Antioxidant Properties and Protective Effect of Aqueous Anti-Ulcer Drug (AQAUD) against Aspirin-induced Gastric Ulcers in Albino Rats

B. A. Mba[1*], C. S. Alisi[1] and A. C. Ene[1]

[1]*Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim: The aim of this study is to evaluate the antioxidant properties and protective effects of aqueous anti-ulcer drug (AQAUD) against aspirin-induced gastric ulcer in albino rats.
Methods: In this study, 30 male albino rats were divided into 5 groups of 6 each. Rats in group I served as normal control and received food and water. Animals in group II received food and water in addition to aspirin (400 mg/kg.b.wt) orally on the 14th day. Rats in groups III, IV and V received "AQAUD" (250 mg/kg.b.wt), (500 mg/kg.b.wt) and Omeprazole (20 mg/kg.b.wt) respectively for 14 days and aspirin (400 mg/kg.b.wt) orally on the 14th day. *In vitro* antioxidant property of "AQAUD" was assessed by its nitric oxide and hydroxyl radicals scavenging properties. The ulcer protective effect of "AQAUD" was assessed by determining the free and total acidity, ulcer index and % protection in the stomach content. The antioxidant potential in animals was evaluated by determining the concentrations of malondialdehyde and reduced glutathione. Superoxide dismutase and catalase activities were assayed in the stomach homogenates to further assess antioxidant potential. Total phenolics and flavonoid compounds were quantified to know the antioxidant content. Histopathological assessment of the gastric mucosa was used to assess the protective potentials of "AQAUD". Data were analyzed using Statistical Package for Social Science (SPSS) version 21.

_____

*Corresponding author: E-mail: blessingamarachi470@gmail.com;

Results: The results revealed that free acidity and ulcer indexes were significantly ($p<0.05$) reduced by "AQAUD". There was a significant decrease in SOD activity of the stomach homogenates when compared to the aspirin group, with values for "AQAUD" 250 mg/kg.b.wt and "AQAUD" 500 mg/kg b.wt as $37.24\pm5.39ux10^{-2}$/mg protein and $23.64\pm2.91ux10^{-2}$/mg protein respectively. Result of acute toxicity testing showed that "AQAUD" is generally safe up to 5000 mg/kg b.wt.
Conclusion: The results revealed that treatment with aspirin caused loss of gland architecture with erosion of epithelial layer, but AQAUD treatment ameliorated the effect of aspirin administration. The study revealed that "AQAUD" has considerable antioxidant potentials and can effectively protect against gastric ulcers.

1. INTRODUCTION

Peptic ulcer is a sharp wound with loss of mucus membrane of mucosa of the stomach, duodenum or any other part of the gastrointestinal tract exposed to pepsin or acidic contents of the gastric juice [1]. Because of its worldwide distribution, peptic ulcer has continued to be a subject of numerous studies, both experimentally and in clinical practices. In this regards, peptic ulcer occupies a place secondary to carcinoma in the field of gastroenterology [2]. A peptic ulcer in the stomach is called gastric ulcer. Gastric ulcers are mostly caused by the imbalance between the mucosal offensive factors (gastric acid, pepsin, ROS, NSAIDs and *H. pylori*) and mucosal defensive factors (gastric mucus and bicarbonate secretion, prostaglandin, nitric oxide, gastric blood flow and innate resistance of mucosal cells) in the upper gastrointestinal tract [3,4].These offensive factors have been shown to be involved in the pathogenesis of gastric ulcer.

The stomach is a major site for large production of Reactive Oxygen Species (ROS), much higher than in other tissues or biological fluids [5]. The generation of these ROS plays a major role in the development of multiple pathologies, such as gastritis, peptic ulcerations or gastric adenocarcinoma [6]. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The major feature of an antioxidant is its ability to scavenge the free radicals due to their redox hydrogen donators and singlet oxygen quenchers [7,8].

There are drugs available for treating gastric ulcer [9]. These drugs have brought about remarkable changes in gastric ulcer therapy but the efficacy of these drugs is still debatable. Reports on clinical studies of these drugs show that there are cases of relapses, adverse drug effects and danger of drug interactions during ulcer therapy [10,11]. Hence, the search for an ideal anti – ulcer drug continues and has also been extended to medicinal plants in search for new and novel molecules, which could give better protection, lesser toxicity, decrease the incidence of relapse and at low cost.

