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Biochemical evaluation of ulcer curative effect of ethanolic and aqueous extracts of *Schwenkia americana* Linn on experimental rats

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Abstract
Gastric ulcers are common and serious diseases in the world. The study assessed the effect of the ethanolic and aqueous extract of *Schwenkia americana* Linn on aspirin-induced gastric ulcers in Wistar rats. 200 mg/kg body weight of aspirin was used to produce ulcers in rats for 3 days. After checking for the presence of ulcers, the ulcerogenic rats were divided in 4 groups of 6 rats. 2 groups were treated with ethanolic or aqueous extract, while a group received Omeprazole in 28 days and the last group was the ulcer untreated control. There were significant increases in free and total acidity, serum alkaline phosphatase and ulcer score in aspirin treated group compared to the control group. Aspirin induced distinct gastric histological changes compared with the control group, while the plant extracts ameliorated these changes which showed that the plant extracts has a regenerative potentials as an antiulcer agent.

Keywords: *Schwenkia americana* Linn, Wistar rats, Ethanolic extract, Aqueous extract, Gastric Ulcer.

1. Introduction
Gastric ulcers are common and serious diseases, which have been a major cause of morbidity and mortality for more than a century [1]. The pathophysiology of gastric ulcer disease is based on an imbalance between aggressive and protective factors in the stomach [2]. Gastric ulcers are caused by psychological and physiological stress, excessive acid, free radicals, alcohol use, side effect of NSAIDs, Helicobacter infection or free radicals or a combination of two or more of these causes [3].
Currently the NSAIDs such as aspirin and indomethacin are preferred drugs for various diseases like arthritis, inflammation, and cardiovascular protection. However, they cause gastrointestinal complications such as ulcers and erosions. NSAIDs also generates oxygen free radicals that are known to play a role in the pathogenesis of mucosal injury [4]. Aspirin exerts its effect through inhibition of cyclooxygenase the enzyme responsible for the synthesis of prostaglandin. The most adverse effect of aspirin is irritation of the gastric mucosa. Various synthetic anti-ulcer drugs are presently available and some of these like misoprostol esomeprazole, omeprazole, lansoprazole, pantoprazole are specifically used to cure the NSAID induced gastric ulcer. However, each of these drugs confers simpler to severe side effects, warranting a search for non-toxic and inexpensive antiulcer medication [5, 6].
Schwenkia americana are found in several parts of the world including Central and South America, East and southern Africa, Nigeria [7]. In Nigeria it is known by the Hausa as "dandana" and igbale odan by the Yoruba [8]. It has been used for the treatment of various ailments. The plant sap is used for the treatment of headache, sinusitis and conjunctivitis [9]. It has been reported to be effective in the treatment of rheumatic pains and swellings, feverish conditions and general weakness of the body, cough medicine for children and chest complaints [10]. Information on the effect of the plant on ulcers are lacking. This study is thus aimed at assessing the effect of the ethanolic and aqueous extract of *Schwenkia americana* Linn on aspirin-induced gastric ulcers in Wistar rats.

2. Materials and Methods
2.1 Plant Material
Schwenkia americana was collected from a farm land located in Lessel, Benue State, Nigeria.

Plant authentication was done at the Herbarium, University of Benin, Benin City. A voucher specimen of the plant was deposited in the Herbarium, University of Benin, Nigeria.

2.2 Preparation of plant Extract

Ethanolic Extract

Schwenkia americana Linn was dried and powdered. The dried powder was soaked in absolute ethanol for 72 hours. The obtained ethanolic extract was filtered, concentrated with rotary evaporator and freeze dried at the Centre for Energy and Environment, of the University of Benin, Nigeria.

2.3 Aqueous Extract

The aqueous extract of *Schwenkia americana* Linn was dried and powdered. The dried powder was soaked in distilled water for 48 hours. The obtained aqueous extract was filtered, concentrated with rotary evaporator and freeze dried also at the Centre for Energy and Environment, of the University of Benin, Nigeria.

2.4 Phytochemical Analysis

Phytochemical analysis of the plant extracts was carried out using methods described by Sofowora; Evans and Treatise [11, 12].

2.5 Determination of Total Phenolic, Tannin, Saponin, Alkaloid and Flavonoids Content in the plant extracts.

Saponin and alkaloid composition was determined using gravimetric method [13, 14]. Tannin by spectrophotometric method [12]. Total phenolic and flavonoid content of the extracts was determined by using the method of Marinova [15].

