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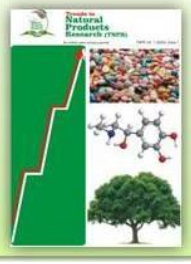

Aqueous Preparation of *Zea mays* L. (Poaceae) Starch Slurry Offer Some Protection Against Experimentally Induced ulcers

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Key words: *Zea mays*, peptic ulcer, stress ulcer, ethanol, indomethacin, pylorus ligation

Abstract: Present modality for the management of peptic ulcer disease with synthetic agents is known to be associated with serious side effects hence the need to look for natural agents with better safety and efficacy profile. *Zea mays* starch (prepared as a slurry) has been used in parts of Nigeria to ameliorate ulcer symptoms. This work aimed to evaluate the antiulcer properties of aqueous preparation of *Z. mays* starch (ZM) slurry in rodents using ethanol-induced ulcer (EIU), water immersion restraint stress-induced ulcer (WIRSIU), indomethacin-induced ulcer (IIU) and pylorus ligation-induced ulcer (PLIU) models. Phytochemical screening and acute toxicity study of the extract were performed. The ZM up to 5000 mg/kg administered orally showed no toxicity or sign of intoxication after 24 hr observation period. The extract gave positive reactions for flavonoids, tannins, saponins, reducing sugars and carbohydrates. The slurry exhibited a non-dose dependent ulcer protection against EIU. The slurry demonstrated a significant and dose dependent ulcer protection in WIRSIU with best activity recorded at 1000 mg/kg (88.03 %). It also elicited a significant ($p < 0.05$) attenuation of ulcer development in IUU. In PLIU, it significantly ($p < 0.05$) reduced the gastric volume. There was a non-dose dependent decrease in total acidity and a significant ($p < 0.001$) and dose dependent increase in ulcer protection. The results demonstrated that the aqueous preparation of *Z. mays* starch (ZM) slurry possesses anti-ulcer properties.

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Page No.: 51-59
Volume: 1, Issue 1, 2020
Trends in Natural Products Research

INTRODUCTION

Peptic ulcer disease is a benign lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to acid and pepsin (Aguwa, 2004). Peptic ulcers also manifest as defects in the gastric or duodenal mucosa that extend through the muscularis mucosa (Anand, 2019). It is a major health hazard in terms of both morbidity and mortality and affects a considerable number of people worldwide (Charisius, 2014). Current clinical recommendations are that treatment should be individualized based on comorbidities and patient preferences among populations at increased risk for certain morbidities. However, knowledge, attitudes and practices regarding peptic ulcer among potential patient populations are largely unknown (Bhattacharya, 2007). Drugs commonly available for the treatment are usually expensive and low and middle income earners find it difficult to adhere to their medications due to their inability to pay for it. Furthermore, these medications present with some troubling adverse effects (like gynaecomastia observed with cimetidine). For over a century ago, management of peptic ulcer disease relied on surgical intervention, with resulting high morbidity and mortality rates. Development in the effective pharmacological suppression of gastric acid secretion in the 1970s with the introduction of histamine H_2-receptor antagonists (H_2RAs) saw surgical interventions in the antiulcer therapy decline by about 85 % in the 1980s (Aguwa *et al.*, 2012). Also, other orthodox pharmaceutical drugs such as anticholinergic drugs, antacids, and more recently, proton-pump inhibitors have been employed in the management of peptic ulcers, but they provoke many adverse effects (Adinortey *et al.*, 2013).It is known that numerous pharmaceutical agents such as proton pump inhibitors, anticholinergics, antacids, antimicrobial agents, H_2-receptor antagonists, sucralfate, and bismuth are not fully effective, and produce numerous adverse effects such as impotence, arrhythmia, hematopoietic alterations, hypersensitivity, and gynecomastia (Chanda *et al.*, 2011, Palle *et al.*, 2018). In view of this, there is therefore the need to screen for natural agents with better safety and efficacy profile. Many plants have been found to possess antiulcer activity (Vimala and Shoba, 2014). Maize plant has been documented to possess various nutritional, medicinal and economic importance (Kumar and Jhariya, 2013). Jadhav (2016) demonstrated the anti-ulcer activity of methanol extract of *Zea* mays. Z. *mays* starch (prepared as an aqueous slurry) has been used in different parts of Nigeria to ameliorate ulcer symptoms. It is pertinent to note that mixing this slurry with sufficient hot water gives what is popularly known in Nigeria as pap (eko-gbona/ogi in Yoruba language, akamu in Igbo language and kunu in Hausa language) which serves as a household breakfast. The indigenes claim that this aqueous slurry preparation gives an immediate relief (very fast onset of action), better efficacy and in some cases, completely cures the ulcer when compared to the conventional antacids and other anti-ulcer drugs. Thus this work aimed to evaluate the antiulcer properties of aqueous extract of *Z. mays* starch (ZM) slurry in rodents' models of ulcer.

