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Pineapple Juice Attenuates Starvation- Induced Ulceration in Wistar Albino Rats via Antioxidant Mechanisms

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Abstract:
This study was carried out to examine the effect of pineapple juice on starvation-induced ulceration and gastric mucosal SOD level in Wistar albino rats. The animals were divided into four experimental groups; the Control group (water only) and those pretreated with different percentage of pineapple juice in their water (100, 50, and 25%). The treatment was carried out for 30 days. The animals were fed with rat cubes. Results showed that full pineapple juice significantly decreased mean ulcer scores, an index for the extent of gastric mucosal damage. It also increased Superoxide dismutase activity; this was significantly higher in full juice treated animals than its activities in the other treated groups. We proposed that this represents one of the underlying mechanisms through which pineapple juice may be conferring gastro-protection against starvation-induced ulceration. We therefore conclude that pineapple juice may be therapeutically beneficial to individuals prone to ulcer development.

Keywords: *Antioxidants, superoxide dismutase, SOD, pineapple juice, starvation, gastric ulceration*

1. Introduction

Gastric ulcer remains a leading gastrointestinal disorder across the world (Elegbe and Bamigbose, 1976; Sato et al., 1995; Ajiboye and Oluwole, 2012). Many theories have been proposed as the main mechanism behind ulcer development including exposure and re-exposure to certain aggressive factors such as prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs), nicotine, corticosteroids, stress, acid secretion within the gastrum, bacteria infestation especially Helicobacter pylori to mention just a few. As the pathophysiological mechanisms underlying ulcer development is diverse, so also have been the treatment protocols employed over the years. While treatment focuses on combating abnormalities in the secretion of gastric acid and pepsin, and wiping bacteria infestation, a new preventive and treatment strategy in recent years have focused on the role of fruits, food crops and other naturally derived elements and their value.

Pineapple (Ananas comosus), named for its resemblance to the pine cone, is a tropical plant with edible multiple fruit consisting of coalesced berries. It is the most economically important plant in the Bromeliaceae family. Raw pineapple is an excellent source of Vitamin A, Vitamin C, Carotene and other trace elements such as Manganese, Potassium, Copper, and Iron etc. Aside from providing these elements to its consumer, it also helps in proper digestion of food and easy elimination of body waste due to the presence of bromelain, its principal constituent (Maurer, 2001). It is now widely accepted that nutrients with antioxidant activity may help to protect the human body against damage by reactive oxygen and nitrogen species (Halliwell, 1997). Frequent consumption of such natural antioxidant containing foods have been associated with a lower risk of cardiovascular disease and cancer (Renaud et al., 1998; Temple, 2000).

The protective effects of natural antioxidants in fruits and vegetables are linked to the major compounds they contain most importantly; Vitamins (which are important for immunity and cardiovascular function, collagen synthesis and antioxidant agent), Phenols, which block the oxidation of free radicals while Carotenoids contains beta-carotene which has antioxidant properties that help neutralize free radicals.

The free-radical scavenging capability and consequent antioxidant properties of the phenolics play an important role in protecting the cells and tissues from oxidative stress and other biological effects associated with chronic diseases (Rimbach et al.,2005). Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. They can cause cell damage by inducing a chain of reaction and when present in high levels, may contribute to chronic conditions such as cancer, diabetes, and heart disease. Consequently, free radicals have been implicated in the pathogenesis of no less than 50 diseases. Though it is impossible to avoid free radicals altogether, antioxidants can minimize their effect by terminating the chain of reactions in cells thereby inhibiting oxidation.

sources of antioxidants is a diet rich in deeply colored fruits and vegetables; the pigments in these foods (beta-carotene, lycopene and alpha-carotene) are responsible for the antioxidant activity. Pineapple, mango, white guava, carrots are some of the fruits with high antioxidant content (Devasagayam et al, 2004). This study was designed to examine the effects of pineapple juice on the level of mucosal antioxidant enzyme, superoxide dismutase (SOD) in experimentally ulcerated albino rats.