2.6 Acute Toxicity (LD_{50}) of Plants

The method described by Aliu and Nwude was followed in the determination of LD_{50} [16]. Different doses of ethanolic and aqueous extracts of *Schwenkia americana* Linn were administered orally to eight (8) groups of rats weighing 170-240 g, consisting of three rats in a group. The rats were subjected to 24 hours fasting before the extract was administered. Food was withheld for further 3-4 hours after administration. The extracts (aqueous and ethanolic) were suspended in distilled water and administered in doses of 400, 800, 1600 and 3200 mg/kg body weight orally. The ninth group serves as the control and received only distilled water. The rats were observed for signs of toxicity and mortality. Animals were observed individually at least once during the first 30 minutes, with special attention during the first 4 hours after dosing. The animals were placed under observation for 24 hours.

2.7 Experimental Design

48 male Wistar rats weighing 100-120 g were used for this study. The rats were purchased from Anatomy Department, University of Benin, Nigeria. The animals were allowed to acclimatize to the laboratory conditions for two weeks. The animals had free access to tap water and normal pellet diet (NPD) until they were assigned to individual groups. The rats (48) were divided into 2 broad groups of animals; the first group consists of 12 rats and served as control, while the other group of 36 rats was given aspirin (200 mg/kg body weight) for 3 days to produce ulcers according to Javed [17]. At the end of final dose of aspirin, five rats were randomly selected and sacrificed to check for ulceration through histology, ulcer indices and biochemical assay. The ulcerogenic rats were again sub-divided into 4 groups of 6 rats each. Two sub-groups were treated with ethanolic or aqueous extract of the plant; while one sub-group received Omeprazole (standard anti-ulcer drug) and the last sub-group was the ulcer untreated control. After confirmation of ulceration and groupings, the treatment with the plant extracts or Omeprazole lasted for another 28 days (4 weeks) at the end of which period the animals were fasted for 24 hours and then sacrificed. This work was carried out in accordance with the guidelines of Faculty of Life Sciences at University of Benin for animal use

2.8 Collection of Gastric Juice

The stomach was excised carefully by keeping the oesophagus closed and opened along the greater curvature and the luminal contents were removed. The gastric contents were collected and centrifuged at 1000 rpm for 10 min; the volume of the supernatant was expressed as ml/100 gram body weight and the centrifuged samples were decanted and analyzed for gastric volume, free acidity and total acidity. The mucosa was flushed with saline and observed in gastric lesions using a dissecting microscope.

2.9 Scoring method of ulceration was carried out according to [18]

0	Normal gray coloured stomach
0.5	Pink to red coloration stomach
1	Spot ulcer
1.5	Hemorrhagic streak
2	Number of ulcer less than 5
3	Number of ulcer more than or equal 5
4	Ulcer with bleeding
5	Perforation of gastric or duodenal wall

Ulcer index was calculated according to the method of Singh [19].

Ulcer index = (severity of ulcer + No. of ulcer)

Healing index (percentage improvement) was calculated according to the method of Okabe [20].

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

2.10 Biochemical assays

Alkaline Phosphatase (ALP) enzyme activity was assayed using a colorimetric method [21] and total protein was assayed by Tietz method [22]. Total and free acidity was estimated by the method of Kore [23]. 1 ml of gastric juice were pipetted into a 100ml conical flask, 2 or 3 drops of topfer's reagent was added and titrated with 0.01N sodium hydroxide until all traces of red colour disappears and the colour of the solution turns yellowish orange. The volume NaOH was noted, which corresponds to the free acidity. Furthermore, 2 or 3 drops of phenolphthalein solution were added and titration was continued until a definite pink colour appears. The total volume of NaOH was noted, which corresponds to the total acidity.

2.11 Statistical analysis
The values are reported as means ± S.E.M. Statistical difference was determined using ANOVA and differences in the means were tested by Duncan's multiple range tests [24].

3. Result
The result of acute oral toxicity (LD_{50}) of *Schwenkia americana* Linn of ethanolic and aqueous extract was found to be greater than 3200 mg/kg body weight and no mortality was recorded in any group of experimental rats.

The preliminary phytochemical screening of the plant extracts revealed the presence of flavonoid, phenols, saponin, alkaloid, glycosides, tannin, phytosterols, terpenoid and steroids (Table 1). Quantitatively however, the aqueous extract of the plant has more flavonoid, phenols, saponin, alkaloid and tannins compared with the ethanolic extract (Table 2).

