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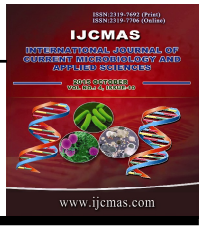

Original Research Article

Evaluation of the Anti-Gastric Ulcer Effect of Methanolic Extract of *Pennisetum purpureum* (Schumach) in Male Wistar Rats

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K e y w o r d s

Pennisetum purpureum, Gastric ulcer, Mucus, Malonialdehyde

A B S T R A C T

The fresh leaf samples of *Pennisetum purpureum* has been shown to be a potential source of dietary protein and antioxidants. The proximate composition and selected micronutrients of young leaf sheaths with their edible inner whorled leafy bundles collected from Napier grass in Nigeria (achara Ibeku variety, with purple sheaths) has been determined from previous studies. There is dearth of information on possible anti-ulcerogenic effect of methanolic extract of edible young leaves of Napier grass (*Pennisetum purpureum*). In this study, the anti-gastric acid effect of fresh leaf samples of achara Ibeku was determined by inducing ulcer with ethanol. The gastric mucus secretion, mucus cells count and malonialdehyde concentration were also determined. The study showed that the methanolic extract of Napier grass (*Pennisetum purpureum*) ameliorated gastric ulcer effect.

Introduction

Pennisetum purpureum (Schumach) commonly known as elephant grass or Napier grass, is a tall grassy perennial plant in Poaceae family (Obi *et al.*, 2008; Cowell, 2009; Feedipedia, 2015). Though a native of sub- tropical Africa, it now exists in most tropical and sub-tropical countries of the world as a weed or planted crop that is largely produced and used for livestock feed (Obi *et al.*, 2008). The apical ends of culms or stems of Napier grass plants usually have whorled tender young leaves before flowering (Cowell, 2009). The common two varieties of the plant found in Nigeria are those with purple stem and light green stem; that are respectively known in local Igbo dialects as Achara Ibeku and Achara Ngwa cultivars. The naturally sheathed tender young leaves of Napier grass which is cherished as a soup vegetable especially amongst Ngwa and Umuahia-Ibeku clans of Igbo people of Abia State, Nigeria (Okaraonye and Ikewuchi, 2009) and some of their surrounding neighbors is also known as "Achara" in the local Igbo dialects. The ethnic soup that is prepared with this leafy vegetable is called "Ofe Achara" (Okaraonye and Ikewuchi, 2009). The Igbo people consume this soup with complimentary ethnic viscous starchy pastes

such as yam "fufu" and "eba" (Ukpabi and Ndimele, 1990; Ukpabi and Oti, 2010). Presently, this perishable leafy vegetable from Napier grass is being marketed in Nigeria. Furthermore, the nutritional data on this leafy vegetable did not indicate varietal effect on its nutritional composition (Okaraonye and Ikewuchi, 2009) and another study indicated that the experimental fresh leaf samples of Achara Ibeku variety are potential sources of dietary protein and antioxidants. The dearth of information on possible gastrointestinal effect viz-a-viz gastric ulcer of this local food and feed stuff led to this study.

Materials and Methods

Chemicals

Histamine acid phosphate (Sigma- Aldrich, St Louis MO), Ketamine Hydrochloride (Rotexmedica, Trittau, Germany), Carbamylcholine chloride (Carbacol: Sigma- Aldrich MO), Pentagastrin (Sigma Aldrich), Trichloroacetic acid, Magnesium chloride (Sigma- St Louis, MO), Sodium chloride (Sigma- St Louis, MO), Hydrochloric acid (Sigma- St Louis, MO), Thiobarbituric acid (Sigma- St Louis, MO), Alcian blue, Sodium acetate(Sigma- St Louis, MO), Diethyl ether (Sigma- St Louis, MO), Sodium hydroxide (Sigma- St Louis, MO). Chemicals and reagents were of analytical grade.

Source of materials and extraction of plant material

Samples of the experimental young shoots of Achara Ibeku variety (with purple colour leaf sheaths) leafy materials covered with tough leaf sheaths were purchased at Orie Ugba market, Umuahia North local government area in Abia state of Nigeria (approximately within latitude 4^{o} 40 and 6^{o} 14 N, and between longitude 7^{o} 10 and 8^{o} E). These botanic shoot samples were authenticated as materials from Napier grass (*Pennisetium purpureum*) by Botany Unit, Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The exterior parts of the sheaths were hand peeled or rolled off to get the soft edible part or matrices also known as "Achara". 1000g of these were placed in Soxhlet extractor and extracted using methanol. The extract was concentrated with the aid of rotary evaporator (Heidolph, model number 4011, USA) to give a yield of 20g of dry extract.

