus precatorius* Linn. could decrease significantly ($p<0.001$) gastric pepsin activity and increase mucin content of gastric juice during indomethacin induced gastric ulcer in albino rats. Results were comparable to that of ranitidine.

Effect of *Abrus precatorius* Linn. leaves on gastric protein and DNA

Results are given in Table 5. In control rats gastric protein and DNA content came 36.16 ± 1.24 and 139.17 ± 5.34 respectively. Indomethacin lowered both the two values which were statistically significant ($p<0.001$). Pretreated group of rats by *Abrus precatorius* Linn. leaves showed increased values of gastric protein and DNA content of gastric mucosa which were more close to the control values. Results were comparable to that of ranitidine.

Table 1: Effect of *Abrus precatorius* Linn. leaves (APL) on indomethacin (INDO) induced gastric ulcer in albino rats.

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
INDO	30.4 ± 1.52	--
INDO+ APL (0.5g/kg)	22.1 ± 1.11*	27.30
INDO+ APL (1.0g/kg)	10.2 ± 1.15**	66.48
INDO+ APL (1.5g/kg)	10.1 ± 1.13**	66.78
INDO+Ranitidine (50mg/kg)	8.8 ± 1.03**	71.05

*Results were in mean ± SEM, Each group had eight rats, *p<0.05, ** p<0.001*

Table 2: Effect of *Abrus precatorius* Linn. leaves (APL) on volume and pH of gastric juice during indomethacin (INDO) induced gastric ulcer in albino rats.

Group	Volume of gastric juice (ml)	pH of gastric juice
Control	1.34 ± 0.04	2.91 ± 0.08
INDO	3.61 ± 0.07**	1.61 ± 0.07**
INDO + APL (1.0 g/kg)	1.39 ± 0.08**	2.71 ± 0.06**
INDO + Ranitidine (50mg/kg)	1.36 ± 0.07**	2.85 ± 0.05**

*Results were in mean ± SEM, Each group had eight rats, ** p<0.001*

Table 3: Effect of *Abrus precatorius* Linn. leaves (APL) on free and total gastric acidity during indomethacin (INDO) induced gastric ulcer in albino rats.

Group	Free acidity (mEq/l/100g)	Total acidity (mEq/l/100g)
Control	11.31 ± 0.49	31.28 ± 0.52
INDO	23.69 ± 1.11**	77.34 ± 2.93**
INDO + APL (1.0 g/kg)	12.12 ± 0.46**	49.71 ± 1.53**
INDO + Ranitidine (50mg/kg)	11.84 ± 0.48**	33.45 ± 1.33**

*Results were in mean ± SEM, Each group had eight rats, *p<0.05, ** p<0.001*

Table 4: Effect of *Abrus precatorius* Linn. leaves (APL) on gastric pepsin and mucin content during indomethacin (INDO) induced gastric ulcer in albino rats.

Group	Pepsin (micromole/ml)	Mucin (microgram/g)
Control	31.62 ± 1.15	5.68 ± 0.31
INDO	46.22 ± 1.35**	1.23 ± 0.15**
INDO + APL (1.0 g/kg)	33.11 ± 1.42**	4.55 ± 0.25**
INDO + Ranitidine (50mg/kg)	32.56 ± 1.73**	4.99 ± 0.17**

*Results were in mean ± SEM, Each group had eight rats, *p<0.05, ** p<0.001*

Table 5: Effect of *Abrus precatorius* Linn. leaves (APL) on gastric protein and DNA content during indomethacin (INDO) induced gastric ulcer in albino rats.

Group	Protein (mg/ml)	DNA (microgram/mg protein)
Control	36.16 ± 1.24	139.17 ± 5.34
INDO	10.88 ± 1.56**	88.78 ± 3.09**
INDO + APL (1.0 g/kg)	30.74 ± 1.44**	126.33 ± 0.02**
INDO + Ranitidine (50mg/kg)	32.54 ± 1.62**	129.59 ± 4.64**

*Results were in mean ± SEM, Each group had eight rats, *p<0.05, ** p<0.001*

Table 6: Effect of *Abrus precatorius* Linn. leaves (APL) on gastric nitric oxide and lipid peroxides during indomethacin (INDO) induced gastric ulcer in albino rats.

Group	Nitric oxide (micromole/g wet tissue)	Lipid peroxides (nm/mg protein)
Control	125.19 ± 7.52	4.39 ± 0.03
INDO	198.44 ± 8.27**	9.94 ± 0.07**
INDO + APL (1.0 g/kg)	129.13 ± 7.56**	4.81 ± 0.06**
INDO + Ranitidine (50mg/kg)	128.78 ± 6.55**	4.59 ± 0.06**

*Results were in mean ± SEM, Each group had eight rats, *p<0.05, ** p<0.001*

Table 7: Effect of *Abrus precatorius* Linn. leaves (APL) on superoxide dismutase and catalase activity during indomethacin (INDO) induced gastric ulcer in albino rats.

