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THE HEALING EFFECTS OF CRUDE FLAVONOID FROM *SCHWENKIA AMERICANA* LINN ON ASPIRIN-INDUCED GASTRIC ULCER IN RATS

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ABSTRACT
Schwenkia americana Linn plant has been used in traditional medicine for the treatment of different pathologies such as cough, headache, fever, sinusitis, including gastric ulcer. This study investigated the healing effect of crude flavonoid on aspirin-induced gastric ulcer in rats. Thirty-six rats were used for the study. Ulcer was induced in experimental animals in group II to VI with 500mg/kg body weight of aspirin for three days. Group I which served as negative control received normal saline and was not induced. The ulcerated animals were treated with different doses of the crude flavonoid, group III, IV and V were treated with 50, 100 and 200 mg/kg body weight of crude flavonoid respectively for14 days. Group II (ulcerated rats) which served as positive control received water. Group VI received omeprazole 20mg/kg body weight (standard anti-ulcer drug). Preliminary phytochemical screening of the extracted flavonoid confirmed the presence of flavonoid compound containing double bonds, with phenolic hydroxyl groups within its structure. There was a significant ($p<0.05$) increase in the activities of superoxide dismutase, catalase and reduced glutathione level in rats treated with different doses of crude flavonoid compared with ulcerated untreated rats (group II). The result shows that crude flavonoid from *Schwenkia american* improved the healing process of aspirin-induced ulcers in rats.

Keywords: *Schwenkia americana*, flavonoid, healing, gastric ulcer, aspirin.

INTRODUCTION

Schwenkia americana Linn belong to the family of Solanaceae, it is a slim vertical herb, woody at the base, grows up to 1m tall, it is generally found in waste places and common in tropical Africa and America including Nigeria (Asusheyi, *et al*., 2010). In Nigeria the Hausa know it as dandana and igbaleodan by the Yoruba (Hutchinson and Dalziel, 1974). *Schwenkia americana*is used for the treatment of rheumatic pains and swelling. The juice of the whole plant is applied as eye drops, and nose drops to treat conjunctivitis and sinusitis respectively, headaches and fungal infections (Iwu, 1993; Jimoh*et al*., 20011). *Schwenkia americana* has been used for the treatment of feverish conditions and general weakness of the body, cough medicine for children, analgesic and anti-inflammatory (Asusheyi*et al*., 2010; Jimoh *et al*., 2011).

Peptic ulcers are open sore in the lining of the stomach, oesophagus or duodenum (Kerdsakundee *et al.*, 2015). Peptic ulcer occur due to imbalance between aggressive factors such as hydrochloric acid, pepsin, refluxed bile, reactive oxygen species (ROS) and protective factors, for example prostaglandins (PGs) and mucosal blood flow (Jaiswal and Rao, 2016). The prevalence of gastric ulcers remains widespread. Factors that contribute to gastric damage are alcohol consumption, use of nonsteroidal anti-inflammatory drugs (NSAIDs), pepsin secretion, *Helicobacter pylori* infections and smoking. Most of the anti-ulcer drugs available for the treatment of ulcer usually have various side effects, therefore the need to find new antiulcerogenic compound(s) with potentially less or no side effects. Medicinal plants are well known source of new drugs for the treatment of gastric ulcer (Vijayakumar *et al.*, 2016). The use of medicinal plants for the prevention and treatment of different pathologies is in continuous growth worldwide (Mota *et al*., 2009).

Despite modern advances, adequate medication for the NSAID induced gastric damage remains intangible, therefore there is need to develop new drugs from plant origin, which do not have side effect, less expensive and easy to access by the rural people in the developing countries like Nigeria. Currently, gastric ulcer therapy is a challenge because most of the drugs used for the treatment of gastrointestinal diseases seems not to be very effective and with a severe side effect (Heibashy *et al.*, 2014). The medicinal properties of many plants are due to the presence of flavonoids, but they may also be influenced by other compounds. The study is therefore designed to evaluate the healing effects of crude flavonoids from *Shwenkia americana* Linn in ulcerogenic rats.

MATERIALS AND METHODS

Plant Materials

Schwenkia americana Linn plant was supplied by Mr Philip Iorliam from Benue State, Nigeria. The plant was authenticated at Herbarium Unit by Dr. Akinnibosun Henry, University of Benin, Edo State, Nigeria. Herbarium specimen with voucher number UBHs0253 was deposited at herbarium of University of Benin, Benin City, Nigeria.

