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The Antioxidant Effect and Safety Assessment of Ethanolic and Aqueous Extracts of *Schwenkia americana* Linn on Aspirin Induced Gastric Ulcer in Rats.

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ABSTRACT

The present study was conducted to investigate the antioxidant effect of ethanolic and aqueous extract of *schwenkia americana* Linn on aspirin-induced gastric ulcers in wistar rats and its safety. Gastric ulceration was induced in wistar rats with 200mg/kg body weight of aspirin for 3 days and was treated with 200mg/kg body weight of ethanolic and aqueous extracts and 20mg/kg body weight of omeprazole for four weeks. Antioxidants parameters and safety assessments involving, organ weight to body weight ratio, lipid peroxidation, kidney function status, liver enzyme activities and heart biomarkers were performed. The extract had no significant effect ($p>0.05$) on organ weight to body ratio, serum levels of Alanine aminotransferase, Aspartate aminotransferase and Alkaline phosphatase (ALP) decreased significantly ($p \leq 0.05$) in extracts and omeprazole treated group compared to ulcer untreated rats. There were no statistical difference ($p > 0.05$) in the value obtained for serum bicarbonate, creatinine, lactate dehydrogenase and heart protein between the normal control and treated groups. The study showed that the extracts corrected the architectural distortions caused by gastric ulceration in the experimental animals and the plant extracts has no adverse effect on liver, kidney and heart function in rats.

Keywords: *Schwenkia Americana* Linn, gastric ulcer, antioxidants, wistar rats.

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INTRODUCTION

Peptic ulcers are open sores in mucous lining of the stomach and duodenum. They persist as a function of the acid or peptic activity in gastric juice. The pathology of gastric ulcers was first described in 1835 [1]. Peptic ulcer is one of the most common, chronic gastrointestinal disorders in modern era. Now it has become a common global health problem affecting a large number of people worldwide and also still a major cause of morbidity and mortality [2]. Peptic ulcer disease can be characterized by inflamed lesions or excavations of the mucosa and tissue that protect the gastrointestinal tract. Damage of mucus membrane which normally protects the esophagus, stomach and duodenum from gastric acid and pepsin causes peptic ulcer [3]. The pathophysiology of this gastro-intestinal disorder is viewed as an imbalance between mucosal defensive factors such as bicarbonate, prostaglandin, nitric oxide, peptides, growth factors and injurious factors like acid, pepsin [4] Ulcer, being a global disease that can cause morbidity and mortality, it has attracted much attention to address this global health problem. Several medicines have been used to cure this disease. The modern approach is to control gastric ulceration by inhibiting gastric acid secretion, to increase gastroprotection, to increase epithelial cell proliferation or to stop apoptosis for effective ulcer healing process [5]. Though different classes of drugs are used in the treatment of peptic ulcer but most of the drugs exhibits serious side effects like arrhythmias, impotence, gynaecomastia, arthralgia, hypergastrinemia and haemopoeitic changes [6]. Owing to these, interest is focused on the use of phyto-constituents as drug therapy to treat major ailments, which has proved to be clinically effective and less relatively toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of peptic ulcer [6]. *Schwenkia americana* is a perennial plant that belongs to the family Solanacae. It is found in several parts of the world including Central and South America, East and southern Africa, Nigeria [7-8]. 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 is reported for effective treatment of rheumatic pains and swellings, feverish conditions and general weakness of the body, cough medicine for children and chest complaints [10]. The study is aimed at assessing the antioxidant effect of ethanolic and aqueous extract of *Schwenkia americana* Linn and its safety on aspirin-induced gastric ulcers in Wistar rats.

MATERIALS AND METHODS

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.

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.

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.

Animalsand Treatment

Thirty male wistar rats weighing 100-120g were used for this study. The rats were purchased from Anatomy Department, University of Benin, Nigeria. All animals were housed in wooden cages and each cage contained 6 rats. The rats were housed for two weeks after their arrival to the animal house for acclimatization. The animals had free access to tap water and normal pellet diet (NPD) until they were assigned to individual groups. This work was carried out in accordance with the guidelines of the Faculty of Life Science at University of Benin for animal use.