Group	Superoxide dismutase (SOD) (Unit/g wet tissue)	Catalase (CAT)(Unit/g wet tissue)
Control	139.44 ± 5.65	25.54 ± 2.34
INDO	89.87 ± 2.43**	9.99 ± 1.15**
INDO + APL (1.0 g/kg)	125.34 ± 4.33**	19.61 ± 2.33**
INDO + Ranitidine (50mg/kg)	130.45 ± 4.42**	21.45 ± 2.48**

*Results were in mean ± SEM, Each group had eight rats, *p<0.05, ** p<0.001*

Anti oxidant defense mechanism

Nitric oxide levels and lipid peroxides were increased significantly (p<0.001) in indomethacin treated rats (values came 198.44 ± 8.27 and 9.94 ± 0.07 respectively) in comparison to that of control rats (values came 125.19 ± 7.52 and 4.39 ± 0.03 respectively). Pretreatment with *Abrus precatorius* Linn. leaves lowered enhanced free radicals as generateed by indomethacin almost to control levels. Ranitidine also decreased nitric oxide levels and lipid peroxides during indomethacin induced gastric ulcers. Results are shown in Table 6.

Gastric glutathione, glutathione peroxidase, superoxide dismutase and catalase activities were found significantly decreased (p<0.001) during indomethacin induced gastric ulcer. Pretreatment with *Abrus precatorius* Linn. leaves could increase activities of these enzymes almost to near normal. Same trend was also noticed for ranitidine (Tables 7 and 8).

The term "Peptic ulcer" refers to an ulcer in the lower oesophagus, stomach or duodenum, in the jejunum after surgical anastomosis to the stomach or, rarely in the ileum adjacent to a Meckel's diverticulum. Ulcer in the stomach (gastric ulcer) may be acute or chronic. Quincke [30] was probably the first to use the term 'Peptic ulcer'. Because of its frequency and worldwide distribution, peptic ulcer continues to be a subject of numerous investigations, both experimental and clinico pathological. In this respect peptic ulcer occupies a place secondary to carcinoma in the field of gastroenterology.

There are medicines for treatment of patients suffering from peptic ulcer [31] . In case, the ulcer is due to infection of *Helicobacter pylori (H. pylori)*, the different medications are usually prescribed. This is

known as "Triple therapy". This includes a proton pump inhibitor viz. omeprazole to reduce acid production and two antibiotics to get rid of the organism. Sometimes, instead of one of the antibiotics, bismuth salicylate may be the third medication recommended. This drug, available over the counter, coats and soothes the stomach, protecting it from the damaging effects of acid. Two, rather than three, drug regimens are currently being developed. For non *H. pylori* ulcers number of drugs are now available for treatment. These drugs are broadly classified into two categories :

1. Those that decrease or counter acid – pepsin secretion viz. ranitidine, famotidine etc. (H2-blockers), pirenzepine, telenzepine etc. (M1–blockers), omeprazole, lansaprazole etc. (proton pump inhibitors).

2. Those that affect cytoprotection by virtue of their effects in mucosal defense factors like sucralfate, carbenoxolone etc. [32].

No doubt the above said drugs have brought about remarkable changes in peptic ulcer therapy, the efficacy of these drugs is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy [33]. Hence, the search for an ideal anti – ulcer drug continues and has also been extended to medicinal plants / herbs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse.

Numerous medicinal plants showed anti gastric ulcer activity. Sanyal *et al.* [34] found that vegetable banana is efficacious not only for experimentally induced gastric ulcers in albino rats, guinea pigs etc. but also for human being suffering from gastric ulcers. Akah *et al* [35] demonstrated anti gastric ulcer activity of the herb *Cassampelos mucronata*. Likewise Shetty *et al* [36], Sairam *et al* [37], Maity *et al* [38, 39] and Dharmani and Palit [40] confirmed anti gastric ulcer activities of *Ginkgo biloba*, *Convolvulus pluricaulis Chois*, tea root extract and *Vernonia lasiopus* respectively. We also reported anti gastric ulcer activities of few medicinal plants in different experimental ulcer models [41-46].