Data for confirmation of the presence of ulceration is presented in Table 3. Aspirin increased the free acidity, total acidity, and number of ulcers compared with the untreated (water treated).

When the rats that were confirmed with ulcer were given the ethanolic or aqueous extract of the plant, some of these indices of ulcer measured (free and total acidity, and gastric volume) were significant ($p<0.05$) decreased to levels comparable with either the control or the Omeprazole treated animals (Table 4) and the severity, number of ulcer present, and ulcer index were reduced with the plant extract (Table 5). There was a significant increase ($p<0.05$) in severity of ulcer and number of ulcer in the ulcer control when compared with the treated group.

The data on the effect of the plant extracts on gastric protein and ALP activity are presented in Table 6. Statistical analysis of the data reveals a significant drop in gastric protein level in ulcer compared with normal control. This trend was reversed with the plant extract and the commercial drug used in this study. On the other hand, ALP level increased in ulcer and was reduced with the plant extract and the drug to levels comparable with the normal control.

Table 1: Phytochemical Constituents Present in various extracts of *Schwenkia americana* Linn.

Chemical constituent extract	Aqueous extract	ethanolic
Alkaloid	+	+
Glycosides	-	-
Tannin	+	+
Phenols	+	+
Flavonoids	+-	+
Saponin	+	+
Phytosterols	-	-
Terpenoid	+	+
Steroids	+	+
Phytosteroids	+	+

Table 2: Quantitative phytochemical Analysis of Aqueous and Ethanolic Extract of *schwenkia americana* Linn Quantitative phytochemical Analysis of Aqueous and Ethanolic Extract of *schwenkia americana* Linn

Chemical constituent) (mg/100 g)	Aqueous extract Mean±SD	Ethanolic extract Mean±SD
Alkaloid	2.07±0.11	0.22±0.03
Tannin	3.87±0.11	2.99±0.02
Phenols	0.16±0.03	0.10±0.01
Flavonoids	9.58±0.05	4.85±0.02
Saponin	1.270.06	0.15±0.03

Table 3: Result for confirmation of ulceration

Treatment	Control (water only)	Aspirin (200mg/body weight)
Free acidity	10.40	20.60
Total acidity	18.50	48.80
ALP	1135.74	1169.14
No. of ulcer	-	46
Severity of ulcer	-	24
Ulcer index	-	70

Table 4: Effect of *Schwenkia americana* Linn ethanolic and aqueous extract on gastric volume, free and total acidity (N=6) of male albino rats

Treatment	Normal control	Ulcer control	SAEE	SAAE	Omeprazole
Volume of gastric juice (ml)	1.38±0.58	2.50±0.13	1.70±0.10	1.50±0.29	1.50±0.06
Free acidity(a\mEq/l)	12.00±0.58	17.00±0.71	13.00±0.71	12.75±0.50	13.75±0.2
Total acidity(mEq/l)	26.00±0.81	45.50±0.50	35.50±0.65	35. 50±0.65	37.00±1.08

Table 5: Effect of schwenkia Americana Linn ethanolic and aqueous on ulcer incidence (N=4)

Treatment	Normal control	Ulcer control	SAEE	SAAE	Omeprazole
Severity of ulcer (S.U)	-	15	7	5	5
No. of ulcer (N.U)	-	36	14	12	10
Ulcer index(S.U+N.U)	-	51	21	16	15
Ulcer index (Mean±SEM)	0.00±0.00	12±0.85	4.75±0.48	4.25±0.25	3.75±0.25
Healing index (% improvement)	-	-	59	69	71

Table 6: Effect of *Schwenkia americana* Linn ethanolic and aqueous extract on serum ALP and Gastric Protein (N=6)

Treatment	Normal control	Ulcer control	SAEE	SAAE	Omeprazole
Gastric protein(g/dl)	0.53±0.04	0.12±0.00	0.52±0.02	0.30±0.08	0.38±0.05
Serum ALP (U/l)	863.30±49.54	1221.30±20.70	759.00±35.06	800.40±36.61	662.40±25.83

4. Discussion

Plants that contain phytochemicals present such as the ones present in this plant most invariably has medicinal value. Also earlier report showing the presence of flavonoid, phenols, saponin, alkaloid Alkaloid, Glycosides, Tannin, Phytosterols, Terpenoid and Steroids in this plant [7, 10]. They had shown the antimicrobial usefulness of this plant. Our study shows that the total amount of phenol, flavonoid, saponin, alkaloid and tannin in the plant extract are more in aqueous extract than the ethanolic extract (Table 2). Phytochemicals especially flavonoids and tannin are strongly linked to anti ulcer benefits. Flavonoids are not only free radical scavengers, but are important increasing mucosal prostaglandin content and can decrease histamine secretion from mast cells by the inhibition of histidine decarboxylase [25]. These effects of flavonoids improve mucus secretion. Tannins prevent ulcer development by improving vasoconstricting effects [26]. It is therefore not surprising when we observed that the plant may be able to ameliorate aspirin induced gastric ulcer.

