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Evaluation of Antiulcer Activity of Ethanolic Extract of Leaf, Bark, Flower and Fruit Pulp of *Cassia fistula* in Wistar Rats

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Abstract

The present study was conducted to investigate the gastro-protective effects of *Cassia fistula* L. extracts on ethanol-induced gastric ulcers in rats. Forty-two male Wistar rats were divided into seven groups with six rats in each group. Group I rats were administered 0.5ml/day of 0.5% Carboxymethyl Cellulose (CMC), Group II received ethanol (0.5ml/day of 50% ethanol), Groups III–VI received ethanol (0.5ml /day of 50% ethanol) and *C. fistula* leaf, bark, flower and fruit pulp ethanolic extracts @ 100 mg/kg/day respectively. Group VII received ethanol (0.5ml /day of 50% ethanol) and ranitidine @ 50mg/kg for two weeks. On day 15, ulceration in the stomach was scored; ulcer index and percentage protection were calculated. Histopathological study was conducted to assess the effect of *C. fistula* extracts on ethanol-induced gastric toxicities. Oral administration of ethanol produced severe gastric lesions in ethanol treated group. All the extracts of *C. fistula* showed protection against ulcers. The maximum protection (52.0%) was shown by bark followed by leaf which showed protection of 49.7%. Histopathological findings further confirmed the protective effect of *C. fistula* extracts. The present study suggests that *C. fistula* extracts have an ameliorative effect against ethanol-induced gastric damage in Wistar rats.

Major Findings: The present study suggested that *Cassia fistula* extracts have an ameliorative effect against ethanol-induced gastric damage in Wistar rats. Ethanolic Extract of Leaf, Bark, Flower and Fruit Pulp of *Cassia fistula* showed protection against gastric ulcers in rats. The maximum protection (52.0%) was shown by bark followed by leaf which showed protection of 49.7%.

Keywords: *Cassia fistula*, Ethanol, Rats, Stomach, Ulcers

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1. Introduction

A breach in the mucosa lining in the stomach can result in a chronic disease called gastric ulcer. The disease is caused by an imbalance between endogenous aggressive factors, such as pepsin, gastric acid secretion, and Reactive Oxygen Species (ROS), and protective factors, such as bicarbonate, mucus, antioxidant defenses, microcirculation, and cell regeneration. Furthermore, the development of peptic ulcers can be caused by exogenous factors such as stress, alcohol consumption, long-term use of Nonsteroidal Anti-Inflammatory Medicines (NSAIDs), and *Helicobacterium pylori* infection[1]. Prolonged alcohol intake can lead to both acute and chronic damage to the stomach mucosa, which can raise the amount of hydrochloric acid in the stomach juice and damage the mucosa. A breakdown in the mucosal barrier can lead to an increase in intragastric epithelial cell proliferation, which can break down tight junctions and allow digestive juices to penetrate deeper regions. It starts as epithelium layer loss, damages deeper tissues, and eventually causes erosion and ulceration[2]. Currently, the two most common treatments for Peptic Ulcer Disease (PUD) are Type 2 histamine receptor antagonists and proton pump inhibitors. In the case of *H. pylori* infection, broad-spectrum antibiotics are recommended. Prolonged antisecretory therapy use, however, can have undesirable side effects, such as gastric hypochlorhydria and hypergastrinemia, which can impair the absorption of calcium, iron, magnesium, and vitamin B12 and increase the risk of infection in patients[3]. Alternative solutions to this global health issue are required due to the rise in the occurrence of stomach ulcers and the side effects associated with some of the synthetic medications used to treat or control them. As a result, there has been a greater need in recent years for novel therapeutic molecules, with natural products being viewed as a particularly potential source. The active ingredients found in medicinal plants are used to cure a variety of diseases[4].

Multiple varieties of cassia plants have characteristics that are useful in wound healing, hepatoprotection, anti-inflammatory, antibacterial, and antitussive. Tannins, flavonoids, glycosides, carbohydrates, stearic acids, oleic, oxalic, linoleic, oxyanthraquinones, and anthraquinones derivatives are abundant in the majority of these species. Many of the active phytochemicals are abundantly present in all plant organs, including leaves, stems, roots, flowers, and fruits. The leaves of *C. fistula* are used to relieve pain, edema, and reduce skin irritation caused by swelling, while the root is used to cure colds and flu. Extracts from the plant are also used as laxatives and to control constipation. Furthermore, fruit and stem bark preparations are utilized to remove toxins from the blood[5].