2. Materials and Methods

2.1. Animals

Twenty-four (24) female albino rats and 24 male albino rats both of the Wistar strain weighing between 150 and 180g were used for this study. They were fed standard rat chow (Ladokun feeds, Ibadan, Oyo-state, Nigeria) and water given ad libitum in the Central Animal House, University of Ibadan, Ibadan. The animals were allowed to acclimatize for a period of two weeks, housed in well ventilated galvanized steel animal cages.

2.2. Experimental Design

The twenty-four male Wistar albino rats were used to investigate the effect of Pineapple juice on starvation – induced ulceration while the twenty-four female rats were used to assess the level of gastric mucosa SOD enzymes. In both studies, the animals were divided into four groups of six rats each:

- GROUP 1: (Control group) The animals were given water and rat chow for thirty consecutive days.
- GROUP 2: Animals were pre-treated with 100ml of pineapple juice for thirty days.
- GROUP 3: Animals were pre-treated with a mixture of 50ml of water and 50ml of pineapple juice for thirty days.
- GROUP 4: Animals were pre-treated with a mixture of 25ml of water and 75ml of pineapple juice for thirty days. After thirty days, the following studies were carried out.

2.3. Starvation-Induced Ulcer Study

In a pilot study on induction of ulcer via starvation, animals fasted for six days without feeds produced visible and measurable ulcers.

In the current study, animals were starved for six days after the pretreatment period. They were sacrificed by cervical dislocation. An abdominal incision was made along the linea-alba to expose the stomach. The stomach was removed by disconnecting the cardiac and pyloric ends. This was cut opened through the greater curvature and rinsed several times in distilled water to remove stomach debris. The ulcers were scored by two independent observers with a magnifying lens (Rao et al., 1997; Minaiyan and Alireza., 2006).

Scoring was computed as follows:

- Score 1 = up to 5 petechial haemorrhages with erosions depth of 1mm
- Score 2 = Up to 10 petechial haemorrhages with erosions of depth above 1mm.

2.4. Index of Ulceration

Paul's Index was used as ulcer index (Martin-Aragon et al., 1994). This index is expressed thus:

Ulcer index = M x N/100

Where M= Mean number of ulcers per rat in the group, N= percentage of rats with ulcer in the group.

Measurement of Superoxide Dismutase (SOD) Activity

Experimental procedures:

Blood was collected from the animal through retroocular route with the use of heparinized capillary tubes into EDTA sample bottles to prevent coagulation. The animal was sacrificed by cervical dislocation, dissected and the organs harvested.

The stomach was removed by cutting at the cardiac and pyloric ends and later opened through the greater curvature. The stomach was flushed using saline solution. The liver, heart, kidneys and uterus of the animal were also harvested. The organs were removed, rinsed in saline solution to wash off excess blood, blotted dried with filter paper, and weighed accordingly.

2.5. Preparation of Tissue Homogenate

The organs were homogenized in four parts of homogenizing buffer (i.e. 1g of organ in 4mL of buffer) and centrifuged at 1000rpm for 15 minutes in an ultracentrifuge at temperature <2oC to get the mitochondrial fraction. The supernatant (post mitochondria fraction) was decanted and stored at <4oC for subsequent analysis. Each time the supernatant was outside the freezer, it was kept in ice bags.

Spectrophotometer was calibrated using a blank solution. Each sample was diluted with 1500mM buffer solution. With the use of cuvettes, the samples were arranged according to groups. XO (absorbance) of each solution was taken using a spectrophotometer at 540nM.

Fundamental principle; SOD mediates decrease in the rate of auto-oxidation of hematoxylin in aqueous alkaline solution. 40µM of each sample solution was added to 920µM phosphate buffer at pH 7.4 which contained 1mM EDTA and mixed thoroughly. The solution was incubated at temperature 25oC for 2 minutes; hematoxylin 40µM was added to the solution. The solution was poured inside cuvettes and placed in a spectrophotometer, absorbance was read at 540nm. The results are expressed as the percentage of inhibition of hematoxyl in auto oxidation rate with respect to the reaction mixture without the test compound.