The result of acute oral toxicity (LD50) of *Schwenkia americana* Linn of ethanolic and aqueous extracts show that the plant extracts are tolerated by rats and would not suffer any adverse effect even when administered at 3200 mg/kg body weight.

The increase in gastric volume of the untreated aspirin group is due to increased production of hydrochloric acid as it is evident from the total acidity of the gastric juice [27]. Significant reduction in total and free acidity, the gastric volume and ulcer index (Table 5) suggest that acid inhibition accelerates ulcer healing [28]. The decrease in free acidity, total acidity and gastric volume in the present study may be attributed to ulcer healing effect of ethanol and aqueous extracts of *schwenkia americana*. Anti-inflammatory drugs like NSAIDS have long been known to cause gastric ulceration in animals and man, which is attributed to the mucosal prostaglandin synthesis deficiency caused by inhibition of key enzyme cyclooxygenase [29]. Endogenous prostaglandins are involved in the regulation of mucus and bicarbonate secretion by the gastric and duodenal epithelium, mucosal blood flow, epithelial cell proliferation, epithelial restitution and mucosal immunocyte function [30]. The ability of an NSAID to cause gastric damage correlates well with its ability to suppress gastric prostaglandin synthesis [31]. This is in an agreement with the result obtained, the decrease in gastric volume, free acidity and total acidity may be attributed to ulcer healing effect of *schwenkia americana* ethanolic and aqueous extracts by maintaining mucosa. Measurement of the ulcer indices of the rats in the study demonstrated that treatment with the plant extracts led to faster ulcer healing compared to the untreated rats. This shows that the extracts have ameliorating effect. The decrease in the protein content of the gastric mucosa in the ulcerogenic group may be due to damage in the gastric mucosa which results, in the leakage of protein into the gastric juice. Treatment with plant extracts increased the mucosal protein which indicates its ability to enhance cell proliferation and stimulates the growth of the gastric mucosa.

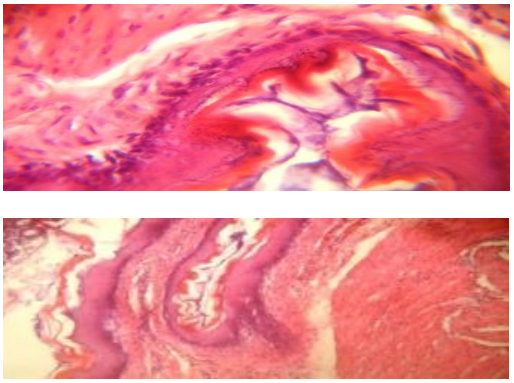
Analysis of the results showed that aspirin produced extensive increase ALP activity in rat serum. The increase of this enzyme activity seems to be a general property of all chemicals which are known to provoke severe ulcer. The release of alkaline phosphatase has been suggested to play a role in tissue necrosis associated with various models of gastrointestinal ulceration [32]. Thus the increased activity of this enzyme in the serum found in an ulcer control group is not surprising. It significantly evident from the results of the present investigation that treatment with aqueous and ethanolic extracts of *schwenkia americana* significantly decreased serum ALP activity at a dose of 200 mg/kg body weight compared to the untreated rats implying mucosal reconstitution and healing of gastric ulcer

5. Conclusion

Our results showed a regenerative potential against aspirin-induced gastric erosions in animal models by *schwenkia americana* aqueous and ethanolic extracts at the dosage of 200 mg/kg/ body weight. The bio-components of the plant extracts may be responsible for the ameliorating effect by increasing the antioxidants in stomach and also the ability to inhibit acid secretion through reduction of gastric volume, free and total acidity and thus leads to the improvement in the ulcer healing process.