Preparation of crude flavonoid

Pulverized plant material of *Schwenkia americana* Linn (1000g) was weighed and soaked in 80% methanol for forty eight hours after which it was filtered (Subramanian and Nagarajan, 1969). The filtrate obtained was concentrated in water bath at 45^{o}C and the aqueous fractions was defatted with hexane, and subjected to fractionation to obtain crude flavonoid in accordance with Woo *et al.* (1980).

Confirmatory test

The extracted crude flavonoid was subjected to various chemical tests in order to detect the presence of different phytoconstituents. The presence of flavonoid (Harborne, 1973); phenolic compounds, double bond, aldehyde and ketone groups (Harborne, 1984) were determined.

Test animals

A total number of 36 male rats weighing between 200-220g were used in the study. The rats were purchased from local breeder within University of Nigeria, Enugu, Nigeria. All animals were kept in the animal house in Biochemistry Department, University of Benin, Benin City, Nigeria, to acclimatize for one week. All experimental protocols involving animals were carried out in accordance with the standard procedure established by National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by Ethic Committee of the Faculty of Pharmacy, University of Benin, Benin city, Nigeria.

Experimental Design

Thirty-six male rats were used for the study. Ulcer was induced in experimental animals in group II- VI with 500mg/kg body weight of aspirin for three days as also performed by Mahmoud and El-Ghffar (2019). Group I which is the negative control received normal saline. The animals in group III to V were treated with different doses of the crude flavonoid (50,100 and 200 mg/kg body weight) for 14 days and group VI received Omeprazole 20mg/kg body weight (standard anti-ulcer drug). Group II (ulcerated rats) which served as positive control received water. At the end of the experiment, rats were fasted for 24 hours and sacrificed under anaesthesia**.** Their stomachs were opened along the greater curvature, flushed with normal saline, stretched out as much as possible by the use of metal pins on carbon paper on a ceiling board. Numbers of bleeding spots were examined using a magnifying glass.

Tissue Preparation

An abdominal incision was done on the rats. The excised clean stomachs were homogenized in phosphate buffered 50 mM pH (7.4) for estimation of superoxide dismutase (SOD), catalase (CAT), GPx activities, GSH, MDA levels and mucinase activity. The crude tissue homogenates were centrifuged at 3500 rpm for 15 minutes, and the resultant supernatant was used for the different estimations.

Antioxidant Activities

Glutathione peroxidase activity was estimated by the method of Nyman (1959). Catalase activity was determined by the method of Cohen *et al*. (1970). The activity of superoxide dismutase was determined according to the methods of Mistra and Fridovich (1972). Reduced GSH was determined by the method of Ellman (1959). Malondialdehyde was determined using the thiobarbituric acid assay (Ohkawa and Ohishi**,** 1979).

Ulcer Index

Ulcer index was calculated according to the method of Nwafor *et al.* (2000).

$$\text{Ulcer index} = \frac{\text{Total ulcer score in a group of rats}}{\text{Number of ulcerated animals in the same group}}$$

Healing index (percentage improvement) was calculated according to the method of Okabe *et al*. (1977).

$$\text{Healing index (\% improvement)} = \frac{\text{control (U.I)} - \text{Drug (U.I) X 100}}{\text{Control (U.I)}}$$

Determination of Mucus Secretion

The hexose content was determined by the method of Winzler (1958). Hexosamine was determined using the method of Dische and Borentreund (1950). The fucose concentration was estimated by the method described by Dische and Shettles (1948), and sialic acid concentration was determined by the method described by Warren (1959). Mucinase activity was determined by the method of Shiau and Chang (1983).

Estimation of Gastric Secretion Parameters

Free and total acidity was measured by method of Hawk (1947), the pH value was noted with the help of pH meter. The mucin concentration of the gastric juice was determined according to the method described by Winzler (1955). The method of Debnath *et al.* (1974) was used for the estimation of pepsin.

Acidity was calculated by using the formula:-

$$\frac{\text{Volume of NaOH X Normality of NaOH X 100}}{0.1}\ \text{mEq/L}$$

Statistical Analysis

The results were expressed as the Mean ± S.E.M. Statistical significance of difference in parameters was determined by one way analysis of variance (ANOVA) followed by Duncan's multiple range test using SPSS software version 22. $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Results on Table 1 shows that extracted flavonoid from *Schwenkia americana* Linn plant consist of flavonoid compounds containing double bonds, with phenolic hydroxyl groups within its structure.

