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# *IN-VIVO* STUDY OF THE HEALING EFFECTS OF *PERSEA AMERICANA* SEED EXTRACTS ON STRESS INDUCED ULCERATION IN RATS

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## ABSTRACT

**Background:** In folkloric medicine, aqueous seed extract of *Persea americana* (ASEPA) is indicated for treating peptic ulcer disease, without much validation of its efficacy. This study evaluates the effects of ASEPA, its n-hexane and water fractions on stress-induced ulcer in albino rats. **Method:** Dried, milled seeds of *P. americana* was soaked in distilled water then filtered and freeze-dried. The extract was fractionated with n-hexane, the residue was the water fraction. Acute toxicity and phytochemical screening tests were determined. Ulcer-protective and ulcer-healing effects of ASEPA and fractions were analyzed, including histopathological examinations of the rats stomachs. Cimetidine and distilled water were the standard drug and negative control, respectively. Outcome parameters were ulcer index, percentage healing of ulcer and effects on biochemical analysis. **Result:** Higher estimated oral median lethal dose (LD50) was obtained for ASEPA. The secondary metabolites (flavonoids, tannins, and saponins) had both ulcer protective and healing effects, which partly explains the pharmacological benefits of ASEPA seeds. ASEPA and fractions conferred dose-dependent ulcer-protective and ulcer-healing effects on the stress ulcer model. Oral treatment with ASEPA and fractions reduced significantly ($p<0.05$) ulcer indices in the treated groups. The reductions were comparable to cimetidine, but more pronounced with ASEPA and n-hexane fraction. Histopathological analyses confirmed their cytoprotective properties. ASEPA and fractions significantly modulated malondialdhyde and catalase levels which probably contributed to their ulcer-protective and ulcer-healing effects. **Conclusion:** ASEP possess stress-ulcer healing effects which may explain their beneficial uses for treating gastric ulcer.

**KEYWORDS:** *Persea americana* seed, ulcer healing, stress ulcer, rats.

## INTRODUCTION

### 1.1. Background

Medicinal plants have continued to attract attention in the global search for effective methods of using plants' parts for the treatment of many diseases affecting humans (Alexandra et al., 2018). Many important drugs used in medicine today are directly or indirectly derived from plants due to their bioactive constituents such as alkaloids, steroids and tannins (Thomford et al., 2018).

Peptic ulcers are sores in the lining of the stomach or small intestine. They occur when the protective factors of the gastro-intestinal tract are overwhelmed by the "aggressive factors" (MacGill 2018). Aggressive factors include Helicobacter pylori, HCl, pepsins, nonsteroidal anti-inflammatory drugs (NSAIDs), bile acids, ischemia, hypoxia, smoking and alcohol and stress. While defensive factors include bicarbonate, mucus layer, mucosal blood flow, prostaglandins and growth factors (Harold et al., 2007). Peptic ulcer disease (PUD) is an illness that affects a considerable number of people worldwide.

The incidence of peptic ulcer has been shown to be common in Africa and South Asia (Wikipedia, 2011).

When these ulcers occur in the stomach, they are called gastric ulcers but when they occur in the first portion of the intestine, they are called duodenal ulcers. "Peptic Ulcer" is the term used to describe either or both of these two types of ulcers. Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide, representing a major burden for health care resources (Tanih et al., 2010). Stress ulcers are multiple, superficial erosion which occur mainly in the fundus and body of the stomach. Stress-induced ulcer is believed to be mediated by the release of histamine which increases gastric acid secretion (Siddiqui et al., 2019). Physical or psychological stress is one of the common causes of upper gastrointestinal ulceration (Hoogerwerf & Pasricha 2006). Although the pathogenesis of gastric lesions due to stress is not completely understood, the production of oxygen free radicals via the xanthine-xanthine oxidase system and neutrophils and lipid peroxidation initiated by the produced reactive oxygen species (ROS) have been used to explain the mechanisms of acute gastric lesion formation associated with stress (Kumar, 2011). Stress ulcers are common among people with immense physical stress such as those in intensive care units. The primary goal for treatment is to reduce stomach acid, lower the risk of serious infection, bleeding and shock. Peptic ulcer is made worse by stress. While both stress ulcer and peptic ulcers cause sores in the lining of the stomach and intestines, a typical peptic ulcer tend to emerge gradually as gastrointestinal lining weaken (Hoogerwerf & Pasricha, 2006). Stress ulcer manifest suddenly usually as a result of physiological stress probably because stress increases stomach acid. The treatment for stress induced ulcer is basically the same as peptic ulcer. Although potent ulcer-healing drugs are available such as omeprazole, they are usually combined with other drugs for them to be effective. This combination increases cost of treatment, drug interactions and side effects. These setbacks are worse for patient with stress ulcer considering the fact that stress ulcer is very common among those who are already ill and most likely on other medications. Therefore, there is need to search for new alternatives (Lavnya *et al*., 2012) for the management and treatment of stress induced gastric ulcer disease.