Abrus precatorius Linn., a plant of Eastern Himalaya, was known for its ethnic use in peptic ulcer [1]. In screening programme we noted anti gastric ulcer activity of *Abrus precatorius* Linn. in ethanol induced gastric ulcers in rats [14]. As one experimental model is not sufficient to prove conclusively anti ulcer activity of a plant we studied anti gastric ulcer activity of *Abrus precatorius* Linn. in indomethacin induced gastric ulcer model in rats. Results showed that *Abrus precatorius* Linn. leaves in the dose of 1g/kg could prevent formation of indomethacin induced gastric ulcer by 66.78%. The result was comparable to that of Ranitidine, the standard drug of ulcer, where inhibition rate was 71.05% (Table 1).

In ulcer research emphasis has been given on rate of gastric secretion and gastric pH [47]. Indomethacin increased gastric secretion in rats. *Abrus precatorius* Linn. leaves could lower the increased gastric secretion to almost normal level. This gastric inhibitory activity of the plant, perhaps, helps to prevent formation of gastric ulcer by indomethacin. The plant leaves also increased pH of gastric juice (Table 2).

Elevated gastric acidity is responsible for ulcer formation [31]. Indomethacin increased gastric free and total acidity. Gastric acidity of rats pretreated with *Abrus precatorius* Linn. leaves showed more or less normal values. This helps to prevent ulcer formation by indomethacin Ranitidine also decreased gastric acidity (Table 3).

Pepsin has relation with development of gastric ulcer. In many cases of gastric ulcers, gastric pepsin was found elevated [31]. The present study showed that gastric pepsin was elevated during indomethacin induced gastric ulcer which came to almost normal level by the pretreatment with *Abrus precatorius* leaves. Gastric mucin which gives cyto protection to stomach [48] was found decreased by indomethacin and reversed back to control level by pretreatment with *Abrus precatorius* Linn. leaves (Table 4). This suggests cyto protective activity of the plant.

In experimental ulcers levels of gastric protein and DNA of gastric mucosa are found to be decreased[48]. Indomethacin could develop ulcer by decreasing gastric protein and DNA of gastric mucosa. Rats pretreated with *Abrus precatorius* Linn. leaves showed increased amount of these materials and thereby could prevent ulcer formation (Table 5).

Gastric mucosal lipid peroxidation has been reported to increase incidence of experimental ulcers [49]. In this study we noted elevated levels of nitric oxide and lipid peroxides in gastric mucosa during indomethacin induced gastric ulceration. Pretreatment of rats by *Abrus precatorius* Linn. leaves could decrease levels of gastric nitric oxide and lipid peroxides thus prevent indomethacin induced ulcer formation (Table 6).

Free radicals scavenging enzymes like superoxide dismutase, catalase,

Table 8: Effect of *Abrus precatorius* Linn. leaves (APL) on glutathione and Glutathione per oxidase activity during indomethacin (INDO) induced gastric ulcer in albino rats.

Group	Glutathione (GSH) (Micro mole/mg protein)	Glutathione per oxidase (Micro mole of GSH consumed/min/mg protein)
Control	4.13 ± 0.07	242.34 ± 7.56
INDO	1.09 ± 0.03**	182.65 ± 5.55**
INDO + APL (1.0 g/kg)	4.88 ± 0.05**	219.02 ± 6.47**
INDO + Ranitidine (50mg/kg)	4.54 ± 0.04**	227.56 ± 6.28**

*Results were in mean ± SEM, Each group had eight rats, ** p<0.001*

glutathione per oxidase are involved in development of gastric ulcer. If generation of free radicals exceeds the ability of free radical scavenging enzymes, gastric mucosa may be injured by the free radicals resulting development of gastric ulcer [50]. Activities of all these free radical scavenging enzymes were found decreased by indomethacin which was reversed by pretreatment with *Abrus precatorius* leaves (Tables 7 and 8).

From the results of this study, it may be stated that indomethacin could increase gastric lipid peroxidation therefore generate reactive oxygen metabolites. This could damage gastric cells. This was reflected by decreased amount of DNA in gastric mucosa which, in turn, was responsible for decreased synthesis of gastric muco substances. In absence of proper protective layer of muco substances , ulcer developed in the stomach. *Abrus precatorius* Linn. leaves, on the other hand, could inhibit gastric lipid peroxidation thereby inhibit generation of reactive oxygen metabolites. This could protect the gastric cells from damage. DNA of gastric mucosa was, thus, found increased with concomitant increase in the level of muco substances. These muco substances gave proper protection in the stomach for which ulcer could not develop.

Conclusion

Leaves of *Abrus precatorius* Linn. could decrease indomethacin induced gastric ulcers in rats and this anti ulcerogenic activity was mediated through anti oxidant defense mechanism.

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