Table 1: Chemical characterization of crude flavonoids (CF).

Test	Result	Colour (Extracted flavonoid)
Flavonoid test	+ve	Yellow precipitate
Double bond test	+ve	Brown
Aldehyde and ketone	-ve	Yellow precipitate
Phenol test	+ve	Green

Administration of extracted flavonoids and omeprazole showed a significant ($p<0.05$) increase in the level of individual mucopolysaccharide compared with untreated ulcerated rats (Table 2).The increased hexose and sialic acid in the gastric mucosa after treatment with the crude flavonoid may contribute towards its antiulcerogenic and healing effect by increasing the viscosity of the gastric mucus.

Table 2: Curative Effect of Crude Flavonoids from *Schwenkia americanan* on Mucus Secretion

Treatments	Hexose (µg/ml)	Sialic (µg/ml)	Fucose (µg/ml)	Hexosamine (µg/ml)
Negative control	72.62 ± 0.05^{a}	130.41±1.20^{a}	147.69±1.11^{a}	34.37±0.32^{a}
Positive control	31.81 ± 0.25^{d}	43.44±0.21^{d}	82.69±1.11^{d}	17.03±0.03^{d}
CF 50	56.52 ± 0.09^{c}	103.45±0.59^{c}	121.02±0.22^{c}	25.17±0.73^{c}
CF 100	62.33 ± 0.24^{b}	108.02±1.34^{b}	126.58±1.11^{b}	28.67±0.67^{b}
CF 200	70.61 ± 0.05^{a}	128.02±1.23^{a}	144.36±1.11^{a}	32.83±1.17^{a}
Omeprazole	71.19 ± 0.05^{a}	129.99±1.74^{a}	146.68±1.78^{a}	33.67±1.33^{a}

Values are given as means ± S.E.M from six rats in each group. Values not sharing a common superscript vertically differ significantly at $p< 0.05$. CF represents crude flavonoid.

Treatment with different doses of crude flavonoid for 14 days reduced the volume of gastric juice significantly at $p<0.05$ compared with untreated rats (group II). There was significant decrease in free and total acidity (acid output) in treated groups compared to untreated rats (group II).The results shows that administration of aspirin increases the acid output in gastric secretion leading to gastric mucosal lesion. Treatment with crude flavonoid for 14 days and omeprazole showed significant anti-ulcer effect by reducing the volume of gastric juice and acid output. Acid suppression is regarded as the main target in most pharmacological treatments designed to heal gastric ulcers (Lehmann *et al.*, 2003).

Table 3: Curative Effect of Crude Flavonoids from *Schwenkia americana* on Gastric Secretion

Treatments (mg/kg)	pH	Volume of Gastric Juice (mls)	Free Acidity (mEq/L))	Total Acidity(mEq/L)
Negative control	5.25±0.10^{a}	2.98±0.10^{c}	30.00±0.82^{c}	61.50±0.29^{f}
Positive control	3.35±0.12^{c}	4.85±0.06^{a}	57.50±0.29^{a}	113.00±0.41^{a}
CF 50	4.53±0.09^{b}	4.03±0.19^{b}	41.75±0.63^{b}	89.25±0.48^{b}
CF 100	4.63±0.10^{b}	3.75±0.10^{b}	41.50±0.65^{b}	86.75±0.25^{c}
CF 200	4.98±0.19^{a}	3.13±0.05^{bc}	30.25±0.48^{c}	66.75±0.48^{d}
Omeprazole	4.98±0.18^{c}	2.58±0.09^{d}	28.50±0.96^{c}	63.25±0.48^{e}

Values are given as means ± SEM from six rats in each group. Values not sharing a common superscript vertically differ significantly at $p< 0.05$. CF represents crude flavonoid.