Studies on the antiulcer activity of one or more parts have been conducted. However; studies on the comparative antiulcerogenic effect of different parts of *C. fistula* are limited. Therefore, the present study was conducted to investigate the protective effects of ethanolic extract of leaf, bark, flower and fruit pulp of *C. fistula* against ethanol-induced gastric toxicities in rats.

2. Materials and Methods

2.1 Preparation of *C. fistula* Extract

The leaves, flowers, bark and fruits of *C. fistula* were collected from different locations in and around District Ludhiana (Punjab), India, identified by CSIR-Institute of Himalayan Bioresource Technology, High Altitude Biology Division, Palampur (H.P) India (voucher No. PLP 15392), and shade dried at room temperature. After drying, all these parts were powdered using a grinding machine. Ethanolic extracts were prepared by maceration technique. In this process, the whole or coarsely powdered 100g of crude sample was soaked in ll of ethanol and allowed to stand at room temperature for at least 48-72 h with frequent agitation until the soluble matter dissolved. After maceration, the extract was initially filtered using a muslin cloth and then re-filtered again using filter paper (Whatman filter paper No 1, Cat no.1001125). The filtrate obtained was evaporated in the oven at a temperature of 40°C. The residue obtained was kept at 4°C in air-tight bottles until use[6].

2.2 Experimental Design, Animals and Treatment Groups

Forty-two male Wistar albino rats (2-3 months old, weighing 100-150 g) were obtained from Disease free small animal house, LUVAS, Hisar, Haryana India. The animals were kept in polypropylene cages (6 animals per cage) and maintained under 12:12 h light:dark cycles in standard laboratory conditions of temperature (27-30 °C) and relative humidity. The animals were fed with water and commercial rat pellets *ad libitum* in accordance with internationally accepted guidelines for the use and care of laboratory animals. The animals were acclimatized to the environment for two weeks before starting the experiment. The experimental protocol was approved by the Institutional Animal Ethics

Committee (IAEC) vide reference no. IAEC/2018/1216-1250 dated 31.11.2018 and was conducted in accordance with ethical committee guidelines. After acclimatization, the rats were randomly divided into six groups (n=6 per group) as follows: Group I: Normal (vehicle control) group, receiving 0.5ml/day of caboxy methyl cellulose (0.5% CMC) by gavage; Group II: Model group, receiving 0.5ml/day of 50% ethanol @3g/kg by gavage; Groups III-VI: Treatment groups, receiving ethanolic extracts of *C. fistula* leaves, bark, flowers and fruit pulp respectively @100mg/kg/day in 0.5ml of 0.5% CMC and 0.5ml of 50% ethanol @3g/kg by gavage. Group VII (positive control) received ethanol (0.5ml/day of 50% ethanol) + ranitidine @50mg/kg. Treatments continued daily for 14 days.

2.3 Determination of Ulcer Index and Percentage Protection

After completion of the treatment, animals were anaesthetized under light ether anaesthesia and incised along the greater curvature. Ulceration was scored; ulcer index and percentage protection were calculated[7].

Scoring of the ulcer was done as follows:

- Normal stomach.....(0)
- Red coloration.....(0.5)
- Spot ulcer.....(1)
- Hemorrhagic streak…….(1.5)
- Ulcers.....(2)
- Perforation.....(3)

The ulcer score for each group was expressed as an ulcer index.

Ulcer index (UI) = UN + US + UP x 10^{-1}

(UI = Ulcer index, UN = Average number of ulcers per animal, US = Average of severity score, UP = Percentage of animals with ulcer)

$$\text{Percentage protection} = \frac{\text{Ulcer index (Control)} - \text{Ulcer index(Test)}}{\text{Ulcer index (Control)}} \times 100$$

2.4 Histopathological Investigation

At the end of the experimentation, six (06) rats from each group were euthanized and a systematic necropsy examination was carried out. The represented tissue samples of the stomach were collected and fixed in 10% neutral buffered formalin solution and fixed for at least 48 h. For histopathological processing, the tissue samples were given overnight washing under tap water and dehydrated in increasing grades of ethyl alcohol, cleared in xylene and embedded in paraffin, 3-4 µ thick tissue sections were cut on glass slides and Hematoxylin and Eosin (H&E) staining was performed. Then H&E stained sections were examined under a light microscope for the pathological alterations in all the treated groups and microphotography was performed using Olympus Microscope, Japan (BX-40)[8].