MATERIALS AND METHODS

Animals

Adult Swiss albino rats (100-120 g) and mice (20-25 g) of either sex bred in the Laboratory Animal Facility of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka (UNN) were used. The experimental protocols were approved by the University Ethics Committee with a certification number (DPTFPSUNN423). All animal experiments were in compliance with the principles for laboratory animal use and care of the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH publication #85-23, revised in 1985).

Collection of Plant Material and Preparation of Extract

The grains of *Zea mays* were collected from the botanical garden of the Faculty of Agricultural Sciences, University of Nigeria, Nsukka in July 2019 and authenticated by Mr Alfred Ozioko of the International Centre for Ethnomedicines and Drug Development (INTERCEDD) Nsukka, Enugu State, where a voucher specimen was deposited (specimen number: INTERCEDD 1576). The grains were air dried for two weeks before further processing. The dried grains were thoroughly washed and soaked in a good quantity of cold water for 3 to 4 days with daily replacement of the water. Afterwards, the grains were grinded until smooth, using electric grinders. A white chiffon cloth was tied over a big bowl and the grinded grains were sieved and rinsed until only the chaff was left. The mixture of water and grinded part was allowed to settle for at least 3 hours. After about 3 hours, the clear water was decanted and the rest of the mixture poured into a muslin bag and left overnight so that excess water could drain off to yield aqueous extract of *Z. mays* starch (ZM). After 24 hours, it was brought out from the muslin bag, and placed in containers and refrigerated till it was ready for use (Abdulraham and Kolawole, 2006).

Determination of Acute Toxicity and LD_{50}

The acute toxicity and lethality (LD_{50}) of aqueous

extract of *Z. mays* starch (ZM) slurry was determined in mice using standard procedure (Lorke, 1983). Briefly, nine mice randomly divided into three groups (n = 3) were orally administered 10, 100, and 1000 mg/kg of ZM slurry, respectively and observed for 24 h for death. Since no death was recorded, 1600, 2900 and 5000 mg/kg of ZM slurry were administered, respectively to a fresh batch of animals (n = 1) and the number of deaths in 24 hr recorded. The LD_{50} was calculated as the geometric mean of the highest non-lethal dose and the least lethal dose.

Phytochemical screening

Phytochemical tests were carried out on ZM using standard procedures (Harborne, 1988; Trease & Evans, 1989).

Indomethacin Induced Ulcer (IIU)

Gastric ulceration was induced in the animals according to the procedure described by Takeuchi *et al* (2007). The animals were deprived of food but had free access to water 48 hr prior to ulcer induction. Twenty-five (25) albino rats were randomized into five groups of five rats each (n = 5) and were treated as follows; group 1 (negative control 5 ml/kg of distilled water), group 2 (positive control 20 mg/kg of omeprazole), group 3 (ZM 250 mg/kg), group 4 (ZM 500 mg/kg) and group 5 (ZM 1000 mg/kg). After 1 hr, indomethacin (25 mg/kg) was administered orally to all the animals. After 8 hr post ulcer induction, the animals were humanely sacrificed using chloroform, the abdomen was opened and the stomach excised and opened along the greater curvature. The stomach was cleaned, pinned to a cork board and were examined for ulcer with the aid of a magnifying lens (x10). The ulcers on the mucosal surface were scored (< 1.0 mm = 1, 1 – 2 mm = 2, ≥ 3 mm = 3), the sum of the scores were divided by 10 (magnification of the lens) to obtain the ulcer index (Main and White, 1975) and the % inhibition against ulceration was determined using the expression:
% Ulcer inhibition/protection = [U.I. in control − U.I. in test] × 100/U.I. in control (Hoogerwerf and Pasricha, 2001).