Group-1 Control
Group-2 Ulcerogenic rats untreated

Group-3 Treated with 200mg/kg body weight of ethanolic extract
Group-4 Treated with 200mg/kg body weight of aqueous extract
Group-3 Treated with omeprazole (standard antiulcer drug)

Aspirin-induced gastric ulcer.

Ulcer was induced in experimental animals with 200mg/kg body weight of aspirin for three days according to Khalil *et al*. [11]. At the end of the last dose of aspirin five rats were randomly selected and sacrificed to check for ulceration through histology, ulcer indices and biochemical assay.

Tissue Sample Preparation

At the end of the experiment, rats were fasted for 24 hours, an abdominal incision was done on the rats. The tissues collected were homogenized in phosphate buffer 50 mM pH (7.4) for estimation of SOD, CAT activities, GSH and MDA level. The crude tissue homogenate was centrifuged at 3500 rpm for 15 minutes, and the resultant supernatant was used for the different estimations.

Blood Sample Preparation

At the end of the experiment, rats were fasted for 24 hours. Blood was collected by cardiac puncture from the rats at fasting state after being anesthetized with chloroform. The blood samples were collected in plain tubes, allowed to coagulate at room temperature and centrifuged at 3500 rpm for 15 minutes at room temperature for separation of serum. The clear, non-haemolysed supernatant was separated using clean dry Pasteur pipette and stored at -20°C. Serum was used for subsequent biochemical measurements as follows: kidney function status, liver enzyme activities related to its function and heart biomarkers.

Alanine Aminotransferase (ALT)

Principle: The method is that of Reitman and Frankel [12]. Alanine Aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

Aspartate Aminotransferase (AST)

Principle: The method is that of Reitman and Frankel [12]. AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Alkaline Phosphatase (ALP)

Principle: Uses a colorimetric method; this is an optimized standard method according to the recommendations of the Deutsche Gesellschaft fur KlinischeChemie [13].

Lactate Dehydrogenase

Principle: Kinetic determination of lactate dehydrogenase according to Klin [14], where pyruvate is converted to L-Lactate by LDH in the presence of NADH.

Creatinine (Colorimetric Method)

Principle: Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration. Creatinine was determined by colorimetric method as described by Bartels and Bohmer [15].

Estimation of Malondialdehyde (MDA)

Principle: This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid producing a pink chromogen, which can be measured spectrophotometrically at 532 nm [16].

Catalase Assay

Principle: The method of Cohen *et al.* [17] was adopted. Catalase is present in nearly all animal cells, plants and bacteria and acts to prevent accumulation of noxious H_2O_2 which is converted to O_2 and H_2O.

Superoxide Dismutase (SOD) Assay

Principle: The levels of SOD activity was determined by the method of Mistra and Fridovich [18] Adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome whose concentration can be determined at 420 nm using a spectrophotometer. The enzyme SOD inhibits the auto-oxidation of adrenaline by catalysing the breakdown of superoxide anions. The degree of inhibition is thus a reflection of the activity of SOD and is determined at one unit of the enzyme activity.

Estimation of Reduced Glutathione (Ellman's Method)

Principle: The principle is based on the reduction of 5,5dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm [19]

Total Protein

Principle: the method is that of Tietz [20]. Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex and its absorbance can be measured at 540nm.

Bicarbonate

Principle: phosphoenol pyruvate carboxylase (PEPC) catalyzes the reaction between phosphoenol pyruvate and carbon dioxide (bicarbonate) to form oxalacetate and phosphate ion. Oxalacetate to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to (NAD; the reaction is catalysed by malate dehydrogenase (MDA). This results in a decrease in absorbance at 340nm that is directly proportional to CO2 concentration in the sample [21].

Albumin

Principle: the measurement of serum albumin is based on its quantitative binding to the indicator 3,3',5',5',-tetrabromo-m cresol albumin-BCG-complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample [22].

Statistical analysis

The experimental results was expressed as the Mean ± S.E.M. Statistical significance of difference in parameters amongst groups was determined by One way analysis of variance followed by Duncan's multiple range test. $P<0.05$ was considered to be significant.