2.5 Histopathological Lesion Score

The intensity of microscopic changes in the stomach tissues was scored as mild (1), moderate (2) and severe (3). The total of individual lesion scores (vascular, degenerative, necrotic and inflammatory lesions) was calculated as the sum of the products of each lesion score of a particular type of lesion with the number of rats showing that particular lesion and severity. The mean was calculated by dividing the total score by the total number of rats in that group. The total lesion score for the stomach of each rat was determined by the sum of the total intensity score of each alteration.

2.6 Statistical Analysis

Statistical analysis was conducted in IBM SPSS version 20.0. One-way Analysis of Variance (ANOVA) was utilized to compare the differences of means between groups, followed by Tukey's *post hoc* test. All results were expressed as mean±SE and $p \leq 0.05$ indicated statistical significance. The Kruskal-Wallis non-parametric test was employed for the analysis of microscopic lesions in the stomach tissues using GraphPad InStat software (San Diego, USA).

2.7 Ethical Statement

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) (Approval number: IAEC/2018/1216-1250) and was conducted in accordance with ethical committee guidelines.

3. Results

3.1 Effects of *C. fistula* Ethanolic Extracts on Ethanol-Induced Ulcers in Rats

Oral administration of ethanol produced severe gastric lesions in Group II (Ethanol, 50%) Pretreatment with, leaf, bark, flower, and fruit pulp extracts decreased gastric

damage represented by a reduction in ulcer index in Groups III, IV, V, VI and VII as compared to Group II. All the extracts of *C. fistula* showed protection against ulcers. The maximum protection (52.0%) was shown by Group IV (bark, 100mg/kg) followed by Group III (leaf, 100mg/kg) which showed protection of 49.7%. However, the protection was less than Group VII (standard) which showed protection of 82.7% (Table 1).

3.2 Histopathological Findings

Histopathological examinations showed *C. fistula* extract effectively improved the structural and functional defects observed in the stomach of ethanol-treated rats.

In the ethanol-treated group, the sections of the stomach showed moderate to severely engorged dilatation of blood vasculature in the glandular and non-glandular portions of the stomach. The focal areas of coagulative necrosis of partial to entire mucosal thickness and the desquamation or sloughing of necrotic mucosa along with inflammatory cell infiltrations (mainly neutrophils) were noted in the mucosa (Figure 1 B1). Sub-mucosal oedema with or without inflammatory infiltration was also seen. The cystic appearance of glandular structures is also noted in the mucosa of the glandular stomach. Degenerated and necrosis of gastric epithelium at the mucosal surface, degenerated or atropied mucosal glands, degenerated and necrotic parietal and chief cells, area of haemorrhages and the infiltration of inflammatory cells (neutrophils and lymphocytes) also seen inside the mucosa and submucosa (Figure 1 B2). The forestomach also showed variable hyperkeratotic changes along with inflammatory cell infiltration in the lamina propria of the mucosa. Treatment with *C. fistula* extract improved these changes (Figure 1 C-F).

3.3 Histopathological Lesion Score

The lesion score comprising vascular changes, degenerative changes, necrotic changes, and cellular infiltration was effectively less as compared to ethanol treated group (Table 2). The mean lesion score of degenerative changes in the stomach was significantly ($p \leq 0.05$) higher in Groups II and III when compared to those in the control group rats (Group I). However, the mean lesion score was found to be significantly ($p \leq 0.05$) decreased in Groups IV, V, VI and VIII when compared to those in the Group II rats.

The mean lesion score of necrotic changes in the stomach was significantly ($p \leq 0.05$) higher in Group II when compared to those in the control group rats (Group I). However, the mean lesion score was found to be significantly ($p \leq 0.05$) decreased in Groups III, IV, V, VI and VII when compared to those in the Group II rats.