As high as 80% of the world population depends on plant-derived medicines for the first line of primary health care (Panda & Sonkamble 2012). The fruit of *Persea americana* is commonly referred to as avocado pear, alligator pear and butter fruit (Morton, 1987). Locally, it is known as Ebenmbakara in Ibibio, Ube bekee in Igbo and Ado in Yoruba (Morton, 1987). It is a widely distributed plant in the lowlands and rain forest areas of Nigeria. The seed has a diverse application in ethno-medicine, ranging from treatment of diarrhea, dysentery, toothache, ulcer, high cholesterol level, prevention of cardiovascular diseases as well as skin beautification (Okafor *et al.*, 2005). The leaves have been popularly used in the treatment of diabetes in countries of Latin America and Africa (Lima *et al*., 2012). *Persea americana* leaves have also been reported to possess anti-inflammatory and analgesic properties (Adeyemi *et al.*, 2002) as well as anti-hypertensive properties (Ozolua *et al*., 2009). Ilozue *et al* (2014) and Omodamiro *et al* (2016) reported the presence of alkaloids, flavonoids, saponins, steroids, tannins, and phenol as phytoconstituents in *Persea americana* seeds. Del Refugio *et al* (2004) reported the elucidation of two glucosylated abscisic acid derivates from avocado seeds. In another study, Francisco *et al* (2018) demonstrated the radical scavenging properties of avocado seed oil. Recently Umeh *et al* (2020) demonstrated the gastric acid inhibitory effect of P*ersea americana* seed extract. Although lot of studies have been done on *P. americana* seed, the healing efficacy on stress-induced ulcer has not been elucidated, thus the need for this study.

## 2. MATERIALS AND METHODS

### Collection and identification of plant material

The fruits of *P. americana* were purchased from Oye-Nimo, Nimo, Njikoka local government area of Anambra State, Nigeria in the fruiting season of April 2016. It was identified and authenticated by a Taxonomist at the Department of Botany, Nnamdi Azikiwe University Awka, Nigeria and a herbarium specimen, number NAUH.13, was assigned to it and kept in the hebarium.

### Chemicals

Potassium chloride (KCl) (1.15 % w/v), 0.2 M hydrogen peroxide (0.01 M), phosphate buffer (7.0) and acetic acid.

### Drugs

Cimetidine (Arochem Enterprises, New Delhi, India) was used as standard (reference) drug.

### Animals

The study was carried out using adult albino rats of 200 to 220 g weight of both sexes bred locally in the Animal House of the Department of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, and University of Nigeria Nsukka, Nigeria. The rats were fed with feed pellets, Top Feed Premier Feed Mills Sapele, Delta State, Nigeria. The animals were given food and water *ad libitum* throughout the experiments. They were kept in specially constructed cages to prevent coprophagia during and after the experiments. All animal experiments were conducted in compliance with NIH Guide for Care and use of Laboratory Animals (Pub. No. 85-23 Revised 1985) as approved by the Nnamdi Azikiwe University, Awka and University of Nigeria Nsukka Ethical Committees for the use of Laboratory Animals.