Acute toxicity study of the extract

Adult albino mice (25- 30g) were divided into five groups each containing 10 mice. The mice were fasted for 6 h with only access to water *ad libitum* before the experimental study. Groups I, II, III and IV animals were administered various doses of methanolic extract of *Pennisetum purpureum* (500, 800, 1000 and 1500 mg/kg) orally and Group V received Tween 80 only. The animals were observed for 72 h for mortality (Ravichandran *et al.,* 2007). There was no death during and after the test.

Ethanol induced ulcer model

The ulcer was induced by administering ethanol. All the animals were divided into four groups each consisting of five rats. Group I received distilled water orally and served as control. Groups II and III rats received *Pennisetum purpureum* extract (EPP) dissolved in Tween 80 at doses of 200 and 400 mg/kg respectively. Rats in Group IV were administered with omeprazole (20 mg/kg) as a standard reference drug. Rats in Group IV were administered with omeprazole (20 mg/kg) as a standard

reference drug. The gastric ulcers were induced in the rats by administering 1ml/200g of absolute ethanol (90%) after 45 minutes of EPP and omeprazole treatment. They were kept in modified cages to prevent coprophagy during and after the experiment. The animals were anaesthetized 1 h later with ketamine (0.2 ml/100g body weight). The stomach was removed and incised along the greater curvature and ulceration scored using the method of Desai *et al.* (1999).

Effect of EPP on gastric mucus secretion

The rats were divided into four sub-groups of five rats and treated for 14 days orally with extract of EPP. Group 1 was the control treated with distilled water and Group IV was administered omeprazole (20 mg/kg). Groups II and III were given EPP at doses 200 mg/kg and 400 mg/kg respectively. The rats were sacrificed by cervical dislocation and their stomachs removed and weighed. The glandular portion of each stomach was opened along the lesser curvature. The everted stomachs were soaked for two hours in 0.1% Alcian blue dissolved in 0.16M sucrose buffered with 0.05M sodium acetate, adjusted to pH 5.8 with hydrochloric acid.

Uncomplexed dye was removed by two successive washes at 15 and 45 minutes in 0.25M sucrose. Dye complexed with mucus was diluted by immersion in 10ml aliquots of 0.5M Magnesium Chloride for 2 hours. The resulting blue solutions were shaken briefly with equal volume of diethyl ether and absorbance of aqueous phase was measured at 605 nm using spectrophotometer (Corney *et al.,* 1974). The absorbance of each solution was then used to calculate the various concentrations of dye and the weight of dye (expressed in mg) deduced, using a standard curve. The weight of dye was then expressed over the weight of the stomach, to give the weight of mucus secreted.

Thus, gastric mucus secretion (mg/g tissue)

$$= \frac{\text{Weight of dye (mg)}}{\text{Weight of stomach (g)}}$$

Effect of EPP on gastric mucus cell count

The rat were divided into four sub-groups of five rats each and treated for 14 days orally with EPP. Group 1 was the control and was treated with distilled water. Groups II and III were given EPP at doses 200 mg/kg and 400 mg/kg respectively, while Group IV was administered omeprazole (20 mg/kg). The rats were sacrificed by cervical dislocation and the stomachs removed. The glandular portion of the each stomach was opened along the lesser curvature. Gastric mucus cell count was done by counting the number of gastric mucus cells that stained with haematoxylin and eosin. The stained slide of the stomach mucosa viewed were indicated as blue patches. These were counted using calibrated microscope in five randomly selected area of the gastric mucosal tissue. Five cubic boxes each with an area of 1 mm^2 were assessed. This method is an improvement over the earlier described approach for counting by Li *et al.* (2002).

Effect of EPP on Malondialdehyde Concentration (MDA)

The rat were divided into four sub-groups of five rats each and treated for 14 days orally with EPP. Group 1 was the control and was treated with distilled water. Groups 2 and 3 were given EPP at doses 200 mg/kg and 400 mg/kg respectively, while Group 4 was administered omeprazole (20 mg/kg). The rats were sacrificed by cervical dislocation and the stomachs removed. The stomach was opened along the lesser curvature,

washed and homogenized to be used for lipid peroxidation. Briefly, lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) produced according to the method of Gutteridge and Wilkins (1982). This method is based on the reaction between 2-thiobarbituric acid (TBA) and malonialdehyde (MDA) which is an end-product of lipid peroxides during lipid peroxidation. On heating in acidic solution, a pink coloured complex was produced that absorbs maximally at 532 nm on the spectrophotometer. 0.1ml of the test sample was mixed with 0.5ml of 10% TCA and 0.5ml of 75% TBA was then added. The mixture was placed in water bath at 80^{o}C for 45 minutes. The absorbance of the resulting pink colour solution was measured against a reference blank of distilled water at 532nm. The test sample was calibrated using the MDA as standard and the result was expressed as the amount of free MDA produced or MDA quantified by using the molar extinction coefficient, C of 1.56 x $10^{5}M^{-1}cm^{-1}$.