RESULTS

The data on gastric tissue antioxidants, lipid peroxidation and total protein are presented on table 1. Statistical analysis of the data reveals a significant decrease ($p<0.05$) in MDA when treated groups were compared with the ulcer control and non-significant when the treated groups were compared with the normal control. There was significant increase ($p<0.05$) in SOD, catalase and GSH activities in extracts and standard treated group when compared with ulcer control and non-significant when compared with normal control. Significant increase ($p<0.05$) in serum total protein when omeprazole and ethanolic extract treated group was compared with ulcer control and non-significant when compared with normal control.

There was a significant decrease in serum AST, ALT and ALP when ulcer control was compared with treated groups and non statistical difference ($p >0.05$) when compared with the normal control (Table 2). The values of total serum protein and liver AST and ALT obtained showed no statistical significant difference (p

>0.05) between the treated groups and normal control. Albumin in the serum was non- significant when ethanolic treated group was compared with the normal control and significant decrease when omeprazole and aqueous extract treated group was compared with the normal control (Table 3).

Table 1: Gastric tissue antioxidants and lipid peroxidation

Treatment	Normal control	Ulcer control	SAEE	SAAE	Omeprazole
SOD Activity (unit/ml)	7.65±0.40	5.83±0.32	7.02±0.55	8.09±0.34	7.26±0.43
MDA (10^{-5} unit/ml)	1.23±0.08	1.91±0.04	1.57±0.03	1.61±0.04	1.52±0.07
Catalase (unit/min)	0.19±0.01	0.16±0.01	0.18±0.01	0.18±0.02	0.19±0.00
Reduced Glutathione (mg/l)	0.49±0.06	0.40±0.02	0.47±0.05	0.53±0.05	0.65±0.10
Protein (g/dl)	0.53±0.03	0.13±0.01	0.53±0.02	0.53±0.03	0.54±0.03

Table 2: Effect of *schwenkia americana* Linn ethanolic and aqueous extract on Liver function profiles (N=6)

Treatment	Normal control	Ulcer control	SAEE	SAAE	Omeprazole
Serum AST (U/l)	35.25±2.69	39.50.00±1.56	28.00±1.22	29.25±2.25	33.50±1.50
Serum ALP (U/l)	863.30±49.54	1221.30±20.70	759.00±35.06	800.40±36.61	662.40±25.83
Serum ALT (U/l)	20.00±1.08	26.50±1.19	21.75±0.75	20.50±0.50	20.25±0.75

Table 3: Effect of *schwenkia americana* Linn ethanolic and aqueous extract on Liver function profiles (N=6)

Treatment	Normal control	Ulcer control	SAEE	SAAE	Omeprazole
Serum Albumin (g/dl)	3.08±0.08	3.01±0.29	2.73±0.32	1.39±0.03	1.96±0.14
Liver AST (U/l)	9.14±0.22	7.72±0.20	9.51±0.34	8.54±0.20	8.65±0.24
Liver ALT (U/l)	41.00±1.15	38.75±1.84	44.25±4.75	41.00±2.97	39.75±3.47
Serum Total protein (g/dl)	7.65±0.53	4.61±0.54	6.98±0.34	7.79±0.34	7.98±0.37

Table 4: Organ weights to body weight ratios

Treatment	Normal control	Ulcer control	SAEE	SAAE	Omeprazole
Liver	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.28±0.00
Kidney	0.005±0.00	0.005±0.00	0.006±0.00	0.005±0.00	0.005±0.00
Heart	0.003±0.00	0.003±0.00	0.003±0.00	0.003±0.00	0.003±0.00

Table 5: Effect of *schwenkia americana* Linn ethanolic and aqueous extract on kidney function profiles (N=6)

Treatment	Normal control	Ulcer control	SAEE	SAAE	Omeprazole
Serum Bicarbonate (mmol/L)	31.50±1.32	35.50±2.06	31.50±1.32	31.25±2.66	31.00±2.80
Serum creatinine (µmol/l)	2.54±0.09	2.54±0.09	2.36±0.24	2.73±0.24	2.91±0.28

Table 6: Effect of *schwenkia americana* Linn ethanol and aqueous extract on Heart function profiles (N=6)

Treatment	Normal control	Ulcer control	SAEE	SAAE	Omeprazole
Serum Lactate dehydrogenase(U/L)	480.90±34.81	536.00±41.11	440.83±40.08	400.75±46.27	480.90±24.62
Heart Protein(g/dl)	1.55±0.11	1.24±0.20	1.50±0.11	1.42±0.08	1.71±0.21

There were no statistical significant difference (p >0.05) in organ weights to body ratios of the liver, kidney and heart between the treated groups and the normal control (Table 4).