### 2.2. Methods

#### 2.2.1 Extraction of *Persea americana* seeds

The seeds of *P. americana* were removed from the fruits and sliced into small pieces, shade-dried for 5 days and protected from environmental contaminations. After drying, they were grounded into fine powder with

Binatone blender (Model BLG-401). The powder (one kg) was soaked in 2 liters of distilled water for 24 h at 25$^{o}$C. Then, it was first filtered by passing it through a cotton plug and further filtered with filter paper (Whatman filter paper, #1) (Debas *et al;* 2011). The aqueous seed extract of *Persea americana* (ASEPA) was freeze-dried to a constant weight. The yield of dark-brown gummy extract was stored at 4°C in an amber-colored bottle until required for experiments.

**2.2.2. Fractionation of ASEPA**

To 100 g of freeze-dried ASEPA in a mortar, 100 ml of distilled water was added and mixed thoroughly. The mixture was then poured into a separating funnel and another 100 ml of distilled water was added and mixed by shaking vigorously. Then portions of 250 ml of n-hexane reagent were used to wash the extract exhaustively until the n-hexane layer became clear. The residue which was the water fraction was then removed from the separating funnel and the two fractions water fraction (WF) and n-hexane fraction (NF) - were dried to a constant weight using rotary evaporator at 40$^{o}$C (Debas *et al;* 2011)**.**

**2.2.3. Acute toxicity study ($LD_{50}$)**

Acute toxicity study of the extract was performed according to Miller and Tainter (1944). Six treatment groups (n=5) of male and female rats, starved for 24 h were administered orally with ASEPA at doses of 250, 500, 1000, 2000 and 5000 mg/kg using an orogastric tube. After treatment, the rats were fed with Top Feed and water and were observed for obvious signs of toxicity and mortality (that may result from the administration of the extract) at hourly intervals for 24 h, and thereafter, for a total of 14 days (Bruce, 1985).

**2.2.4. Phytochemical screening of the extract and fractions**

To determine the presence and concentrations of the secondary metabolites, qualitative and quantitative phytochemical screening of ASEPA and fractions were carried out according to the procedures outlined by Harborne (1998).

**2.2.5.1 Effects of ASEPA and fractions against stress-induced ulcer**

The animals were grouped into 8 (n=5; six treatment and two control groups) and treated orally for 14 days (Alpine & Woods, 1999) as follows:

Group 1: Distilled water 10 ml/kg (negative control)
Group 2: Cimetidine 150 mg /kg (positive control)
Group 3: ASEPA 250 mg/kg
Group 4: ASEPA 500 mg/kg
Group 5: n-hexane fraction (NF) 250 mg/kg
Group 6: NF 500 mg/kg
Group 7: WF 250 mg/kg
Group 8: WF 500 mg/kg

After 24 hours fasting, stress ulcers were induced by forced-swimming the rats in glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25 °C for 3 hrs. The animals were humanely sacrificed after 3 hrs period using excess anesthetic ether and the stomachs excised and opened along the greater curvature. The degree of ulceration was recorded with the aid of hand-held lens (10x) and the ulcer grading was recorded. Each stomach was given a severity rating (Ganguly & Bhatnagar, 1973). The degree of ulcer protection for each treatment group was calculated as a percentage with respect to the mean ulcer index of the negative control group.

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

**The Ulcer index (UI) and percentage protection were calculated as shown below**

UI = US x 10-1 / UN

UI= Ulcer Index; UN = total number of ulcers per animal; US = total number of severity score for each animal.

$$\% \text{ Protection/Healing (\% H)} = \frac{\text{Control mean ulcer index - Test mean ulcer index multiply by 100}}{\text{Control mean ulcer index}}$$

**2.2.6 Biochemical analysis**

**Estimation of catalase (CAT) and malondialdehyde (MDA)**

The rat stomachs obtained after stress-induced ulcer were used for biochemical analysis. The excised stomach from the control and treated rats were weighed and chilled in ice cold saline. A stomach homogenate prepared in KCl (1.15 % w/v) was further utilized for biochemical analysis. Estimation of MDA (Koracevic et al., 2001) and CAT (Clairborne et al., 1985) levels in the stomach homogenates were determined.