2.6. Calculation of Superoxide Dismutase

SOD activity was calculated by determining the % inhibition using the following formula:
%inhibition= [(control XO rate –sample rate) / (control XO rate)] ×10

3. Statistical Analysis

Data were expressed as Mean ± SEM. Statistical difference between test groups and control group was calculated using the student's t-test. $p<0.05$ was considered as significant.

4. Results

Starvation-induced ulceration study, the results of this study are shown in Table 1

Groups	Mean Ulcer Score ± SEM	Mean Ulcer Index
Control	8.40 ± 0.15	-
Pineapple only	3.75 ± 0.25*	6.97
50% Pineapple + 50% Water	10.0 ± 0.00	10.00
25% Pineapple + 75% Water	10.0 ± 0.00	10.00

Table 1: Mean Ulcer Score in Different Pre-Treated Groups
N= 6
**- Significant at P< 0.05 When Compared with Control*

Group	Stomach	Serum
Control	51.86 ± 3.15	16.67 ± 0.52
Full juice	55.63 ± 0.72	17.75 ± 0.50
Half juice	43.89 ± 0.60*	13.50 ± 1.24*
Quarter juice	43.90 ± 1.98*	13.90 ± 1.52*

Table 2: Estimation of Gastric and Serum SOD Level
N= 6
**= P<0.05 Full Juice Vs 50/25% Juice*

5. Discussion

` The quest for an ulcer cure has led a search into the various mechanisms by which ulcers are formed resulting in a wide range of treatment protocols which are available today. Yet none of these protocols serve as a perfect solution to the ulcer problem. Reactive oxygen species are a major factor in the establishment of visceral damage. Thus, antioxidants that prevent this kind of damage such as superoxide dismutase (SOD) and catalase are normally considered a first line of defence against these reactive species (Bafna and Balaraman, 2005). In the current study, ulcer formation was significantly lowered by 100% pineapple juice administration when compared with Control group and the 25% and 50% pineapple juice administration.

Oxidative stress plays an important role in the pathogenesis of various diseases, including gastric ulcer disease, with antioxidants reported to play a significant role in the protection of the gastric mucosa against various necrotic agents (Dursun et al., 2009). SOD, which plays an important role in protecting gastro intestinal mucosa, owes its antioxidant properties to its ability to scavenge superoxide anions (Dursun et al., 2009). Subcutaneous administration of SOD has been shown to significantly reduce the gastric damage induced by indomethacin (Yoshikawa et al., 1993; Naito et al., 1998).

In this study, pineapple juice was orally administered at different doses and it effect on SOD activity was determined. Table 2 showed that the full pineapple juice caused a significant increase in SOD activities in both stomach and serum samples. However, SOD activity of the stomach of animals that received 50ml and 25ml of pineapple juice for thirty consecutive days was not significantly elevated. This may be accountable for the less ulceration observed in the group that received full pineapple juice, when compared to the rest of the groups.

High levels of tissue SOD in the group given 100% juice clearly point to minimized oxidative activity with the explanation of an underlying antioxidant mechanism in its gastro protective action, while the ability of the pineapple juice to prevent lipid peroxidation in another in vitro study reinforces its potential use as a therapeutic fruit for free radical pathologies (Ajitha and Rajnarayana, 2001). Elevated SOD activities had also been reported following mixed fruit wine administration at high doses (Olas et al., 2002).

We therefore conclude, with respect to this study, that pineapple juice administered in large and pure quantities will significantly enhance SOD activity as evident in the stomach and serum samples. This fruit juice supplement will be therapeutically useful for the prevention of peptic ulcer in ulcer prone subjects.

