

# Gastro-protective Effects of Green Banana (Musa cavendishii Lamb.) Pulp Powder on Aspirin-induced Gastric Ulcer in Albino Rats

Motasim A Mohammed<sup>1</sup>, Amna E El-Hadi<sup>1</sup>, Esmat A Mohammed<sup>1</sup>, Fatima S Abbas<sup>1</sup>, Khadiga O Musa<sup>1</sup>, Mustafa Y Al-Ameen<sup>1</sup>, Alia M Ahmed<sup>2</sup>, Mona Timan Idriss Gassab<sup>3</sup>, Hozeifa M Hassan<sup>4</sup>, Abdelgadir A Abdelgadir<sup>5\*</sup>

<sup>1</sup>Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>2</sup>Department of Pathology, Faculty of Medicine, University of Gezira, Wad Medani, Sudan; <sup>3</sup>Department of Pharmaceutics, Imperial University College, Elriyad, Khartoum, Sudan; <sup>4</sup>Department of Pharmacology, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>5</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>5</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>6</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>6</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>6</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>6</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>6</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>6</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>6</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan; <sup>6</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan

# ABSTRACT

Green banana (*Musa cavendishii*) is used in the traditional medicine in Sudan to treat peptic ulcer disease. The aim of this study was to evaluate the gastro-protective effect of aqueous extract of green banana pulp powder on aspirin-induced ulcer in albino rats. The effect was studied using aspirin-induced gastric damage in rats and compared between groups which were: group 1 (negative control) received water only, group 2 (positive control) received aspirin. Groups 3 and 4 were received 0.5 g/kg and 1 g/kg of banana extract before aspirin 100 mg/kg, respectively. Groups 5 and 6 were received 0.5 g/kg and 1 g/kg of banana extract of banana extract after aspirin 100 mg/kg, respectively. The results indicate that there was a significant difference (p<0.05) in ulcer index between *M. cavendishii* extract treated groups 3, 4, 5, 6 with ulcer index (15.0  $\pm$  1.31), (09.5  $\pm$  0.64), (09.0  $\pm$  0.79) and 0 respectively when compared to the control group with ulcer index (25.5  $\pm$  1.45). The histopathological assessment showed significant improvement as partial erosion of mucosa with groups 3 and 4, while group 5 and 6 showed total gastric aspirin induced-ulcer protection. The study concluded that banana pulp powder suspension showed significant gastro-protective effect in all groups of rats, promotes ulcer healing and strengthens the mucosa. This study confirmed the use banana in folk medicine for the management of gastric ulcer. The mechanism of protection afforded by bananas has to be further elucidated.

Keywords: Gastro-protective; Green banana; Aspirin-induced ulcer; Rats

# INTRODUCTION

Gastric ulcer is one of most common diseases of the upper gastrointestinal tract that affects millions of people worldwide [1]. It is an erosion in the gastric wall that develops through the muscles of mucosa into the deep layers of the wall and this ulcer may affect a major blood vessel lead to potentially life-threatening bleeding [2]. The incidence of gastric ulcers is related to a many causing factors such as non-steroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* infection and drinking alcohol [3]. The most clinically used drugs for management of gastric ulcers include antacids, acid inhibitory agents, cytoprotective agents, histamine (H<sub>2</sub>) receptors antagonist, muscarinic receptors (M<sub>1</sub>) antagonists, *H. pylori* eradication drugs and triple therapy regimen [4]. Most of these drugs are chemical and/or synthetic drugs with known adverse effects such as bowel upset, damage of the gastric mucosal tissues, stimulation of the gastro-intestinal tract, gynaecomastia and other adverse effects [5]. Natural products as vegetables, fruits, spices, medicinal plants and crude drug materials are provides a potential source of active constituents to manage gastric ulcers in an *in vitro*, *in vivo* and clinical ulcer models that mediated by different mechanisms of action with less adverse effects and relative low cost [6]. Banana, *Musa cavendishii Lamb*. of the family Musaceae, common name: Dwarf Cavendish; is an edible banana plantain widely distributed worldwide and the shortest used for banana large scale production [7]. It is used in the traditional medicine to treat peptic ulcer disease. This plantain banana is collected green and cooked as a food in the most world developing countries. Previous studies were indicated that banana plantain (*M. sapientum*) pulp and peel extracts have been reported to protect the gastric mucosa