**Determination of catalase level**

Stomach homogenate (0.3 ml) was gently mixed with 1.2 ml of 0.2 M hydrogen peroxide and 3 ml of 0.01 M phosphate buffer (7.0) and allowed to stand at room temperature for 5 minutes. One milliliter of the reaction mixture was withdrawn and introduced into 2 ml of acetic acid reagent at 1 minute time interval. It was mixed and incubated in boiling water bath for 10 minutes, then cooled and the absorbance measured at 570 nm using spectrophotometer (Clairborne et al., 1985).

**Determination of Malondialdehyde (MDA) level**
Assessment of oxidative stress was measured by determining the lipid peroxidation end product of MDA, using Thiobarbituric acid (TBA) (Koracevic et al, 2001). Tissue homogenate (2 ml) obtained as stated above was combined with 2 ml of 1 % TBA in 20 % NaOH and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath and allowed to cool. The flocculate precipitate was removed by centrifugation at 1000 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 nm against a blank that contains all the reagents except the stomach homogenate. The MDA concentration of the sample was calculated using the extinction coefficient of 1.56x (105M-1cm-1).
**MDA concentration (M) =Abs/1.56 x 105**

**2.2.7. Histological study of the stomach.**
Histological studies of the stomach specimen from stress-induced ulcer model were performed as described by Drury & Wallington (1980). The harvested stomach tissues which were preserved in 10 % formaldehyde solution were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections (6 mm in thickness) of the tissues were prepared and stained with Haematoxylin and Eosin, and subsequently examined under the microscope. The photomicrographs of the stomach tissues were obtained.

**2.2.8. Statistical analysis**
The data were analyzed by statistical package for Social Sciences (SPSS version 20) using one way ANOVA, followed by post-hoc turkey's test for multiple comparisons. The data were expressed as Mean ± Standard error of mean (SEM). Graphical representation was done using Microsoft excel 2010. The differences between mean were considered significant at $p<0.05$.

## RESULTS

**3.1. Acute toxicity study ($LD_{50}$)**
Following 24 h of oral administration of up to 5000 mg/kg dose of ASEPA, no lethality or any other signs of acute toxicity were observed or noted. The LD50 was therefore greater than 5000 mg/kg.

**3.2. Phytochemical studies**
The qualitative phytochemical study of ASEPA and fractions revealed the presence of some bioactive substances which were mostly concentrated in ASEPA that its fractions (Table 1). ASEPA contained alkaloids, saponins, flavonoids, tannins, terpenoids, cardiac glycosides and reducing sugars while NF (n-hexane fraction) and WF (water fraction) contain all the bioactive substances in ASEPA at lesser concentration. The quantitative analysis of ASEPA showed that reducing sugars had the highest concentration (14.2 %) followed by saponins (12.25 %) while flavonoid was the least with concentration of 0.49 % (Table 1).

**Table 1: Result of the Phytochemical Screening.**

| Sample | Alkaloid | Saponin | Tannin | Flavonoid | Steroid | Terpenoid | Cadiac glycosides | Reducing sugar |
|---|---|---|---|---|---|---|---|---|
| **Constituents (%)** | **4.7** | **12.25** | **3.8** | **0.49** | **-** | **4.7** | **11.6** | **14.2** |
| **ASEPA** | + | +++ | + | + | _ | + | ++ | +++ |
| **NF** | + | ++ | + | + | _ | + | + | + |
| **WF** | + | + | + | + | _ | + | + | ++ |

Key: + = trace or mildly present, ++ = moderately present, +++ = abundantly present, - = absent

**3.3.1 Effects of ASEPA and fractions on stress-induced ulcer index**
Stress resulted in increased ulceration in the control groups. ASEPA and fractions exhibited a dose-dependent ulcer protective effects against stress-induced ulcer. At 250 mg/kg, only ASEPA and n-hexane fraction resulted in significant ($p<0.05$) reduction in ulcer index in the treated groups, whereas at 500 mg/kg, ASEPA and all the fractions caused significant ($p<0.05$) reduction in ulcer indexes in all the treated groups (Table 2). Furthermore, at 500 mg/kg, the ulcer indexes for NF treated group (2.01 ± 0.20) and ASEPA treated group (1.66 ± 0.19) were lower than that of Cimetidine (2.06 ± 0.05) (Table 2).