The mean lesion score of inflammatory changes in the stomach was significantly ($p<0.05$) higher in Groups II, III and VI when compared to those in the control group rats (Group I). However, the mean lesion score was found to be significantly ($p<0.05$) decreased in Groups IV, V and VII when compared to those in the Group II rats.

The mean total microscopic lesion scores of the stomach were significantly ($p<0.05$) higher in Groups II and III when compared to those in the control group rats (Group I). However, the mean total lesion score was found to be significantly ($p<0.05$) decreased in Group IV when compared to those in the Group II rats.

Table 1. Effect of ethanolic extracts from different parts of *C. fistula* on ethanol-induced ulcers in rats

Group	Treatment	Dose	*Ulcer index	Percentage protection
Group I	Control	---	0.00 ± 0.00^{a}	---
Group II	Ethanol (50%)	3g/kg	14.42 ± 1.12^{b}	----
Group III	Leaf	100mg/kg	7.25 ± 0.78^{c}	49.7
Group IV	Bark	100mg/kg	6.92 ± 0.65^{c}	52.0
Group V	Flower	100mg/kg	9.51 ± 1.04^{c}	34.1
Group VI	Fruit pulp	100mg/kg	10.34 ± 1.2^{c}	28.3
Group VII	Ranitidine	50mg/kg	2.5 ± 0.46^{a}	82.7

*Data are means ± S.E (n=6).
Means bearing superscripts a, b, and c differ significantly ($p \leq 0.05$) among the different groups within a column.