**Correspondence to:** Abdelgadir A Abdelgadir, Assistant Professor, Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Al-Razi Campus, Wad Medani, Sudan, Tel: +249912554791; E-mail: gadora-313@hotmail.com, gadora313@uofg.edu.sd

Received: January 18, 2021, Accepted: January 25, 2021, Published: January 31, 2021

Citation: Mohammed MA, El-Hadi AE, Mohammed EA, Abbas FS, Musa KO, Al-Ameen MY, et al. (2021) Gastro-protective Effects of Green Banana (*Musa cavendishii Lamb.*) Pulp Powder on Aspirin-induced Gastric Ulcer in Albino Rats. Med Aromat Plants (Los Angeles) 10: 367.

**Copyright:** © 2021 Mohammed MA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

#### Mohammed MA, et al.

from erosions and ulcer healing effect [8-11]. Therefore this study aimed to evaluate the gastro-protective potentials of unripe banana (M. *cavendishii*) pulp powder on aspirin-induced gastric ulcer in albino rats.

# MATERIALS AND METHODS

## Plant materials

The green banana fruits (10 kg) were purchased from the local market in Wad-Medani, Sudan. It was authenticated by Agricultural Research Corporation, department of Horticultural in Wad-Medani. The pulp was cut into small slices and then dried in an air-open shade at room temperature for 7 days. The dry slices were powdered using electrical blender (Moulinex Blender the genuine 400 W, France) and was stored in well-closed glass container until use.

## **Experimental** animals

Thirty six healthy adult albino rats (150-250 g) were selected from the animal house, housed in clean cages in animal house with standard environmental condition (temperature 23-27°C, with dark and light cycles of 14 hours and 10 hours respectively), with standard chow feeding and drinking water and were left to accommodate under daily observation for one week prior to experiments.

## Acute toxicity studies of banana powder extract

The acute toxicity study was carried for banana aqueous powder suspension using randomly selected three albino rats. The dose (3 g/kg body weight) of the plant sample was administered to rats using intra-gastric tube for three days and then observed for 24 hours for behavior as well as mortality [12].

## Experimental design

Rats were divided into six groups, each one contain five rats. The modified experiment was carried out daily for 30 days. Group 1 were set (negative control) received water only, group 3 (positive control) were received oral daily dose of aspirin 100 mg/kg of body weight administered by intra-gastric feeding tubes. Group 3 were received daily dose of 0.5 g/kg of banana extract and 100 mg/kg of aspirin two hours before the extract, also group 4 were received 1 g/kg of banana and 100 mg/kg of aspirin two hours before received 0.5 g/kg of banana extract and 100 mg/kg of aspirin two hours after the extract and group 6 were received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after the extract and group 6 were received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of banana and 100 mg/kg of aspirin two hours after the extract and group 6 were received 1 g/kg of banana and 100 mg/kg of aspirin two hours after received 1 g/kg of ba

## Ulcer index and toxicity score

The stomachs were washed with normal saline then put in Whatman filter paper (Whatman<sup>®</sup> glass microfiber filters, 90 mm Grade GF/B; Aldrich, Germany). Ulcers were scored using bench magnifier under 5X magnification using the ulcer scoring criteria, the numbers of ulcer spots per stomach were calculate. The ulcer index is a method to determine the degree of gastric mucosal erosion (number and depth of the ulcers) and the following criteria were used to grade the incidence or severity of the lesions as: (0=No ulcer, normal gray colored stomach); (1=Superficial ulcer as spot ulcer); (2=Deep ulcer) or (3=Perforation) [14,15]. Ulcer index was calculated from severity score by using the equation as follows:

Ulcer index (UI) = (UN + US + UP)  $\times$  10<sup>-1</sup>

Where:

UN=Average number of ulcer per rat. US=Average severity scores.