**3.3.2. Ulcer-healing effects of ASEPA and fractions on stress-induced ulcers**
ASEPA and fractions conferred a dose-dependent healing and reduction in ulcer index in the stress-induced ulcer. At 500 mg/kg ASEPA and fractions showed significant ($p<0.05$) reduction in ulcer index when compared to control. Also, at 500 mg/kg dose ASEPA (56%) and NF (46 %) exhibited higher percentage healing on stress ulcers than Cimetidine 150 mg/kg (44%) (Figure 2).

**Table 2: Ulcer Indexes (UI) for the Ulcer-Protective effects of ASEPA and fractions on stress-induced ulcer.**

| Treatment | Distilled water/ml/kg | Cimetidine/mg/kg | ASEPA/mg/kg | | NF/ mg/kg | | WF/ mg/kg | |
|---|---|---|---|---|---|---|---|---|
| | 10 | 150 | 250 | 500 | 250 | 500 | 250 | 500 |
| Ulcer index | 3.63 ± 0.16 | 2.06 ± 0.05* | 2.13 ± 0.51* | 1.66 ± 0.19* | 2.24± 0.59* | 2.01± 0.20* | 2.95± 0.48 | 2.47± 0.17* |

Values are represented as mean ± standard error of mean (n=5). *p<0.05: Statistically significantly different from the control group. Key: ASEPA=aqueous seed extract of *P. americana*, NF=n-hexane fraction, WF=water fraction.

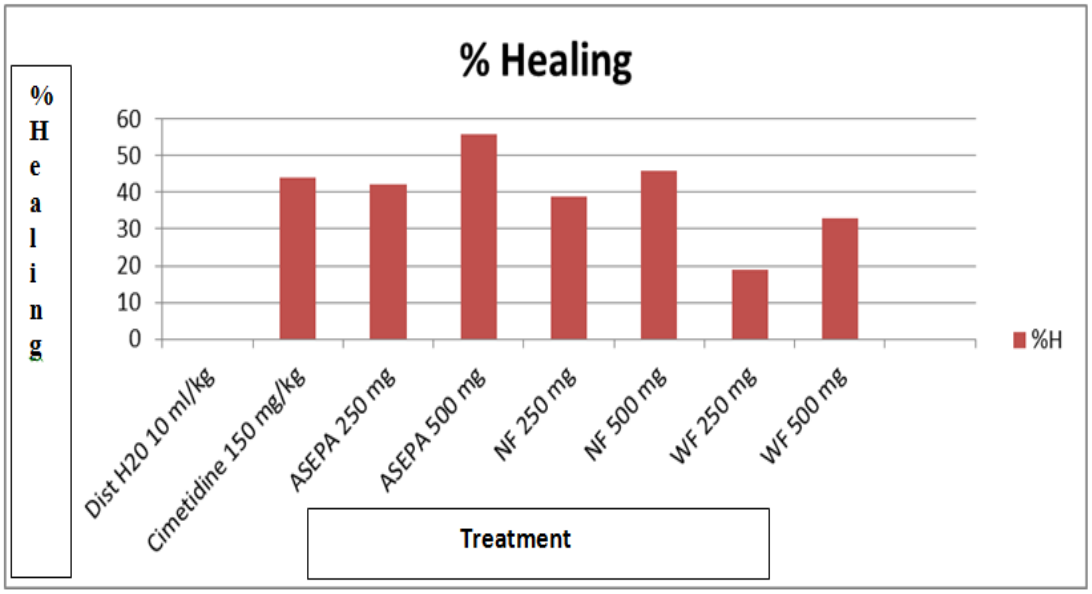


**Figure 1: Percentage healing (% H) on the stress induced ulcers.**

Key: ASEPA=aqueous seed extract of *P. americana*, NF=n-hexane fraction, WF=water fraction, H% = Percentage healing

### 3.4. Effects of ASEPA and fractions on biochemical parameters

***Effect on catalase (CAT) levels***

The extract and fractions exhibited a dose-dependent increase in CAT levels. At 500 mg/kg ASEPA, NF and WF exhibited a significant ($p<0.05$) increase in CAT levels when compared to the control (Table 3).