Pylorus Ligation Induced Ulcer (PLIU)

Rats were randomised into five groups (n = 5). Group I received 5 ml/kg of distilled water (negative control), group II received omeprazole (20 mg/kg) as the positive control, group III received ZM (250 mg/kg), groups IV received ZM (500 mg/kg) and groups V received ZM (1000 mg/kg). Thereafter (1 hr post treatment), animals were anesthetized for about 3 min before pylorus ligation, and awoke few hours after the ligation After 24 hr, rats were sacrificed and their abdomen opened, and stomach excised and opened along the greater curvature. The stomach was cleaned, pinned to a cork board and were examined for ulcer with the aid of a magnifying lens (x10). The ulcers on the mucousal surface were scored (< 1.0 mm = 1, 1 – 2 mm = 2, ≥ 3 mm = 3), the sum of the scores were divided by 10 (magnification of the lens) to obtain the ulcer index (Main and White, 1975) and the % inhibition against ulceration was determined using the expressions:
% Ulcer inhibition/protection = [U.I. in control − U.I. in test] × 100/U.I. in control (Hoogerwerf and Pasricha, 2001).
The gastric content was collected in a measuring cylinder and the volume, pH and total acidity were determined.

Determination of Total Acidity and pH

The stomachs were removed and the content was subjected to centrifugation at 2000 rpm for 10 min, and the supernatant decanted and used. The total acidity of the gastric secretion was determined by titration with 0.01 N NaOH using phenolphthalein as indicator. The total acidity was expressed as milliequivalent using the following formula:
Total Acidity = n×0.01×40×1000
Where n = volume of NaOH quantified, 40 is the molecular weight of NaOH, 0.01is normality of NaOH and 1000 is the factor represented in litre.
The pH of the gastric secretion was recorded with a calibrated pH meter.

Ethanol Induced Ulcer (EIU)

Adult albino rats of either sex (100-140 g) were randomly grouped into five groups with each group containing five rats (n = 5). The rats were fasted for 24 hr. One group was orally administered with distilled water (negative control) and another with omeprazole 20 mg/kg (positive control). For the remaining groups, 250, 500 and 1000 mg/kg of ZM were administered respectively. After an hour, 1 ml of absolute ethanol was administered orally to all the rats. Two hours thereafter, the rats were sacrificed with overdose of chloroform and their stomach were carefully removed and rinsed with distilled water. Each rat was cut open through the greater curvature with a scalpel blade and again rinsed with distilled water. Each stomach was pinned to a white background on a wooden board for examinations and assessment of ulcer. The stomachs were examined for ulcer with the aid of a magnifying lens (x10). The ulcers on the mucosal surface was scored (< 1.0 mm = 1, 1 – 2 mm = 2, ≥ 3 mm = 3), the sum of the scores were divided by 10 (magnification of the lens) to obtain the ulcer index (Main and White,1975) and the % inhibition against ulceration was determined using the expressions:

% Ulcer inhibition/protection = [U.I. in control − U.I. in test] × 100/U.I. in control (Hoogerwerf and Pasricha, 2001).

Water Immersion Restraint Stress Induced Ulcer (WIRSIU)

This was carried out as described by Thabrew and Mrawwala (2002) with some modifications. Twenty (20) adult albino rats were fasted for a period of 24 hr prior to the experiment. They were then randomized into five groups of 4 animals each (n=4). Group I was orally administered with 5 ml/kg of distilled water (negative control) and group II received 100 mg/kg of cimetidine (positive control). Groups III, IV and V received 250, 500 and 1000 mg/kg of ZM. After 30 minutes, the animals were placed individually in each compartment of a stress cage and immersed vertically up to xyphoid level in a water bath and kept for 4 hr which resulted in induction of ulcers. After 4 hr, the animals were sacrificed, the stomach was dissected. The stomach was cleaned, pinned to a cork board and were examined for ulcer with the aid of a magnifying lens (x10). The ulcers on the mucousal surface were scored (< 1.0 mm = 1, 1 – 2 mm = 2, ≥ 3 mm = 3), the sum of the scores were divided by 10 (magnification of the lens) to obtain the ulcer index (Main and White, 1975) and the % inhibition against ulceration was determined using the expressions:
% Ulcer inhibition/protection = [U.I. in control − U.I. in test] × 100/U.I. in control (Hoogerwerf and Pasricha, 2001).