MDA (units/g tissue) =

$$\frac{\text{Absorbance of sample}}{\text{Molar extinction coefficient}}$$

Results and Discussion

Effect of *Pennisetum purpureum* on alcohol-induced gastric ulceration

In the control animals, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of the stomach which appeared as elongated bands of thick, black and dark red lesions. The figure 1 shows the graph of ulcer scores against dose of EPP. The ulcer scores are EPP 200 mg/kg (5.00 ± 0.13), EPP 400 mg/kg (3.38 ± 0.16) and omeprazole 20 mg/kg (2.25±0.19) compared to the control (6.25 ± 0.43). It shows a graded and dose-dependent decrease in the mean ulcer scores with increases in dose of EPP. These decreases in the EPP and omeprazole-treated animals are significantly different compared to the control ($p \leq 0.05$), although the reference standard drug omeprazole showed higher anti-ulcer activity.

Effect of EPP on gastric mucus secretion

In figure 2, there was significant increase in gastric mucus secretion (mg/g tissue) in the 200 mg/kg EPP (0.51±0.03), 400 mg/kg EPP (0.65±0.06) and omeprazole 20 mg/kg (0.68±0.06) pretreated rats compared to the control rats 0.39± 0.02 ($p \leq 0.05$). These increases in gastric mucus secretion are dose-dependent.

Effect of EPP on gastric mucus cells count

In figure 3, the effect of EPP on gastric mucus cells count showed graded increase with the increasing doses. There is a significant increase in the mucus cells count with 200 mg/kg EPP treated rats (92.5± 5.53 cells/cm^2) compared to the control rats (68.2± 2.97 cells/cm^2). Similarly the doses of EPP 400 mg/kg (111.4± 6.83) and omeprazole 20 mg/kg (122.4 ± 4.11 cells/cm^2) produced increases in gastric mucus cells count that are significantly different compared to the control rats group ($p<0.05$)

Effect of EPP on malonialdehyde (MDA) concentration

The figure 4 shows the effect of EPP on MDA concentration. There was no significant decrease in MDA with 200 mg/kg EPP treated rats (0.22 ± 0.011) as against the control rats (0.25 ± 0.008)

($p \leq 0.05$). However, there are significant decreases of MDA concentration in the EPP 400 mg/kg (0.20 ± 0.007) and the omeprazole 20 mg/kg (0.14 ± 0.012) treated rats compared to the control ($p \leq 0.05$). This decrease exhibits dose dependence.

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanism (Piper and Stiel, 1986).