***The effect on malondialdehyde level (MDA).***

ASEPA and fraction showed a dose-dependent reduction on MDA levels. NF and WF (500 mg/kg) produced significant ($p<0.05$) reduction in MDA levels when compared to control (Table 3).

**Table 3: Effects of ASEPA and Fractions on Biochemical parameters.**

| Treatment | Dose mg/kg | CAT level (u/l) | MDA level (nm/mg) |
|---|---|---|---|
| Distilled water | 10 ml | 25.95 ± 2.69 | 1.02 ± 0.01 |
| Cimetidine | 150 | 70.20 ± 0.53* | 0.54 ± 0.03* |
| ASEPA | 250 | 28.45 ± 0.33 | 0.88 ± 0.01 |
| | 500 | 63.86 ± 0.49* | 0.60 ± 0.01* |
| NF | 250 | 29.42 ± 2.00 | 0.78 ± 0.01 |
| | 500 | 65.62 ± 2.54* | 0.52 ± 0.01* |
| WF | 250 | 26.82 ± 0.70 | 0.95 ± 0.03 |
| | 500 | 54.19 ± 1.89* | 0.87 ± 0.02 |

Values are represented as mean ± standard error of mean (n=5). P<0.05: Statistically significantly different from the control group. Key: ASEPA= aqueous seed extract of *P. americana*, NF = n-hexane fraction WF = water fraction, (MDA) = malondialdehyde, CAT = catalase

### 3.5 Histological effects of extract and fractions on rat stomach

The stomachs of the rats of stress-induced ulcer, revealed sub-mucosal inflammation in the negative control rats (Figure 2A). Two weeks of oral daily administration of ASEPA, NF and WF (500 mg/kg) before the induction of stress resulted in normal gastric mucosa (M), normal sub mucosa (SM)) and muscularis mucosa (MM) of their stomachs (Figures 2B, 2C, 2D respectively).