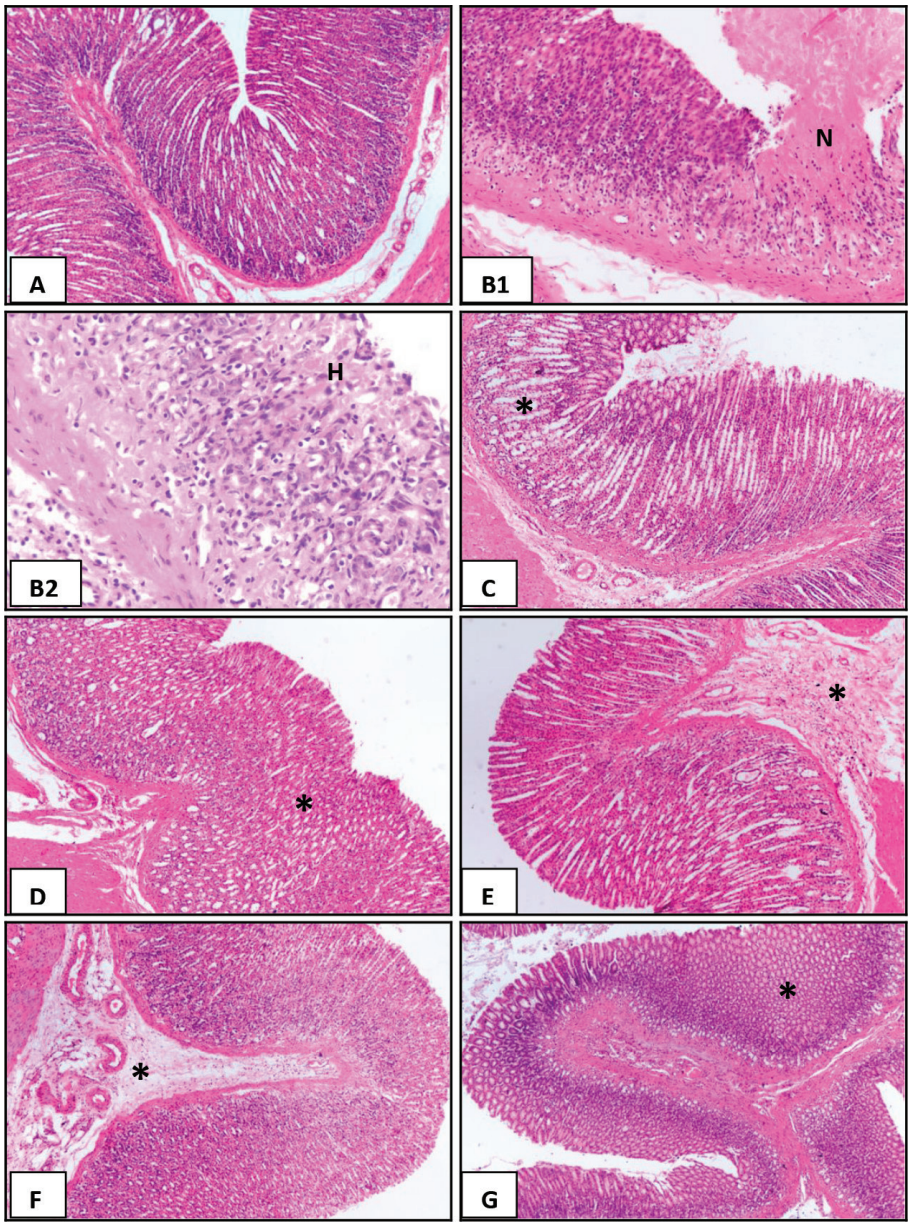


Figure 1. Histopathological microphotographs of the stomach in rats. **(A)** The control group showed normal structure of gastric mucosa and submucosa. H&E staining, 40x. **(B1-B2)** Ethanol-treated group. **(B1)** Stomach showing focal area of coagulative necrosis (N) and the desquamation of necrotic mucosa along with inflammatory cell infiltrations. H&E staining, 100x. **(B2)** Stomach showing degenerated and necrosis of surface gastric epithelium, mucosal glands, parietal and chief cells, area of haemorrhage (H) and the infiltration of inflammatory cells in the mucosa and submucosa. H&E staining, 200x. **(C)** Leaf-treated group showing degenerated and desquamated mucosal surface epithelium, dilated mucosal glands (*) and increased mucous cell secreting activity in the mucosa. H&E staining, 40x. **(D)** Bark-treated group showing dilation of mucosal glands (*) and oedema in the submucosa. H&E staining, 40x. **(E)** Flower-treated group showing dilation of mucosal glands and vascular congestion, oedema (*) and infiltration of inflammatory cells in the submucosa. H&E staining, 40x. **(F)** Fruit pulp-treated group showing vascular congestion, oedema (*) and infiltration of inflammatory cells in the submucosa. H&E staining, 40x. **(G)** Ranitidine treated group showing dilation of mucosal glands and increased mucous cell activity in the gastric mucosa (*).

Table 2. Mean lesion scores (mean±SE) and Mean total lesion scores (mean±SE) of the stomach tissue in rats

Group	Treatment	Dose (mg/kg)	*Lesion score				**Total lesion score (A+B+C+D)
			Vascular changes (A)	Degenerative changes (B)	Necrotic changes (C)	Cellular infiltration (D)	
Group I	Control	----	0.33^{a}±0.21	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.33^{a}±0.21
Group II	Ethanol (50%)	3g/kg	2.33^{c}±0.33	2.00^{b}±0.37	1.83^{b}±0.48	1.67^{b}±0.33	7.83^{c}±1.08
Group III	Leaf	100	1.50^{ac}±0.22	1.67^{b}±0.21	0.67^{a}±0.21	1.33^{b}±0.21	5.17^{c}±0.17
Group IV	Bark	100	1.00^{ab}±0.0	1.00^{a}±0.00	0.50^{a}±0.22	1.00^{a}±0.00	3.50^{ab}±0.22
Group V	Flower	100	1.33^{ac}±0.21	1.17^{a}±0.17	0.83^{a}±0.17	1.00^{a}±0.26	4.33^{abc}±0.33
Group VI	Fruit Pulp	100	1.00^{ab}±0.00	1.00^{a}±0.00	0.50^{a}±0.22	1.50^{b}±0.22	4.00^{abc}±0.45
Group VII	Standard drug (Ranitidine)	50	1.00^{ac}±0.37	1.00^{a}±0.00	0.83^{a}±0.17	1.00^{a}±0.00	3.83^{abc}±0.48

[1]Data are means±S.E (n=6)

Means bearing superscripts a, b, and c differ significantly ($p \leq 0.05$) among the different groups within a column.

*__Mean lesion scores__: Sum of the intensity scores (Mild=1; Moderate=2; Severe=3) and the number of rats showing that particular intensity; and divided by the number of total rats within a group.