UP=Percentage of rats with ulcers.

Then ulcer index was calculate and compared the treated groups with control groups.

Also, the toxicity score was measured for each rat then the means were calculated.

## Histopathological study

Histopathological study was conducted after rat scarifying then the stomachs randomly were taken (two from each group) and preserved in 10% neutral buffered formalin solution for 24 hours [16]. Then biopsies were taken in 10% normal saline and examined grossly for size and adequacy of sampling. One block was taken from each specimen, placed into a tissue cassette and prone to tissue processing under standard conditions using an automatic tissue processor machine then placed in paraffin. The blocks were constructed with embedded paraffin and sections were cut at 4-6 µm thickness using standard microtome. The sections were counterstained with hematoxylin and eosin stain, Mayer's hematoxylin (H&E) and then mounted on cover slip using DPX (Distyrene, Plasticizer and Xylene) mounting media (Sigma-Aldrich). The findings of sections were reviewed using light microscope (Olympus Corp., Tokyo, Japan), and results were reported [17].

## Data analysis

All the calculated data were presented as means  $\pm$  Standard Error of Means (SEM) and analyzed using analysis of variance (ANOVA). Comparisons with the control groups were made using One-way ANOVA. The level of statistical significance was set at p<0.05.

# **RESULTS AND DISCUSSION**

## Acute toxicity study

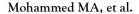
In clinical toxicity study, no any signs of abnormalities or death before and after treatment with aqueous extract of *M. cavendishii* fruit pulp (3 g/kg body weight for 24 hours) were recorded. Hence, according to the Hodge and Sterner toxicity scale, *M. cavendishii* was in non-toxic herbal drug category [18].

## Effect on body weight

The body weights mean  $\pm$  SEM of animals in all the six groups were measured at 0 day showed a range of 174.3  $\pm$  3.2 to 184.4  $\pm$  2.9 g/rat. There was a significant decrease in the body weight in group that received banana extract (P<0.05) when compared to control group (Figure 1). Group 4 was showed higher decrease in body weight followed by group 3, Group 5 and group 6 as show in Figure 1. However, control groups (group 1 and 2) were showed no increase in their body weight. This result was similar to study carried out by Escobar et al. on Wistar rats treated with flour M. *cavendishii* which showed a potential weight control [19].

## Effect on ulcer index and toxicity score

Aspirin inhibitory effect on cyclooxygenase (COX) enzyme leading to decreased prostaglandin production and increase gastric acidity which can greatly increase the risk of ulceration [20]. Ulcer index is a visual indicator used to measure the gastric ulcer and/or injury in the model or evaluation method for the extent of ulcer, which is



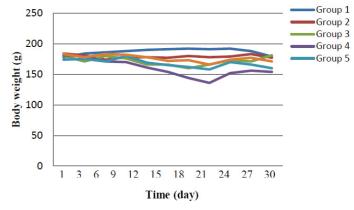


Figure 1: Effect of banana extract on body weight.

commonly used in the ulcer pharmacology studies [21]. The gastroprotective effect of M. cavendishii extract of different doses on ulcer in albino rats before and after two hours aspirin administration is shown in Table 1. The oral administration of aspirin damages the mucosal lining resulting in gastrointestinal bleeding with ulcer index of  $25.5 \pm 1.45$  in group 2. This results revealed that there was significant difference (p<0.05) in ulcer index between group 1 (negative control) with ulcer index 0 and M. cavendishii extract treated groups (treated before administration of aspirin) 3 and 4 with ulcer index  $15.0 \pm 1.31$ ,  $09.5 \pm 0.64$ , respectively. Also, there was significant difference (p<0.05) in ulcer index between group 1 (negative control) with ulcer index 0 and M. cavendishii extract treated groups 5 and 6 (treated after administration of aspirin) 09.0  $\pm$  0.79 and 0 respectively.