This study seeks to discover if Aqueous Anti-Ulcer Drug (AQAUD) herbal formulation can be used as an alternative anti-ulcer drug with minimal drug to drug interactions, less adverse effects and providing a permanent cure for gastric ulcers. The herbal formulation Aqueous Anti-Ulcer Drug (AQUAD) is one of the widely consumed medicinal formulations in the eastern parts of Nigeria. It is used locally in treating gastric ulcers. The medicament consists of aqueous root extract of *Strophantus hispidus* and *Hippocratea welwitschii,* juice from *Citrus aurantifolia* and gbogbonise (A Yoruba herbal formulation).

Strophanthus hispidus is a medicinal plant widely used in traditional African medicine in the treatment of rheumatic afflictions, ulcers, conjunctivitis, leprosy and skin diseases [12]. *Strophanthus hispidus* belongs to the family Apocynaceae and is called "osisikagiri" in Igbo language. It is found all over Africa and in the eastern, western and southern parts of Nigeria [12].

Hippocratea welwitshcii is a medicinal plant used in Igbo (Nigeria) ethnomedicine. It is locally known as ovuru mgbede in Igbo language and its root is used locally in the treatment of gastro-intestinal problems. It is a climber and belongs to the family of celastrales. Phytochemical screening on the root extract indicated the presence of triterpenes, glycosides and saponins.

Citrus aurantifolia (Lime) is a citrus fruit, which is typically round, green in colour, about 3-6cm in

diameter and containing acidic juice vesicles [13]. It belongs to the family of Rutaceae and it is called Oroma nkirisi in Igbo language.

Limes are an excellent source of vitamin C, and are often used to accent the flavours of foods and beverages. Also limes contain eight different liminoids, which are compounds that promote the activity of an enzyme in the liver called glutathione-s-trasnferase or GST, according to Hatherill, [14]. This liver enzyme detoxifies a variety of cancer-causing chemicals, turning them into harmless chemicals that are then removed from the body through the urine.

The Yoruba formulation called Gbogbonise Aporo Epa-ijebu is a well-known medicinal formulation and it is widely consumed in the rural areas of Nigeria. It is used in treating many disease conditions including stomach troubles, rheumatism, fresh cut wound, convulsion, cough, malaria fever, chest pain, worm, pile, chicken pox, measles, dysentery, tooth ache, snake bites and scorpion stings. It is composed of both plants and animal parts [15].

The recipes include juice from *Citrus aurantifolia, Citrus aurantum* and fruits of *Aframonium melegueta* (Grains of paradise, melegueta pepper or alligator pepper) as well as animal parts including a type of rat (*Rattus norvegium),* snake heads and scorpions. This study is therefore aimed at evaluating the antioxidant properties and protective effects of aqueous anti-ulcer drug (AQAUD) against aspirin-induced gastric ulcer in albino rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Quercetin dihydrate (Sigma-Aldrich Mo USA), 2-Thiobarbituric acid (Sigma-Aldrich Mo USA), Sodium dodecyl Sulphate (Fluka-Chemie, Switzerland), Acetic acid, Trichloroacetic acid, Sodium dihydrogen orthophosphate, Disodium hydrogen orthophosphate, Potassium Chloride, Glutathione ((Fluka-Chemie, Switzerland), Protein test kit, All other chemicals and reagents used were of analytical grade.

2.2 Aqueous Anti-Ulcer Drug (AQAUD)

The Aqueous Anti-Ulcer Drug (AQAUD) was obtained as prepared by a traditional medicine practitioner in Owerri, Imo state. Briefly, the herbal medicament was prepared by mixing clean and sorted portion of the plants' roots (100 g of *Hippocratea welwitschii* + 50 g of S*trophantus hispidus*) in a pot. These were boiled with potable water (1.0 litre) for 5 hours.

The aqueous extract obtained was cooled to room temperature (25±2°C). Thereafter, the *C*itrus *aurantifolia* fruit juice (20 mL) and Gbogbonise (10 g) were added to the extract, thoroughly mixed and volume made up to 1 litre to obtain the AQAUD. The drug was collected in plastic containers and preserved in the freezer for further studies.

2.3 Aspirin and Omeprazole

Aspirin (used to induce gastric ulcer) and omeprazole (the standard anti-ulcer drug) were purchased from Bright Way Pharmacy at No 25 Mbaise road, Owerri, Imo state. They were kept hygienically for the studies.

2.4 Qualitative Phytochemical Screening

Qualitative phytochemical screening of AQAUD was done according to the methods of AOAC [16]. Total phenolics and flavonoid content were determined quantitatively according to the methods of Wattashinghe and Shaidi, [17].

2.5 Free Radical Scavenging Determination

Nitric oxide radical scavenging ability was determined according to the methods of Marcocci, et al. [18] with minor modification by Alisi and Onyeze, [19]. Hydroxyl radical scavenging ability was determined according to Halliwell, et al. [20].