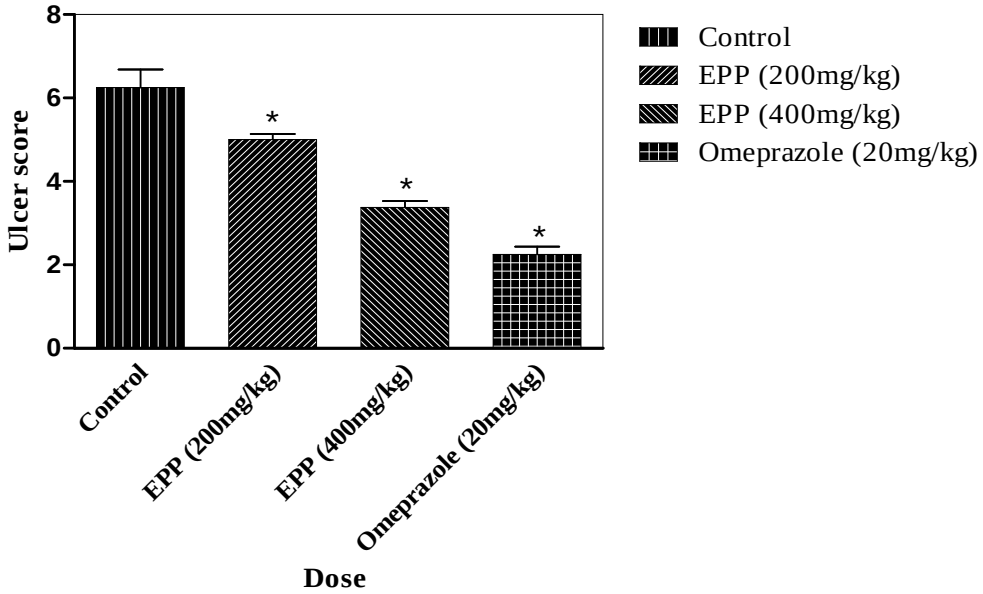

Fig. 1. Effect of *Pennisetum purpureum* on alcohol-induced gastric ulcer

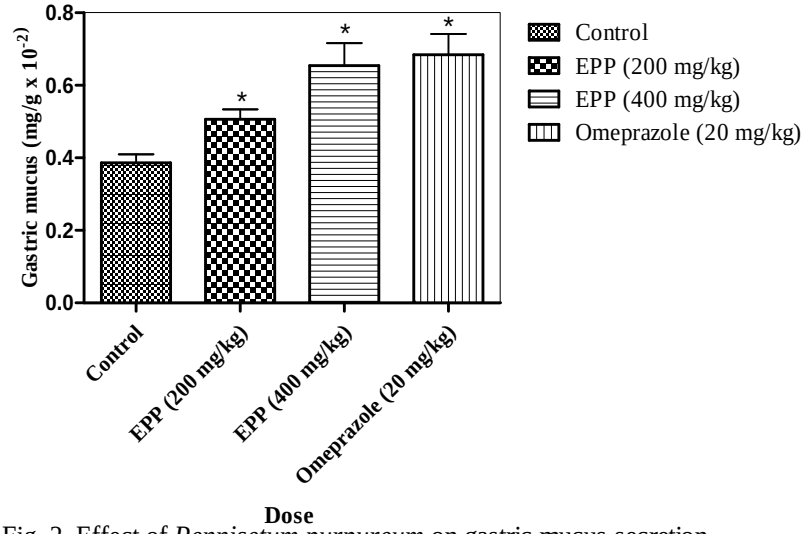

Fig. 2. Effect of *Pennisetum purpureum* on gastric mucus secretion

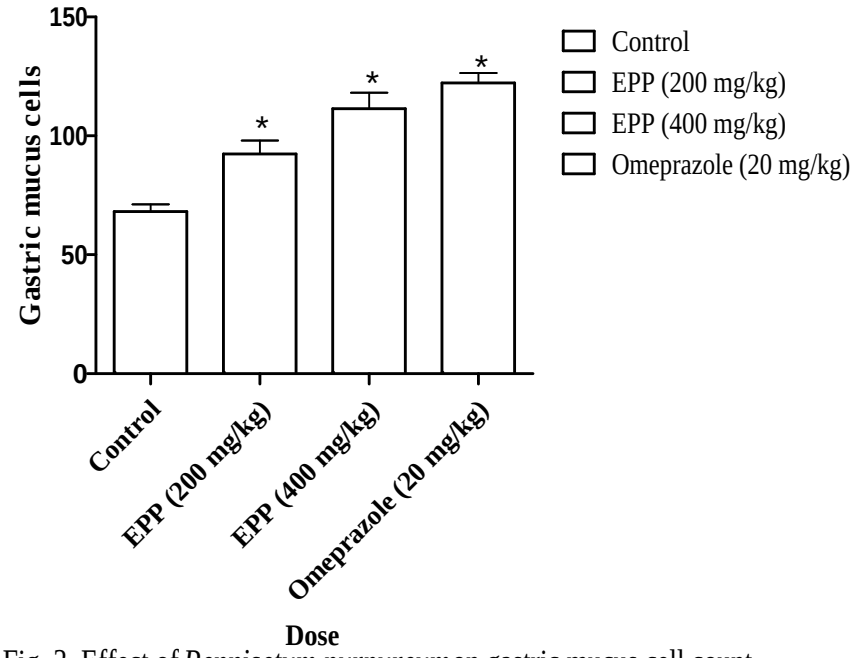

Fig. 3. Effect of *Pennisetum purpureum* on gastric mucus cell count

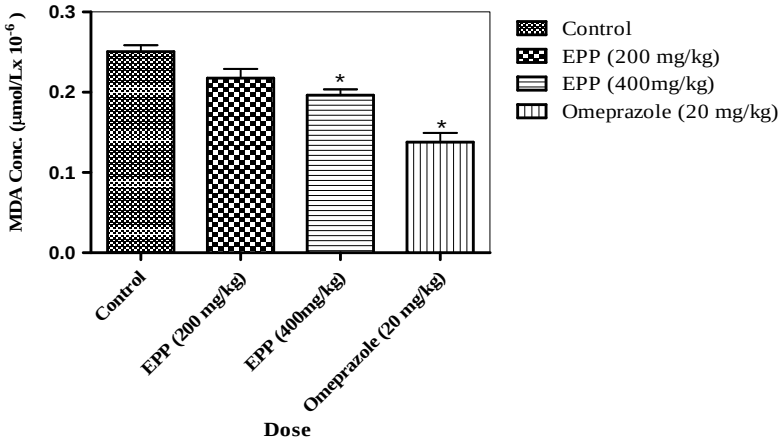

Fig. 4. Effect of *Pennisetum purpureum* on MDA

Natural products exhibiting a wide range of biological properties has been exploited for medical application in combating human diseases for thousands of years (Newman *et al.*, 2003). Natural products are more accessible to most of the population with a wider acceptability among the people and cheaper than those manufactured by modern health technology in most cases. As far as life is concerned the subject of priority is health. But despite efforts to maintain good health, man and animals alike still confront disease conditions which are due to exposure to physiopathological agents

(Sofowora, 1982). The administration of methanolic extract of *P. purpureum* up to 1000 mg/kg did not produce any mortality in the animals, showing its relative safety. The following study shows protective potential of *P. purpureum* while employing ethanol as ulcerogenic agent. In this study, *P. purpureum* which is a natural feed stuff could be exhibiting anti-ulcer effect, though not as effective as the standard drug, omeprazole in its ability to cause reduction of ulcer, the production of mucus and increasing mucus cells of the gastric mucosal. Ethanol induced gastric ulcer was employed to study the cytoprotective effect and antioxidant activities of the extract. Ethanol produces severe gastric erosions when given intragastrically. Oxygen-free radicals which leads to an increased lipid peroxidation and damage to cell and cell membranes, are implicated in the pathogenesis of ethanol-induced gastric mucosal injury, solubilization of mucus constituents and depressing of tissue levels of proteins (Galati *et al.,* 2001). The action of *P. purpureum* in this study indicates reduction in the incidence of gastric ulcer. The mode of action of *P. purpureum* may be similar to those of known drugs such as omeprazole, sucralfate, PAR-2 agonist, NO donors and misoprostol which are reported to increase gastric mucus production in vivo. Sucralfate has been reported to increase gastric mucus production *in vivo* (Slomiany *et al*, 