This obtained results showed that the percentage of gastroprotectice effect of M. cavendishii extract two hours before administration of aspirin was  $41\% \pm 1.5\%$  and  $64\% \pm 2.2\%$  for groups 3 and 4, respectively. This activity of the extracts is a dose-dependent at measured doses which showed a significant increase (p < 0.05) when administered two hours after, groups 5 (0.5 g/kg) and 6 (1.0 g/kg) with gastric lesion protection of  $66\% \pm 1.8$  and 100% respectively. The result of this study support the findings of Onasanwo et al. in 2013, they found that the ulcer healing potentials is dose-dependent for M. sapientum peels extract on aspirin-induced ulcer in rats [11]. Also, gastro-protective against toxicity of aspirin was calculated as shown in Table 1 and Figure 2. Group 2 had mucosal erosion, mild glandular dysplasia, inflammation of epithelial and squamous metaplasia noted when compared to extract treated groups (Groups 3, 4, 5 and 6).

#### Histopathological effects

Microscopic studies of gastric mucosa were performed on all groups to prove the results. Group 1 (negative control) showed organized glandular structure and normal submucosa (Figure 3A). Group 2 (positive control) that administered asprin 100 mg/kg, showed severe ulceration seen as epithelial cell loss, necrosis and bleeding (Figure 3B). Groups 3 and 4 treated with M. cavendishii extract two hours before aspirin showed significant improvement as partial erosion of mucosa induced by asprin as shown in Figure 3C and Figure 3D, respectively. While, group 5 and 6 that treated with

## OPEN ACCESS Freely available online

Table 1: Gastro protective effect of banana extract on aspirin induced ulcer in rats

Group	Ulcer index ± SEM	Toxicity score ± SEM		
		Inflammation	Hyperplasia	Necrosis
		Group 1		
(Negative control)	$0.0 \pm 0.00$	$0.0 \pm 0.00$	$0.0 \pm 0.00$	$0.0 \pm 0.00$
		Group 2		
(Treated with aspirin 100 mg/kg)	25.5 ± 1.45	2.80 ± 0.15	1.90 ± 0.10	2.6 ± 0.21
		Group 3		
(Treated with extract 0.5 g before aspirin 100 mg/kg)	15.0 ± 1.31	0.25 ± 0.09	0.25 ± 0.06	0.25 ± 0.1
		Group 4		
(Treated with extract 1 g before aspirin 100 mg/kg)	09.5 ± 0.64	0.10 ± 0.01	0.05 ± 0.01	0.1 ± 0.01
		Group 5		
(Treated with extract 0.5 g after aspirin 100 mg/kg)	09.0 ± 0.79	0.05 ± 0.001	0.10 ± 0.01	0.05 ± 0.001
		Group 6		
Treated with extract 1 g after aspirin 100 mg/kg)	0.0 ± 0.00	0.005 ± 0.001	0.01 ± 0.001	0.05 ± 0.001
SEM: Standard Erro	or of Means			
3 2.5 2 1.5 1.5 1			— Ну	lammation perplasia crosis

Figure 2: Gastroprotective effect of banana extract on aspirin toxicity score.

5

4

**Group number** 

6

0.5

0

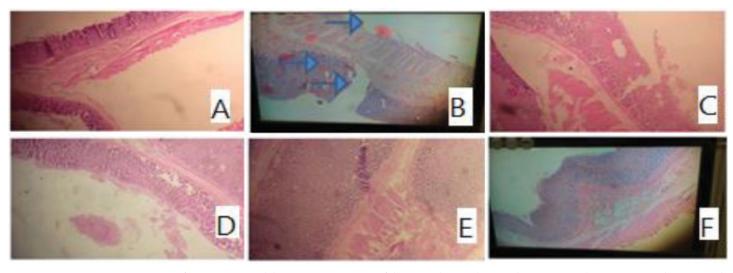
1

2

3

M. cavendishii extract two hours after aspirin showed the higher protection effect for gastric mucosa with no ulcer or necrosis and normal mucosal layer as shown in Figure 3E and Figure 3F, respectively. The results of this study show that unripe banana has more protective effect for peptic ulcer when administered after aspirin because of the possibility of wash the banana from the stomach when administered before aspirin.