The protein content of the gastric juice in the treated rats were low compared with untreated rats (group II), different doses of the extracted flavonoid significantly ($p< 0.05$) reduced the protein content of the gastric juice. Administration of the crude flavonoid and omeprazole significantly reduced the activities of pepsin and mucinase (Table 4). The mucin content of the gastric juice in treated rats was high compared with group II, treatment of rats with different doses of crude flavonoid significantly increased mucus secretion that may be responsible for the healing of gastric ulcer. Administration of crude flavonoids decreased gastric acid secretion, thus led to the healing of gastric ulcer. Studies also shows that

augmented production of the gastric mucin within the mucosal epithelium may further enhance the thickness of the mucus layer and increase the content of mucin within gastric juice (Singh and Guha, 2012). Aspirin reduce mucus secretion, its viscosity and mucus glycoproteins biosynthesis (Chatterjee and Bandyopadhyay, 2014). Mucus secreted by the mucous neck cells is a crucial factor in the protection of gastric mucosa from gastric damage, it is an important factor in the gastric mucus barrier which provides first line of defense against ulcerogenesis. The results from the study shows that there was increased acid secretion and decreased pH following treatment with aspirin, which may be possibly due to inhibition of prostaglandin synthesis mediated through COX-1, leading to ulceration. Treatment with flavonoid maintained the normal pH of the gastric internal milieu by decreasing the production of acid, this may also be due to trapping of bicarbonate ions by the mucosal layer.

In addition, the group treated with omeprazole had the highest value for calculated healing index followed by rats treated with 200 mg/kg body weight of crude flavonoid and 100 mg/kg body weight, the group treated with 50mg/kg body weight of crude flavonoid had the least ulcer healing index (Table 4).

Table 4: Curative Effect of Crude Flavonoids from *Schwenkia americana* on Protein, Pepsin and Mucin.

Treatments (mg/kg)	Protein (g/dl))	Mucin (mg hexose %)	Pepsin (µg/ml))	Ulcer index	% improvement
Negative control	0.26 ± 0.01^{c}	76.13 ± 1.20^{a}	1.53 ± 0.18^{d}	0.00 ± 0.00^{e}	-
Positive control	1.045 ± 0.04^{a}	37.60 ± 0.93^{d}	10.09 ± 0.24^{a}	5.95 ± 0.06^{a}	-
CF 50	0.40 ± 0.01^{b}	53.63 ± 1.36^{c}	5.31 ± 0.19^{b}	3.53 ± 0.05^{b}	59
CF 100	0.36 ± 0.01^{b}	55.15 ± 1.68^{c}	4.315 ± 0.11^{c}	2.03 ± 0.10^{c}	68
CF 200	0.27 ± 0.01^{c}	70.63 ± 1.79^{b}	2.22 ± 0.12^{d}	1.55 ± 0.10^{d}	75
Omeprazole	0.22 ± 0.01^{c}	70.28 ± 1.80^{b}	1.90 ± 0.15^{d}	1.55 ± 0.06^{d}	79

Values are given as means ± S.E.M from six rats in each group. Values not sharing a common superscript vertically differ significantly at $p< 0.05$. CF represents crude flavonoid.

The gastroprotective effect of flavonoids has been reported by El Souda *et al*. (2015). Free radical scavenging ability of flavonoids protect the gastrointestinal tract from gastric lesion (Bhattacharyya *et al.,* 2014). Crude flavonoids from *Schwenkia americana* produced a significant ($p<0.05$) and dose dependent increase in gastric tissue antioxidant activities and decreased MDA level in aspirin induced gastric ulcers as compared with the ulcerated rats. Post treatment of rats with different doses of crude flavonoid from *Schwenkia americana* orally for 14 days showed significantly improved preservation of antioxidant enzymes (SOD, CAT, GPX and reduced glutathione) and subsequent reduction in LPO.

Glutathione (GSH) act as a defence against oxidative stress as a scavenger of reactive oxygen species (ROS). GSH also plays an important role in maintaining cellular redox homeostasis, detoxifying reactive intermediates of noxious chemicals and scavenging lipid peroxides (Udeanu *et al*., 2011). Crude flavonoids significantly reduced lipid peroxidation in rat gastric mucosa when the treated groups were compared with group II (positive control). The anti-oxidant activity in gastric mucosal homogenates observed from decrease in lipid peroxidation (LPO) may be due to increase in SOD and CAT activities.

Mucinase is an enzyme present in the intestinal microflora, which hydrolyzes the protective mucins in the stomach. Mucin also protect exposed surface of the GI tract and other mucosal tissue against bacteria, viruses, and toxins (Yu *et al.,* 2016). Significant ($p<0.05$) increase in mucinase activity in the untreated group led to decreased protection of the underlying tissues, which resulted in ulceration. From the study, the ulcer healing property of the extracted crude flavonoid may be due to decrease in mucinase activity which may be assumed that the phytochemical used in the study has coating protective property on the gastric mucosa.