The data for kidney function profiles are presented in Table 5, there were no statistical significant difference (p >0.05) in the value obtained for serum bicarbonate and creatinine between the normal control and treated groups and no statistical significant difference (p >0.05) in the value obtained for serum Lactate dehydrogenase and heart protein between the normal control and extract treated groups as shown in Table 6.

DISCUSSION

Lipid peroxidation can be used as an index for measuring the damage that occurs in membranes of tissue as a result of free radical generation [23]. In the present study, oral administration of aspirin significantly increased the LPO level. Significant elevation of LPO level observed in aspirin treated experimental-group is possibly due to the generation of free radicals via auto-oxidation or through metal ion or superoxide catalyzed oxidation process. The significant decrease in MDA in the treated groups is due to the scavenging activity of the extract to reduce lipid peroxidation.

The activity of CAT was found to be decreased in aspirin treated rats. The inhibition of CAT activity during aspirin induced ulcer may be due to the increased generation of reactive free radicals, which can create an oxidative stress in the cells. SOD and CAT enzymes are highly specific in their catalytic mode of actions and it decreases the gastric mucosal damaging effect of aspirin [24]. Salim *et al*. [25] also investigated the influence of free radical scavengers on the healing of gastric and duodenal ulcers resistant to therapy and found that antioxidative therapy stimulates the healing of therapy resistant ulcers. The increase in SOD and CAT activities in the treated groups may also be attributed to anti-ulcer properties of the plant extracts. The decreased level of GSH in aspirin- treated experimental group indicates that there was an increased generation of free radicals and the GSH was depleted during the process of combating oxidative stress [26]. This has probably been possible either from the low level of reactive oxygen species (ROS) production or through a rapid dissolution of ROS that has further been strengthened by the elevated activities of important antioxidant defence enzymes CAT and SOD, studied in this experiment.

AST, ALT and ALP are the most sensitive tests employed in the diagnosis of ulcer disease [27]. Analysis of the results showed that aspirin produced extensive increase in AST, ALT, and ALP activities in rat serum. A significant reduction in ALP level implies antiulcerogenic property [28]. The increase of these enzymes activities 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 [29]. The increased activity of this enzyme in the serum found in ulcer control group is in agreement with the above statement. It is evident from the results of the present investigation that treatment with aqueous and ethanolic extracts of *schwenkia americana* significantly decreased serum AST, ALT, and ALP activities at a dose of 200 mg/kg body weight compared to the untreated rats implying mucosal reconstitution and healing of gastric ulcer. The synthetic ability of the liver was maintained judging from total protein and albumin values. There was also no hepatocellular damage as revealed by liver AST and ALT values.

Organ weights are widely accepted in the evaluation of test agent-associated toxicities [30]. The organ weight to body weight ratios of all treated groups was not statistical significant difference (p >0.05) with normal control. This indicates that the extract had no effect on weight of the organs and therefore would possibly have no organ related toxicity while in ulcer control group it signifies that the state of the disease might not be enough to change the weight of the organs in rats.

The result of serum bicarbonate, creatinine, lactate dehydrogenase and heart protein between the normal control and extract treated groups were not statistical difference (p >0.05). Also from the present study, it is established that treatment of ulcer with *schwenkia americana* ethanolic and aqueous extract at a dose of 200 mg/kg body weight did not affect the kidney and heart.

CONCLUSION

This study demonstrates that ethanolic and aqueous extract of *schwenkia americana* Linn corrected the architectural distortions caused by gastric ulceration by reducing the production of ROS due to the presence of antioxidants compound in the plant extracts. It was established that treatment with *schwenkia american*Linn ethanolic and aqueous extract has no adverse effect on liver, kidney and heart function in rats. Therefore, we can say from the present study that *schwenkia americana* Linn plant is safe at 200mg/kg body weight for the treatment of gastric ulcer in experimental animals.

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