However, gastro-protective effect could be attributed to reduce the gastric acid secretion, protect the mucosal tissues that line the stomach or to eliminate H. pylori. This gastro-protective effect of M. cavendishii extract may a fucntion of bioactive compounds which have shown anti-ulcer properties include carotenoids, flavonoids, saponins, tannins, and flavonoids [22-24]. Moreover, M. cavendishii extract improved mucosal tissues protection and promote healing of ulcers due to the presence of aqueous polysaccharides which is one of the major mucosal coating agents.



**Figure 3:** Microscopic appearances of gastric mucosa: (A) Microscopic section of the normal control group showing normal gastric mucosa (100x, H&E). (B) Microscopic section of aspirin treated group showed well marked severe ulceration, necrosis and hemorrhage. (C) and (D) Microscopic sections of Groups treated with M. *cavendishii* extract two hours before aspirin showed significant improvement. (E) and (F) Microscopic sections of Groups treated with M. *cavendishii* extract two hours After aspirin showed normal gastric mucosa.

# CONCLUSION

The result revealed that *M. cavendishii* pulp powder is an effective gastro-protective agent against aspirin induced ulcer which promotes ulcer healing and strengthens the mucosa against the damaging effects of aspirin. The study confirmed the application of the plant in folk medicine for the management of gastric ulcer.

# ACKNOWLEDGMENTS

The authors would like to acknowledge University of Gezira for allowing the use of the laboratory facilities.

# AUTHOR CONTRIBUTIONS

Motasim A. Mohammed, Amna E. El-Hadi, Esmat A. Mohammed, Fatima S. Abbas, Khadiga O. Musa and Mustafa Y. Al-Ameen devised the idea, drafted the proposal, collected the plant material and carried out all experiments. Alia M. Ahmed, Hozeifa M. Hassan Abdelgadir A. Abdelgadir supervised and provided the final analysis of the experiments. All authors discussed the results and contributed to the final manuscript.

# DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

# **FUNDING**

This research received no specific grant from any funding agency in the public, commercial, or not for-profit sectors.

# ETHICAL APPROVAL

The animals used in these experiments were handled according to guidelines for the care and use of laboratory animals and the ethical clearance was obtained from the Ethics Committee at University of Gezira, Faculty of Medicine prior to the study.

# REFERENCES

- 1. Malik TF, Gnanapandithan K, Singh K. Peptic ulcer disease. In: StatPearls. Treasure Island (FL): StatPearls Publishing, UK, 2020.
- 2. Yeomans ND, Naesdal J. A systematic review: Ulcer definition in

NSAID ulcerprevention trials. Aliment Pharmacol Ther. 2008; 27: 465-472.