Statistical Analysis

Results obtained were analyzed using one-way ANOVA in SPSS version 23.0 and the data were expressed as Mean ± Standard Error of Mean (S.E.M). Differences between treated and control groups were evaluated further using LSD Post hoc test and considered significant at $p < 0.05$.

RESULTS

Acute Toxicity and LD_{50} of *Z. mays* Starch (ZM) Slurry

The aqueous preparation of *Z. mays* was found to be relatively safe since no death was recorded at 5000 mg/kg after 24 hr observation period.

Phytochemical constituents

The phytochemical screening of ZM gave positive reactions for flavonoids, tannins, saponins, reducing sugars and carbohydrates.

Effect of *Z. mays* Starch (ZM) Slurry on Indomethacin Induced Ulcer

The extract (1000 mg/kg) showed a dose dependent and significant ($p < 0.05$) attenuation of the indomethacin-ulcer and has a better ulcer protection (49.43 %) when compared to the control. The ZM (1000 mg/kg) elicited antiulcer activity similar to the positive control, omeprazole (20 mg/kg) (Table 1).

Effect of *Z. mays* Starch (ZM) Slurry on Pylorus Ligation

The extract elicited a dose dependent and significant ($p < 0.001$) reduction of ulcer index in pyloric ligated animals when compared to the control. The best activity was recorded at 1000 mg/kg which was comparable to the effect produced by the standard treatment (Figure 1).

Effect on Gastric Volume

There was a non-dose-dependent reduction in gastric volume. The reduction was significant ($p < 0.05$) only at 250 mg/kg (Figure 2).

Effect of *Z. mays* Starch (ZM) Slurry on Ethanol Induced Ulcer

The extract (1000 mg/kg) elicited a significant ($p < 0.05$) ulcer protection against ethanol-induced ulcer. The percentage ulcer protection (52.66) was better than the effect produced by the positive control (Table 2).

Effect on Total Acidity

There was a non-dose dependent and non-significant ($p < 0.05$) decrease in total acidity (Figure 3).

Effect of *Z. mays* Starch (ZM) Slurry on Water Immersion Restrain Stress Induced Ulcer

The extract elicited a dose dependent and significant ($p < 0.05$, $p < 0.01$)) protection against stress-induced ulcer compared to the control and this was even better at 1000 mg/kg than the protection offered by the positive control (Figure 4).

Table 1: Effect of Aqueous Preparation of *Z. mays* Starch (ZM) Slurry on Indomethacin Induced Ulcer

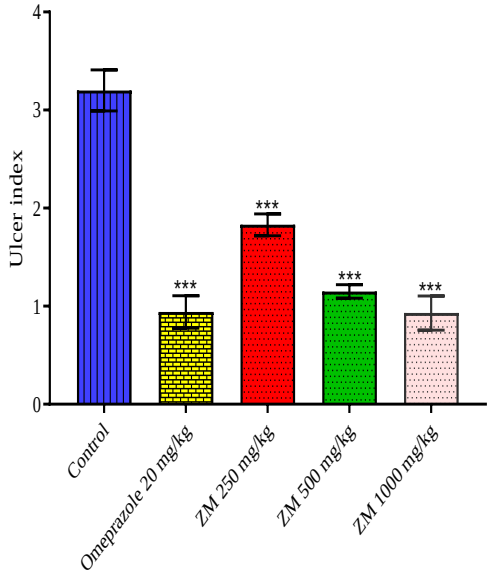
Treatment	Dose (mg/kg)	Ulcer index	Ulcer protection (%)
Distilled water	-	1.74 ± 0.09	-
Omeprazole	20	0.86 ± 0.37*	50.57
ZM	250	1.66 ± 0.23	4.60
	500	1.28 ± 0.12	26.44
	1000	0.88 ± 0.15*	49.43

*$p < 0.05$ compared to control, ZM = aqueous preparation of *Z. mays*, n = 5.