Table 5: Curative Effect of Crude Flavonoids from *Schwenkia americana* on Gastric Tissue Antioxidant and Mucinase Activities and MDA Levels.

Treatments (mg/kg)	SOD (Unit/mg of tissue)	GPX (U/mg protein)	Catalase (unit/min)	Mucinase (mg of glucose liberated/min/mg protein)	GSH (unit µmol/mg of tissue)	MDA (unit µg/mg of tissue)
Negative control	0.08 ± 0.00^{a}	0.35 ± 0.01^{a}	0.96 ± 0.06^{a}	4.69 ± 0.41^{d}	4.24 ± 0.26^{a}	3.85 ± 0.45^{c}
Positive control	0.05 ± 0.00^{c}	0.23 ± 0.01^{c}	0.17 ± 0.06^{c}	15.78 ± 1.30^{a}	1.46 ± 0.05^{d}	8.89 ± 0.66^{a}
CF 50	0.07 ± 0.00^{b}	0.3 ± 0.01^{b}	0.52 ± 0.06^{b}	7.11 ± 0.24^{b}	2.9 ± 0.65^{bc}	4.53 ± 0.20^{b}
CF 100	0.07 ± 0.00^{b}	0.3 ± 0.01^{b}	0.57 ± 0.07^{b}	6.41 ± 0.24^{c}	2.96 ± 0.65^{bc}	4.50 ± 0.20^{b}
CF 200	0.08 ± 0.00^{a}	0.34 ± 0.00^{a}	0.79 ± 0.06^{a}	5.30 ± 0.33^{d}	4.09 ± 0.39^{a}	4.29 ± 0.54^{bc}
Omeprazole	0.08 ± 0.00^{a}	0.34 ± 0.01^{a}	0.9 ± 0.00^{a}	5.29 ± 0.41^{d}	4.06 ± 0.43^{a}	4.29 ± 0.22^{bc}

Values are given as means ± S.E.M from six rats in each group. Values not sharing a common superscript vertically differ significantly at $p< 0.05$. CF represents crude flavonoid.

Histological results of normal rats (Plate1) shows that the general gastric morphology was maintained. Rats treated with graded doses of crude flavonoid and omeprazole for 14 days after induction of ulcer ameliorated the effect of aspirin-induced gastric damage.

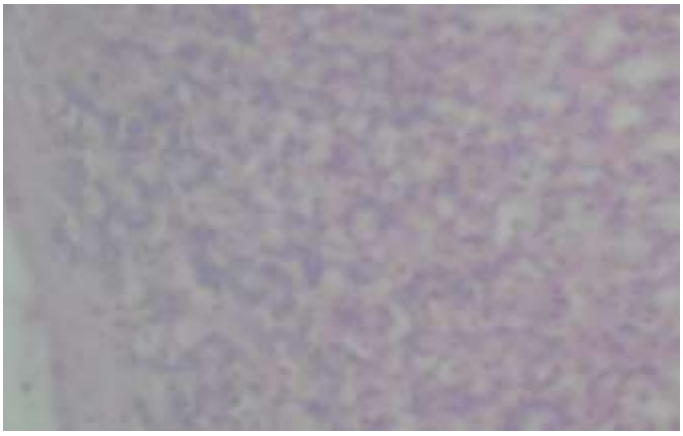

Plate 1: Photomicrograph of negative control: showing normal mucosa.