- Katzung BG. Basic and clinical pharmacology, (14th edn). 2018, McGraw-Hill Companies, New York, USA, 1009.
- Henry DA, Langman MJS. Adverse effects of anti-ulcer drugs. Drugs. 1981; 21: 444–459.
- Awaad AS, El-Meligy RM, Soliman GA. Natural products in treatment of ulcerative colitis and peptic ulcer. J. Saudi Chem Soc. 2013; 17: 101–124.
- Harsha C, Banik K, Bordoloi D, Kunnumakkara AB. Antiulcer properties of fruits and vegetables: A mechanism based perspective. Food Chem Toxicol. 2017; 108(Pt A): 104-119.
- Ploetz RC, Kepler AK, Daniells J, Nelson SC. Banana and plantainan overview with an emphasis on Pacific Island cultivars [Musaceae (banana family)]. Species Profiles for Pacific Island Agrogorestry, 2007.
- Best R, Lewis DA, Nasser N. The anti-ulcerogenic activity of the unripe plantain banana (Musa species). Br J Pharmacol. 1984; 82: 107-116.
- Dunjić BS, Svensson I, Axelson J. Green banana protection of gastric mucosa against experimentally induced injuries in rats. A multicomponent mechanism?. Scand J Gastroenterol. 1993; 28: 894-898.
- Prabha P, Karpagam T, Varalakshmi B, Packiavathy AS. Indigenous anti-ulcer activity of Musa sapientum on peptic ulcer. Pharmacognosy Res. 2011; 3: 232-238.
- 11. Onasanwo SA, Emikpe BO, Ajah AA, Elufioye TO. Anti-ulcer and ulcer healing potentials of Musa sapientum peel extract in the laboratory rodents. Pharmacognosy Res. 2013; 5: 173-178.
- 12. Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. Toxicol Int. 2013; 20: 224-226.
- Goel RK, Gupta S, Shankar R, Sanyal AK. Anti-ulcerogenic effect of banana powder (Musa sapientum var. paradisiaca) and its effect on mucosal resistance. J Ethnopharmacol. 1986; 18: 33-44.
- Gupta SK. Drug Screening Methods (Preclinical Evaluation of New Drugs), 2nd Edition. New Delhi: Jaypee brothers Medical Publishers Pvt. Ltd. 2009; 511-519.
- Gerhard Vogel H. Drug Discovery and Evaluation. 2nd edition Germany: Springer - Verlag Berlin Heidelberg. 2002; 825-946.

#### Mohammed MA, et al.

- Guzmán-Gómez O, García-Rodríguez RV, Quevedo-Corona L. Amelioration of Ethanol-Induced Gastric Ulcers in Rats Pretreated with Phycobiliproteins of Arthrospira (Spirulina) Maxima. Nutrients. 2018; 10: 763.
- Song SH, Kim JE, Sung JE. Anti-ulcer effect of Gallarhois extract with anti-oxidant activity in an ICR model of ethanol/hydrochloride acidinduced gastric injury. J Tradit Complement Med. 2018; 9: 372-382.
- Hodge HC, Sterner JH. Tabulation of toxicity classes. Am Ind Hyg Assoc Q. 1949; 10: 93-96.
- Escobar A, Rocha-Gomes A, Reis CG, Herrera KNS, Guedes TJ, Silva AA, et al. Unripe banana flour (Musa cavendishii) promotes decrease in weight gain and elimination of fecal cholesterol in Wistar rats. Food Sci Nutr. 2019; 50: 157-167.
- 20. Cryer B, Mahaffey KW. Gastrointestinal ulcers, role of aspirin,

and clinical outcomes: pathobiology, diagnosis, and treatment. J Multidiscip Healthc. 2014; 7: 137-146.

- Shuai W, Yong-rui B, Peng DY, Sheng MX, Guo KT. Evaluation of gastric ulcer model based on gray-scale image analysis. Afr J Microbiol Res. 2011; 5: 1285-1290.
- Farzaei MH, Abdollahi M, Rahimi R. Role of dietary polyphenols in the management of peptic ulcer. World J Gastroenterol. 2015; 21: 6499-6517.
- 23. Pereira A, Maraschin M. Banana (*Musa spp*) from peel to pulp: ethnopharmacology, source of bioactive compounds and its relevance for human health. J Ethnopharmacol. 2015; 160: 149-163.
- 24. Lewis DA, Fields WN, Shaw GP. A natural flavonoid present in unripe plantain banana pulp (*Musa sapientum L.* var. paradisiaca) protects the gastric mucosa from aspirin-induced erosions. J Ethnopharmacol. 1999; 65: 283-288.