Table 2: Effect of Aqueous Preparation of *Z. mays* Starch (ZM) Slurry on Ethanol Induced Ulcer

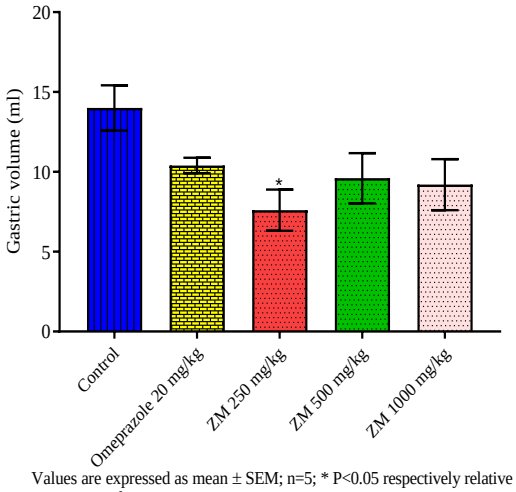
Treatment	Dose (mg/kg)	Ulcer index	Ulcer protection (%)
Distilled water	-	19.00± 4.52	-
Cimetidine	100	12.60 ± 3.72	33.68
ZM	250	15.00 ± 2.97	21.05
	500	11.60 ± 4.82	38.95
	1000	9.00 ± 1.81*	52.66

*$p < 0.05$; n = 5, ZM= aqueous preparation of *Z. mays* starch



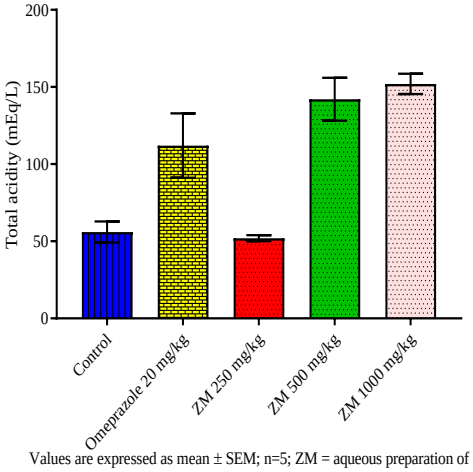
Values are expressed as mean ± SEM; n=5; ***P<0.001 relative to control, ZM = aqueous preparation of *Z. mays*

Figure 1: Effect of Aqueous Preparation of *Z. mays* Starch (ZM) Slurry on Pylorus Ligation



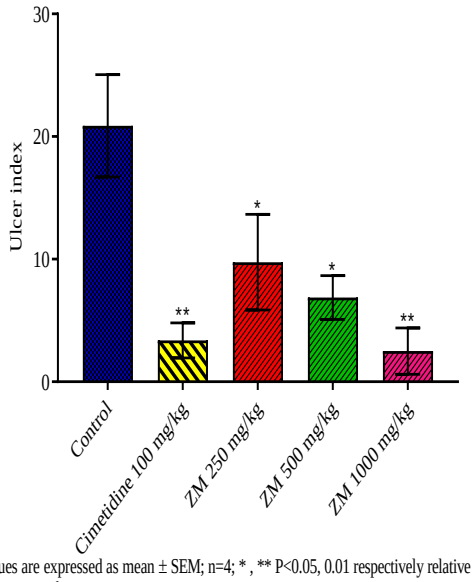
Values are expressed as mean ± SEM; n=5; * P<0.05 respectively relative to control, ZM = aqueous preparation of *Z. mays*

Figure 2: Effect on Gastric Volume of Pylorus Ligated Animals



Values are expressed as mean ± SEM; n=5; ZM = aqueous preparation of *Z. mays*

Figure 3: Effect of Aqueous Preparation of *Z. mays* Starch (ZM) Slurry on Total Acidity



Values are expressed as mean ± SEM; n=4; * , ** P<0.05, 0.01 respectively relative to control, ZM = aqueous preparation of *Z. mays*

Figure 4: Effect of Aqueous Preparation of *Z. mays* Starch (ZM) Slurry on Water Immersion Restrain Stress Induced Ulcer in Rat