2.6 Animals Treatment

Thirty male albino rats (*Rattus norvegicus*) weighing between 200 and 250 g and fifteen Swiss albino mice, weighing between 20 and 30g were used for the study. The animals were purchased from an animal breeding station (Animal friend) at No. 92 Royce road Owerri, Imo state. The animals were maintained under standard laboratory conditions of light, temperature (21±2°C) and relative humidity (55±5%) in a stainless steel cages with free access to standard animal feed (Pelletised, Vital finisher) and tap potable water. Albino mice were used for the determination of the acute toxicity of the "AQAUD" and the albino rats were used for the ulcero-protective study. The animals were

acclimatized in the laboratory for two weeks before the commencement of the study.

2.7 Grouping of Animals for Acute Toxicity Study

The acute toxicity study was carried out in two phases according to the methods of Lorke, [21].

Phase 1: In this phase 12 mice were divided into 4 groups of 3 animals each. Each group of animals were administered different doses (10, 100 and 1000 mg/kg.b.wt.) of the AQUAD and then observed for 24 hours to monitor their behavior as well as if mortality will occur.

Group 1 or control group received normal rat diet and water orally.

Group 2 received 10 mg/kg b.wt. of the AQUAD orally once in addition to normal rat diet and water.

Group 3 received 100 mg/kg b.wt. of the AQAUD orally once, in addition to normal rat diet and water.

Group 4 received 1000 mg/kg b.wt. of the AQAUD orally once, in addition to normal rat diet and water. Phase one result was a determinant to the number of animals and drug dose used in phase 2.

Phase 2: In this phase 3 mice were divided into 3 groups of one animal each. Each group of animals was administered different doses (1600, 2900 and 5000mg/kg.b.wt.) of the AQUAD and then observed for 24 hours to monitor their behavior as well as if mortality will occur.

Group 1 received 1600 mg/kg.b.wt. of the AQUAD orally once, in addition to normal rat diet and water.

Group 2 received 2900 mg/kg.b.wt. of the AQUAD orally once in addition to normal rat diet and water.

Group 3 received 5000 mg/kg.b.wt. of the AQAUD orally once, in addition to normal rat diet and water.

Then the LD_{50} was calculated thus:
$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

D_0 = Highest dose that gave mortality.
D_{100} = Lowest dose that produced mortality.

2.8 Grouping of Animals for Ulcer-Protective Study

A total of thirty healthy albino rats weighing between 200 g and 250 g were divided into five groups with six animals in each group according to their weight.

Group I or normal control group received normal rat diet and water orally for 14 days.

Group II or intoxicated control group received aspirin 400 mg/kg body weight orally as a single dose on the 14th day in addition to normal rat diet and water for 14 days.

Group III or intoxicated test group 1 received 250 mg/kg body weight of the AQAUD for 14 days and aspirin 400 mg/kg body weight orally as a single dose on the 14th day in addition to normal rat diet and water.

Group IV or intoxicated test group 2 received 500 mg/kg body weight of the AQAUD for 14 days and aspirin 400 mg/kg body weight orally as a single dose on the 14th day in addition to normal rat diet and water.

Group V or intoxicated standard group received omeprazole 20 mg/kg body weight orally for 14 days and aspirin 400 mg/kg body weight on the 14th day in addition to normal rat diet and water.

After administration of AQAUD, aspirin and omeprazole for 14 days, all the animals were fasted for 24 hours and sacrificed under light anaesthesia with dichloromethane to bring out the stomachs which were used to check for the ulcer parameters. They include ulcer index, free and total acidity of the stomach content. Nitrite, glutathione, malondialdehyde and total protein concentration of the stomach homogenate was also determined. Also superoxide dismutase and catalase activities in the stomach homogenate were assayed.

2.9 Preparation of Stomach Homogenate

After the gastric contents were removed, the gastric mucosa were homogenized in phosphate buffer (pH 7.4) in 4 parts of homogenizing buffer i.e. 1:4 ratio using a tissue homogenizer and centrifuged at 12,000 rpm for 30 mins. The supernatant was collected and kept in the freezer

at -4ºC and used to assay for the activities of catalase and superoxide dismutase. Malondialdehyde, glutathione, nitrite, and protein concentrations were also estimated in the homogenate.

2.10 Biochemical Analyses

Free and total acidity determination of the gastric content were carried out according to Kulkarni, et al. [22], ulcer index was done according to Suzuki, et al. [23], total protein concentration was done according to Gornall, et al. [24], reduced glutathione (GSH) concentration was done according to Raja, et al. (2007), nitrite/nitrate concentration was done according to Green, et al. [25] and Kumar and Kumar, [26], catalase (CAT) enzyme assay was done according to Sinha [27], superoxide dismutase (SOD) assay was according to Vijayalakshmia and Kumar, [28] and histological studies according to Culling, [29].