1991) and *in vitro* (Takahashi and Okabe, 1996). Misoprostol increases gastric mucus secretion by increasing the thickness of mucus layer, enhancing mucosal blood flow as a result of direct vasodilatation, stabilization of tissue lysosomes or vascular endothelium and improvement of mucosal regeneration capacity. PAR-2 agonist administered parenterally or orally triggers secretion of gastric mucus through the release of endogenous CGRP and tachkinnins. *P. purpureum* caused an increase in gastric mucus and mucus cells (Figs. 2 and 3), and may be acting like these drugs.

The MDA level which is a measure of lipid peroxidation was decreased by *P. purpureum* in like manner as omeprazole in a dose dependent form (Fig. 4). The reduction of MDA concentration in this study may be due to the ability of *P. purpureum* to increase anti-oxidant activity. Phytochemical analysis of *P. purpureum* showed that it contains anthocyanins (Ukpabi *et al.,* 2015). Anthocyanins which are also strong plant antioxidants are known to impart red, purple and blue colorations to plant parts (when present in sufficient quantities) under certain chemical conditions (Gould *et al*, 2002) and this is seen achara Ibeku used in this study. Also, the Nigerian *P. purpureum* varieties have appreciable quantities of pro-vitamin A (carotene) and vitamin C (ascorbic acid). In addition to their biochemical vitamin activities, carotenes and ascorbic acid are recognized as antioxidants in human nutrition (Okaka *et al.,* 2002). These plant antioxidants scavenge systematic damaging oxygen radicals and may be responsible for the decrease in MDA level as observed in this study. This therefore supports other studies that demonstrated a reduction in lipid peroxidation of the gastric mucosa shown to be associated with increased activities of anti-oxidant enzymes (Melchiorri *et al.,* 1997; Dela and Motilva, 1999). Other studies have also shown that the protective activity of gastric mucus is due to the anti-oxidant activity conferred on it by its rich glycoprotein content (Oluwole and Saka, 2001). Allen (1981) has earlier reported that gastric mucus contains glycoproteins at concentrations as high as 50 mg/ml.

The study showed that the methanolic extract of Napier grass (*Pennisetum*

purpureum) ameliorates gastric ulcer effect and could serve as a good source of dietary protein for human and ruminant nutrition in Nigeria.

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