**__Mean total lesion scores__: Sum of the total lesion scores (*i.e.*, vascular changes, degenerative changes, necrotic changes and cellular infiltration); and divided by the total number of rats within a group.

4. Discussion

In the present study, oral administration of ethanol produced severe gastric ulcerations. Ethanol-induced gastric ulcer is a common animal model to investigate the new anti-ulcer drugs[9]. Ethanol-induced gastric ulcer in animal models resembles the pathophysiology of human ulcer[10]. Ethanol causes necrotic lesions of the gastric mucosa in a multifactorial way. It can reach the mucosa by disruption of the mucus-bicarbonate barrier and cause cell rupture in the wall of blood vessels. These effects are probably due to biological actions, such as lipid peroxidation, formation of free radicals, intracellular oxidative stress, changes in permeability and depolarization of the mitochondrial membrane prior to cell death[11].

Very few studies have been conducted to evaluate the antiulcer activity of *C. fistula*. In one of the studies, the Ethanolic Leaf Extract (ELE) of *C. fistula* Linn. at doses of 250, 500, and 750mg/kg b.w was evaluated for antiulcer activity against pylorus ligation-induced gastric ulcer. The antiulcer activity of ELE was evidenced by the significant attenuation of gastric volume, pH, free acidity, and total acidity in the gastric juice of pyloric-ligated rats in a dose-dependent manner[12]. Our results are in consonance with this study as we also found the gastroprotective effect of leaf, bark, flower and fruit pulp of *C. fistula* @100mg/kg. Studies conducted on other plants like *Cuphea ignea* extract @250mg/kg and 500mg/kg showed a potential gastroprotective effect on ethanol-induced gastric ulcer[13]. *Curcuma xanthorrhiza* @250mg/kg and 500mg/kg also showed a potential gastroprotective effect on ethanol-induced gastric ulcer [14].

In the present study, it was found that all the extracts of *C. fistula* showed protection against ulcers. The maximum protection (52.0%) was shown by bark, followed by leaf (49.7%), flower (34.1%) and fruit pulp (28.3%). In our earlier study conducted on *C. fistula*, it was found that the leaf, bark, flower and fruit pulp of *C. fistula* have high phenolic content and good antioxidant activity. The total phenolic content of the ethanolic extract of *C. fistula* bark was found to be the highest (43.1 ± 1.68) mg of GAE/gm of extract followed by leaf, flower, and fruit pulp. In comparison to leaf, flower and fruit pulp bark extract also showed strongest antioxidant activity as confirmed by ABTS scavenging activity (IC_{50}= 90.94µg/ml /ml), DPPH scavenging activity (IC_{50}=177.55µg /ml) and superoxide radical scavenging activity (IC_{50}=241.03µg/ml)[15]. In our earlier studies, we have also detected a high diversity of flavonoids in ethanolic extracts of bark, flower and pod pulp. The major flavonoids detected were hesperidin, tangeritin, nobiletin, apigenin and quercetin. Hesperidin was recorded in the highest quantity in bark extract. Pod pulp exhibited a high concentration of nobiletin and flower extracts were rich in quercetin[16]. The antiulcer activity found in our study might be due to the antioxidant activity and phenolic and flavonoid compounds present in the extract and the effect is proportional to the concentration of compounds and the antioxidant activity of different parts of *C. fistula*.

The stomach lesion healing action of this plant is attributed to its ingredients, which may also work in

conjunction to achieve the desired outcome. Numerous studies have shown that the primary mechanism underlying the ulcer-healing properties of flavonoids, particularly quercetin, rutin, and garcinol, is their antioxidant activity. This is because the flavonoids contain hydroxyl groups at positions 3, 5 and 7 in their structures, a double bond in conjugation with a 4-oxo function at the 2,3 position, and an o-dihydroxy in the B ring[17].

5. Conclusion

In conclusion, the present study revealed that the ethanolic extract of *C. fistula* possessed promising antiulcer activity in ethanol induced ulcer model. The antiulcer activity may be related to antisecretory as well as cytoprotective activities of one or more phytoconstituents. The plant parts especially bark and leaves were highly effective and can be used as protective agents in case of gastric ulcers in the stomach. However, further research should be taken to further explore the underlying mechanisms of action.

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