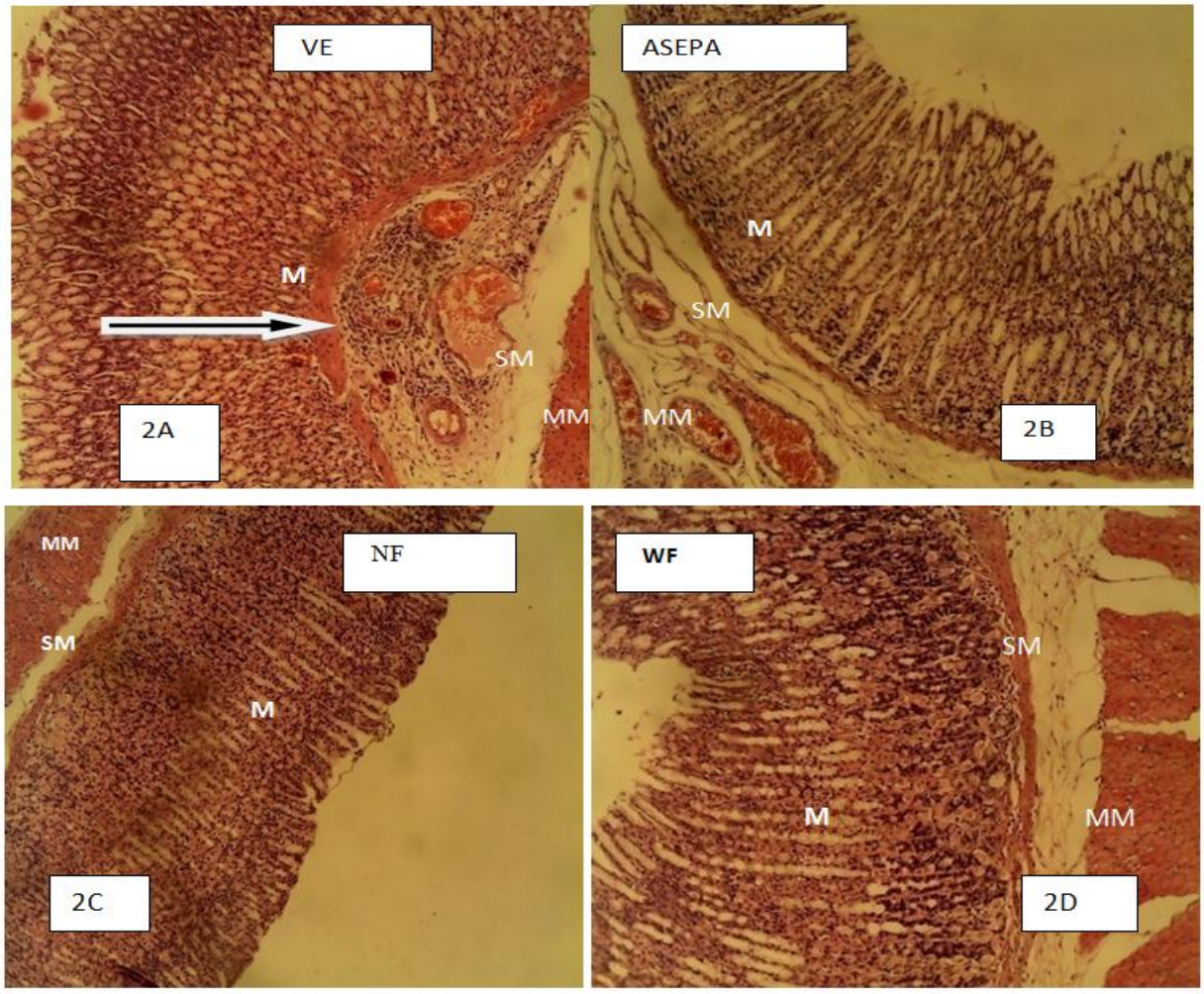


**Figure 2: Photomicrograph of stomach of rats from stress induced ulcer.**

Black arrow shows sub-mucosal inflammation in the negative control (2A**)** and apparently normal gastric mucosa (M), submucosa (SM)) and muscularis mucosa (MM) in ASEPA, NF and WF (Figures 2B, 2C and 2D, respectively)**.** Key: ASEPA = aqueous seed extract of *P. americana*, NF = n-hexane fraction, WF = water fraction

### 4.1 DISCUSSION

Various physical and psychological stressors cause gastric ulceration in humans. However, the etiology of gastric ulcer is assumed in most cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the endogenous defense mechanisms (MacGill, 2018). To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretions or to boost the mucosal defense mechanisms by increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis (Mohod & Bodhankar, 2011, Al-Radahe *et al*., 2013, Sharath *et al.,* 2015). Plants are some of the most attractive sources of new drugs and some have shown promise for the treatment of gastro-duodenal ulcer with minimum side effects (Alexandra *et al.,* 2018). In this study, oral administration of ASEPA was safe up to 5000 mg/kg. This attests to the relative safety of the extracts with very remote chances of acute toxicity. This finding agrees with the report by Ozolua *et al* (2009).

Phytochemical screening of ASEPA and fractions revealed the presence of bioactive compounds that have been previously associated with gastro protective activities. They include alkaloids, saponins, flavonoids, terpenoids, tannins, cardiac glycoside and reducing sugars. Idris *et al.* (2009) and Omodamiro *et al*, (2016) also reported the presence of these secondary metabolites in *P. americana* seed extract. Anti-bacterial and anti-fungal effects of the saponins (which were in abundance in the extract) have been documented (Lanzotti *et al.*, 2012). Saponins are believed to activate mucous membrane protective factors (Borrelli and Izzo, 2000). The ability of flavonoids to increase microcirculation in the gastric mucosa has also been reported (Jarial *et al* 2018).