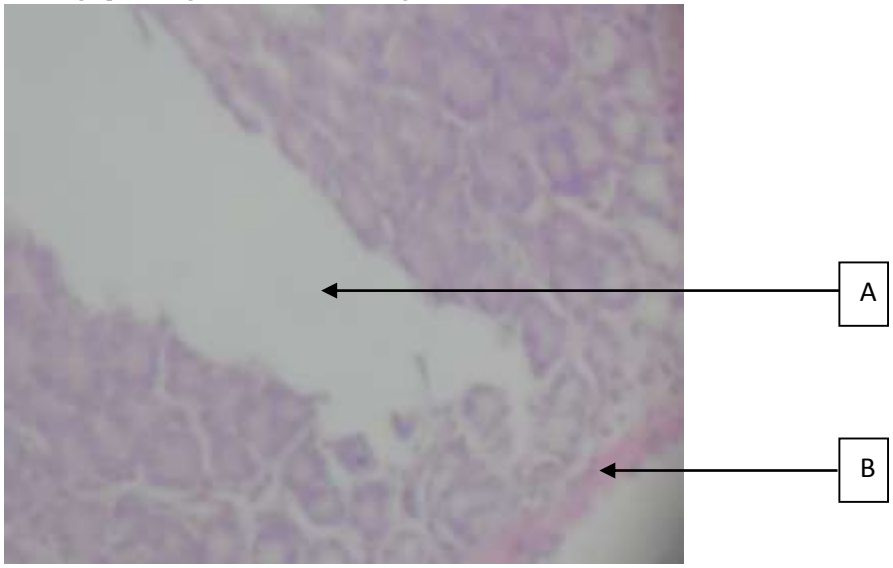

Plate 2: Photomicrograph of rat stomach given aspirin showing A, linear ulcers and B, muscularis mucosa (H&E x 40)

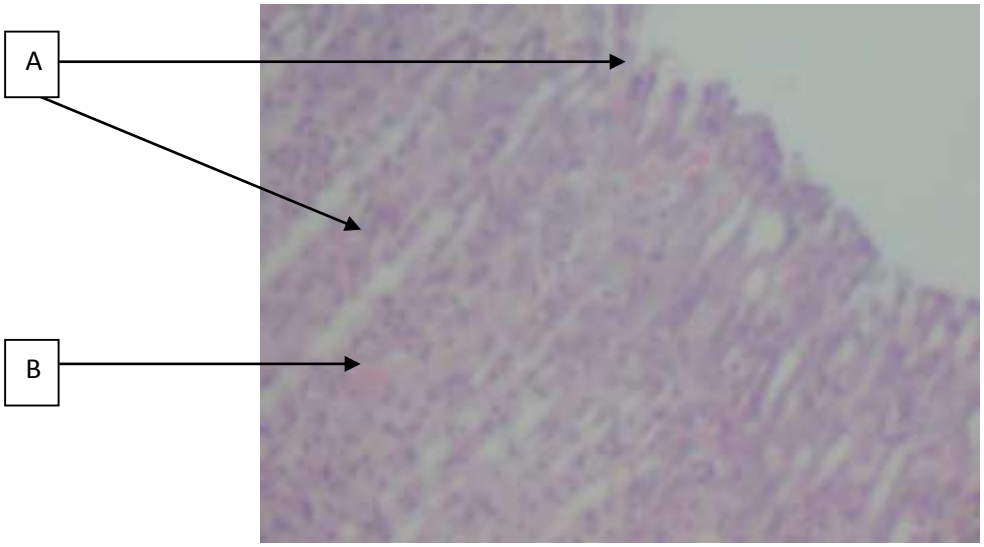

Plate 3: Photomicrograph of rat stomach induced and treated with Omeprazole for 14 days showing A, fairly normal mucosa and B, mild mucosal congestion (H&E x 40)

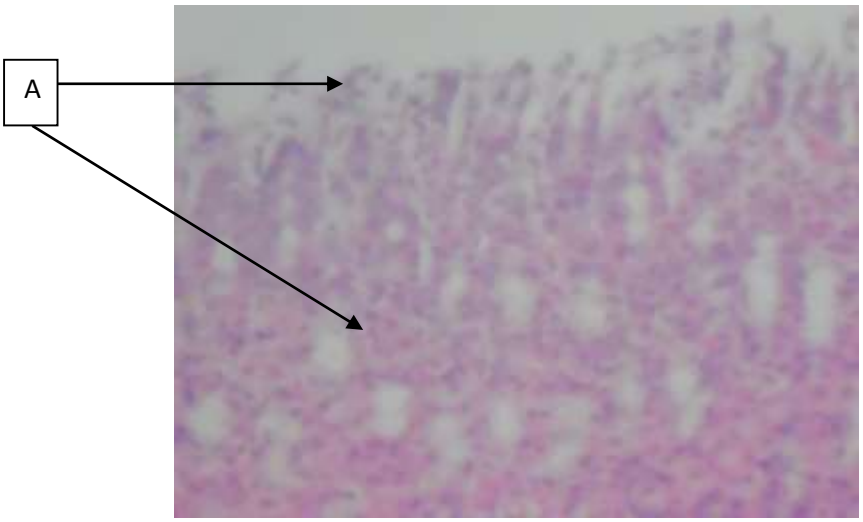

Plate 4: Photomicrograph of rat stomach induced and given 50mg of crude flavonoids showing A, normal mucosa (H&E x 40)