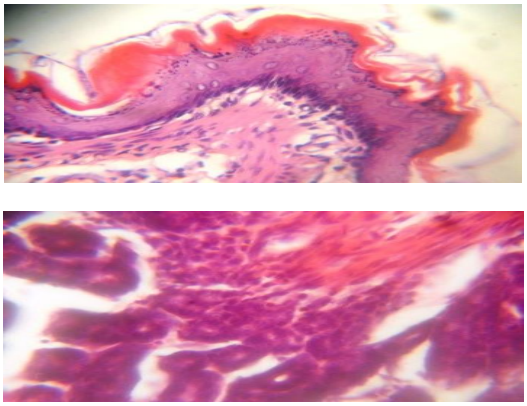
6. Histological Studies

Result of the first stage of the study



X40 and X10

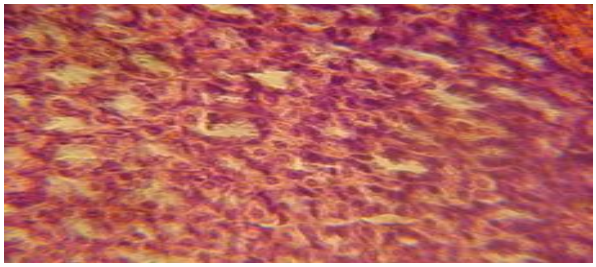
Control group: Feature of the stomach appear distinct and well demonstrated.



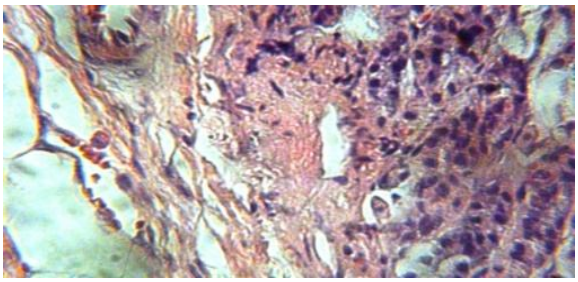
X40 and X10

Aspirin group: Feature of the stomach appears quite distinct with evidence of mild dysphonia and enlarged nuclei at higher magnification.

Histological result after treating with the plant extracts

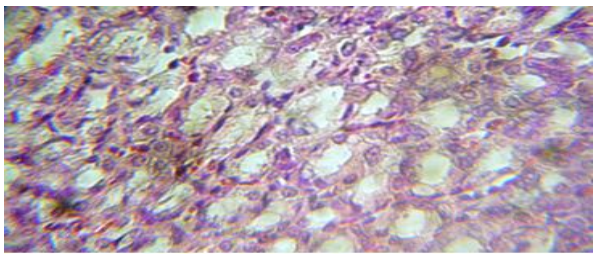


Control rats showed normal gastric mucosa with normal glands, Nucleus appears distinct at.

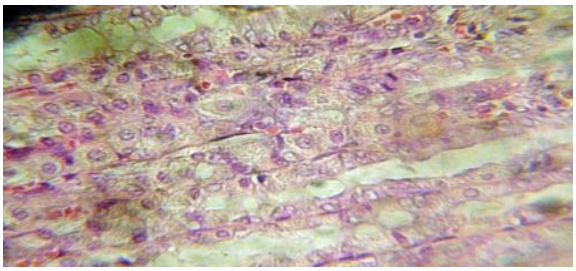


Ulcer group

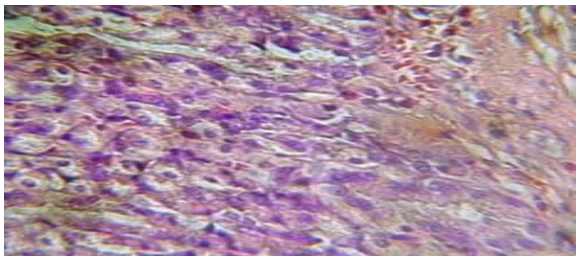
Ulcer group showed mucosal ulceration with sub mucosal edema, inflammation and polymorphonuclear infiltrate at the ulcer site as well as in the oedematous submucosa at lower magnification.



Aqueous extract treated group: disruption to the surface epithelium and edema of the submucosal layer with leucocytes infiltration are quite reduced



Ethanolic extract treated group: disruption to the surface epithelium and edema of the submucosal layer with visible leucocytes infiltration are reduced.



Treated with standard drug (omeprazole).

Omeprazole treated group: There is a mild disruption to the surface epithelium and mild infiltration and haemorrhages to the submucosal layer.

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