DISCUSSION

Modern peptic ulcer therapies show moderate efficacy against mucosal lesions but are often associated with several side effects. There is need for more herbal/traditional based therapy for peptic ulcer disease, exploiting natural products of plant source which are believed to be non-toxic, efficacious and affordable. Studies are conducted on natural products which can either treat peptic ulceration or reduce hyperacidity to a normal level. Excessive gastric acid can increase the incidence of peptic ulcer disease, and maintaining secretion at a normal level is the main therapeutic target. *In vivo* models offer cost effective way to evaluate the efficacy and potency of novel anti-ulcer drugs and

their mechanisms. In this study, four *in vivo* models for ulcer induction were employed to evaluate the antiulcer efficacy of *Z. mays*. The data obtained suggest that the aqueous preparation of *Z. mays* starch slurry was able to attenuate the ulcer formation.

In indomethacin induced ulcer model, healing of mucosa epithelia cells was prominently displayed by the aqueous preparation of *Z. mays* starch slurry through the reduction of ulcer index depicting a good ulcer healing capacity. The Inhibitory action of indomethacin on prostaglandin synthesis coupled with free radicals' formation has been opined as the critical biochemical events in the pathogenesis of peptic ulceration (Lichtenberger, 2005, Inas *et al.*, 2011, Ajani *et al*., 2014). Decreased prostaglandin level has been attributed to impaired gastroprotection and increased gastric acid secretion which are important events in the aetiology of mucosal ulceration. This agrees with the reports (Bech *et al*., 2000, Biplab *et al*., 2011, Muhammed *et al*., 2012) where indomethacin was reported to have caused alterations in gastric secretions of rats. Pre-treatment with *Z. mays* starch slurry which facilitated ulcer healing process is indicative of enhanced cytoprotective potential and is suggestive of their significant role in ulcer healing process.

Pylorus ligation induced ulcers are due to auto digestion of gastric mucosa and breakdown of the gastric mucosal barrier (Khushstar *et al.,* 2009). These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and haemorrhage. Prostaglandin E_2 and I_2 are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus (Khushstar *et al.,* 2009). From the results, we can infer that the aqueous preparation of *Z. mays* starch slurry is anti-secretory and this is beneficial in preventing ulcer induction.

Ethanol is one of the common causes of gastric ulcer in human. Ethanol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane (Neda *et al.,* 2019). Also, ethanol produces necrotic lesions by direct necrotizing action which in turn decreases defensive factors (the production of mucus and secretion of bicarbonate), increases lipid peroxidation, and decreases superoxide dismutase, GSH levels, and catalase (Neda *et al.,* 2019). It is possible that aqueous preparation of *Z. mays* starch slurry induced both mucous and HCO3- secretion to protect the stomach lining against alcohol assault.

Stress is a physiological response caused by the disruption of homeostasis. Stress-induced gastric ulcer is a typical sample of a stress-associated organ injury (Soni *et al.,* 2014). Water immersion induces stress causes gastric ulcers by a stimulation of gastric acid secretion and a reduction in mucosal microcirculation and mucus content (Rujjanawate *et al.*, 2005). Due to the critical role that mucus plays in protecting the stomach and also enhancing healing in the stomach walls, WIRSIU model is recommended for use when evaluating mucosal and cytoprotective agents (Adinortey *et al.,* 2013). Aqueous preparation of *Z. mays* starch slurry significantly protected against gastric ulceration in the water immersion restraint stress induced ulcer which suggests that the main gastro-protective activity of ZM is related to the protection of gastric mucus.

Phytochemical screening of aqueous preparation of *Z. mays* starch slurry revealed the presence of flavonoids, tannins, saponins, reducing sugars and carbohydrates. Studies have shown that these phytoconstituents play a crucial role in managing ulcer (Saikat *et al.,* 2009). Though no specific phytoconstituent could be linked to the activity of ZM at this stage of the work, the presence of these phytoconstituents could be responsible for the antiulcer activity of aqueous preparation of *Z. mays* starch slurry.

CONCLUSION

The aqueous preparation of *Z. mays* starch slurry significantly decreased the acid secretion in the gastric mucosa and offered protection in all the ulcer models used.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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