Flavonoids act as free radical scavengers and are powerful antioxidants (Galleano *et al* 2010). Flavonoids and their derivatives act by decreasing lipid peroxidation via improving vascularity, slowing down the progress of

cell necrosis and strengthening of collagen fibers (Sharath, 2015). Anti-bacterial effects of flavonoids have also been demonstrated (Jarial *et al.,* 2018). Tannins on the other hand, are noted for their antioxidant effects (Kaisarun *et al.,* 2016) and astringent properties (McGee 2004). They render the outermost layer of the mucosa less permeable to chemical irritants due to their astringent properties. Tanins can also hasten the healing of wounds and inflamed mucous membrane due to their anti-inflammatory effects (Cheng *et al*., 2002) and their ability to form a protective layer over the exposed tissue, hence keeping the wound from being infected (Stéphane et al., 2004). Terpenoids have shown antibacterial activities and wound-healing activities (Mai *et al.,* 2003). They have also been reported to possess potent activity against gastric ulcers (Mitra *et al.,* 2014). Isolated pure forms of alkaloids and their synthetic derivatives are used as medicinal agents due to their analgesic, anti-inflammatory and anti-nociceptive properties (Noureddine *et al.,* 2015, Noureddine Bribi *et al.,* 2017). There was abundance of reducing sugars in this seed extract. Wang *et al.,* (2017) reported that reducing sugars significantly decreased malondialchehyde (MDA), decreased free radical activity and enhanced the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Therefore, the presence of these bioactive substances may be an indication of the ulcer-healing effects associated with ASEP extract. ASEPA and fractions caused significant decrease in MDA level. Increase in MDA levels in the stomach of the control rats suggests enhanced peroxidation leading to tissue damage and failure of the antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with ASEPA and fractions at 500 mg/kg significantly reversed these changes.

The antioxidant property of *P. americana* seeds has been reported in several studies (Ikpeme *et al.,* 2014, Owusu *et al*., 2015, Francisco *et al.,* 2018 and Nurdin *et al.,* 2018). Antioxidants accelerate wound healing (Yen *et al*., 2018) and compounds that act as antioxidants or activate the redox system are important for restoring gastric tissues (Hussain *et al.,* 2015). This may have contributed to the stress-ulcer healing effects of the extract and fractions. Likewise, the extract and fractions were able to preserve the catalase in the stomach cells of the treated rats presumably by enhancing antioxidant potentials of the gastric mucosa thereby preventing mucosal damage. The use of the antioxidant, Zinc carnosine, is a novel therapeutic option in the management of peptic ulcer disease (Hiraishi *et al*., 1999).

The photomicrographs of the stomachs (Figure 2) of stress-induced ulcer went further to confirm the ulcer healing effects of *P. americana* seeds on stress-induced ulcers. The epithelium of the stomachs from the negative control group revealed discontinuity in mucosal epithelium, inflammation of the mucosa and submucosa. However, treatment with ASEPA and fraction revealed relative healing of the ulcers in the stomachs of the rats. Hence, normal mucosa, submucosa and muscularis mucosa of their stomachs were observed post treatment.

At 500 mg/kg dose, ASEPA exhibited the highest ulcer healing effect which was higher than the standard drug, cimetidine. Melese *et al.,* (2011) had reported that the aqueous extract of *Plantago lanceolata* showed a better ulcer inhibition profile than ranitidine, another anti-ulcer drug. Such findings strengthen the search for novel agents by tapping the rich herbal drugs used in folk medicine. This could also be attributed to the presence of more of the active components in the crude extract (ASEPA). The *in vivo* stress-ulcer healing activity of the aqueous extract and fractions of *Persea americana* seeds, as was observed in this study, could therefore be attributed to the combined effects of its bioactive substances.

### 4.2. CONCLUSION

The extract of *Persea americana* seeds is relatively safe. The folkloric use of the extract in the management of stress-induced peptic ulcer disease may therefore be justified. The stress-ulcer healing effects could be related to the evidence of its protective activities on the stomach histology, which could in turn be attributed to the combined effects of the phytochemicals present in the seed. We, therefore, recommend further chronic toxicity and pharmacological studies on the seed extract of *P. americana* with an aim of developing a novel anti-ulcer agent.

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