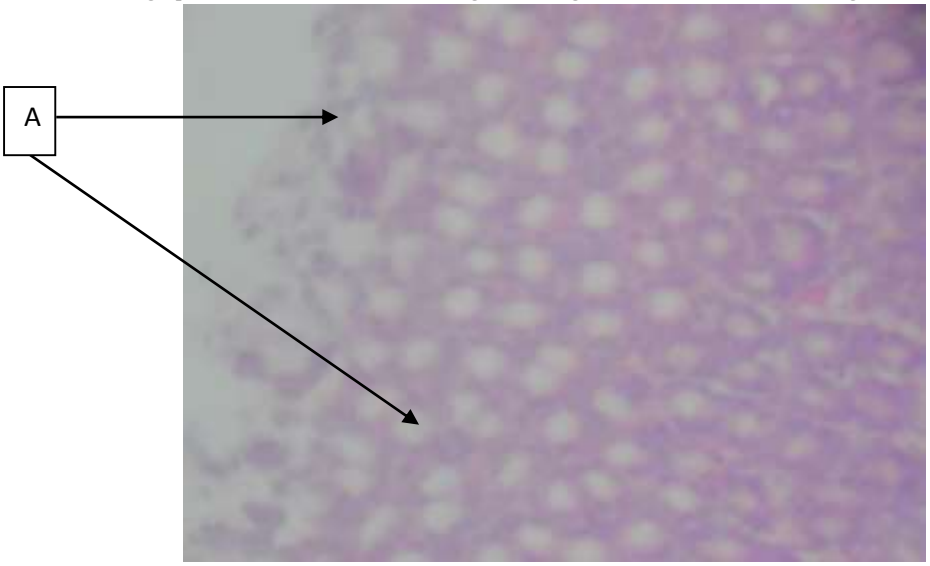

Plate 5: Photomicrograph of rat stomach induced and given 100mg of crude flavonoid showing A, normal mucosa (H&E x 40)

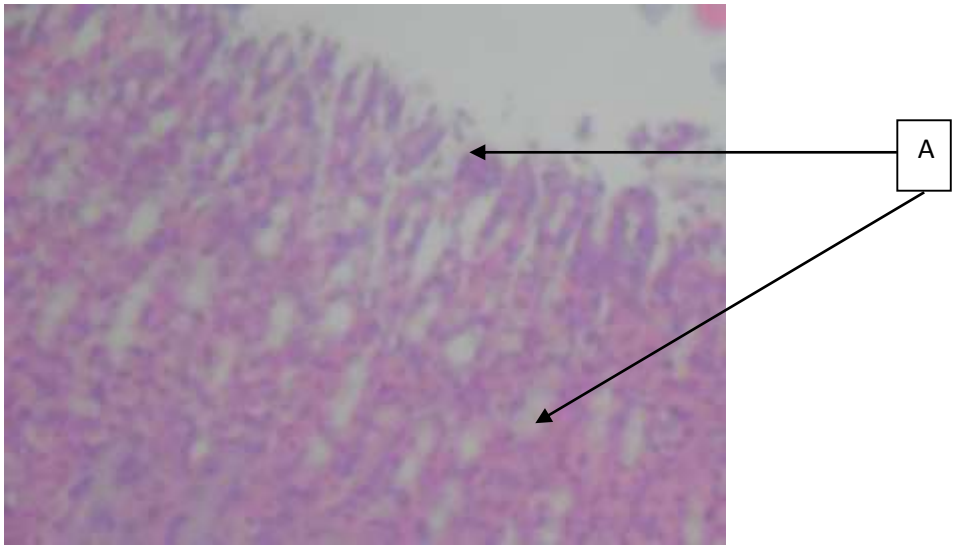

Plate 6: Photomicrograph of rat stomach induced and given 200mg of crude flavonoids showing A, normal mucosa (H&E x 40).

CONCLUSION

This research has focused on the healing effect of crude flavonoid on aspirin-induced gastric ulcer in rats. The study has shown that crude flavonoid from *Schwenkia americana* Linn may ameliorate gastric ulcers. Therefore, crude flavonoid from *Schwenkia americana* Linn has a therapeutic potential for the treatment of gastric ulcers.

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