6. References

i. Ajitha M, and Rajnarajana A. (2001): Role of oxygen free radicals in human disease. Indian Drugs; 38: 545-546.
ii. Ajiboye K.I and Oluwole F.S. (2012): Effects of a Type V Phosphodiesterase Inhibitor (Tadalafil) on Indomethacin-Induced Gastric Ulceration in Rats. Intl J of Tropical Medicine; 7 (3): 111-116.
iii. Bafna PA and Balaraman R. (2005): Antioxidant activity of DHC-1, an herbal formulation, in experimentally-induced cardiac and renal damage. Phytother. Res; 19: 216-221.
iv. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS and Lele RD. (2004): Free Radicals and Antioxidants

in Human Health: Current Status and Future Prospects. J. Assoc. Phys. Ind.; 52:796

v. Dursum H, Bilici M, Albayrak F, Ozturk C, Salglam MB and Suleyman H. (2009): Anti-ulcer activity of fluroxamine in rats and its effects on oxidant and antioxidant parameters in stomach tissue. BMC Gastroenterology; 9: 36.
vi. Elegbe RA, Bamgbose SOA. (1976): Protective dieting factors in experimental ulceration-study of some Nigerians cereal and tubers. Postgrad. D J; 52; 258-263.
vii. Halliwell, B. (1997): Antioxidants and human diseases: A general introduction. Nutr, Rev.; 55: 544-549.
viii. Martin-Aragon S, Benedi J and Villar A. (1994): Studies on the anti-inflammatory and anti-ulcerogenic activities of Tuberaria lignose extracts in experimental animals. Int. J. Pharmacog.; 32(1): 27-32.
ix. Maurer HRO. (2001): Bromelain: Biochemistry, Pharmacology and medical use. Cell. Mol. Life Sci., 58:1231-1245
x. Minaiyan AG and Alireza K. (2006): Anti-ulcerogenic effect of ginger (rhizome of zingiber officinale Roscoe) on cystemine induced duodenal ulcer in rats, DARU; 14: 2.
xi. Naito Y., Yoshikawa T., Yoshida N. and Kondo M. (1998): Role of oxygen radical and lipid peroxidation in indomethacin-induced gastric injury. Digestive Diseases and Sciences. 43: 30S –34S.
xii. Olas B, Wachowicz B, Salukjuszczak J Zielinski. (2002): Effect of resveratrol, a natural polyphenolic compound on platelet activation induced by endotoxin or thrombin. Thromb. Res; 107 (3-4): 141-145.
xiii. Rao AM, Baskaya MK, Maley ME, Kindy MS and Dempsey RJ. (1997): Beneficial effects of S-adenosyl-c-methionine on blood-brain barrier breakdown and survival after transient cerebral ischemia ingerbils. Mol. Brain Res; 44:134-138.
xiv. Renaud SC, Guenguen R, Schenker J and d'Houtand A. (1998): Alcohol and mortality in middle-aged men from Eastern France. Epidemiology; 9:184-8.
xv. Rimbach G, Fuchs J and Packer L. (2005): Application of nutrigenomic stool to analyze the role of oxidants and antioxidants in gene expression. Nutrigenomics (1-12) Bocs Ration, FL. Taylor and Francis.
xvi. Sato N., Kawwano S., Tsuji S., Ogihara T. and Yamada S. (1995): Gastric blood flow in ulcer diseases. Scandinavian Journal of Gasteroenterology; 30 (208): 14-20
xvii. Sies H. (1992): Antioxidant Function of Vitamins. Ann NY Acad Sci.; 669:7-20.
xviii. Temple, N. J. (2000): Antioxidants and disease: more questions than answers. Nutrition Research; 20, 449-459.
xix. Yoshikawa T., Naito Y., Kishi A., Kaneko T., Linuma S., Ichikawa H., Yasuda M., Takahashi S. and Kondo M. (1993): Role of active oxygen, lipid peroxidation, and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. Gut; 34, 732–737.