2.11 Statistical Analysis

Data were analyzed using Statistical Package for Social Science (SPSS) version 21. Results were presented as mean ± S.D. of six observations and statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey post Hoc test. The degree of statistical difference was accepted at $P < 0.05$.

3. RESULTS

The results of the present study are presented in Tables 1-7 while the histopathological assessment is presented in Plates 1-5.

Table 1. Qualitative phytochemical screening of AQAUD

Phytochemicals	Result
Tannins	+
Glycosides	+
Saponins	+
Flavonoids	+
Phenols	+
Alkaloids	+
Cyanogenic Glycosides	-
Steroids	-

+ means present while – means absent

Table 2. Quantitative phytochemical content of AQAUD

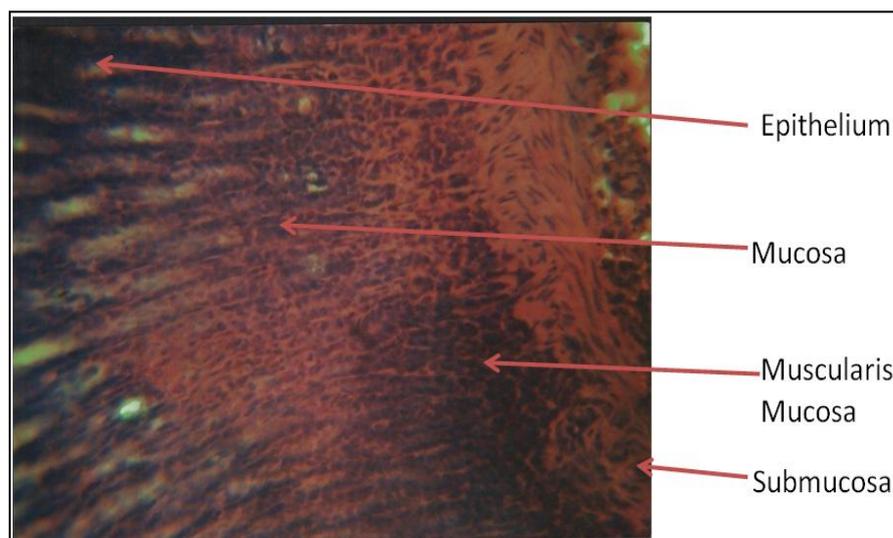| Total phenolic content (mg Tannic acid equivalent /g extract)	Flavonoid content (mg quercetin equivalent /g extract)
1.90 ± 0.190	1.04 ± 0.14

Plate 1. (Normal control): The gastric mucosa consists of a surface epithelium that invaginates to various extents into the lamina propria, forming gastric pits. Emptying into the gastric pits are branched, tubular glands. The lamina propria of the stomach is composed of loose connective tissue. Separating the mucosa from the underlying submucosa is a layer of smooth muscle, the muscularis mucosa. The morphological features of this section are in line with that of a normal stomach (x400), (stain: Hematoxylin (H) and Eosin (E))
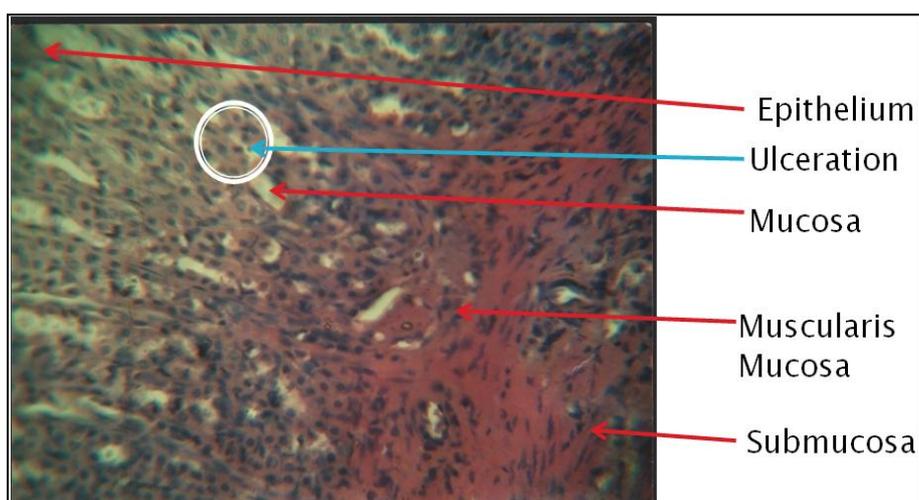
Plate 2. (Stomach of rat intoxicated with Aspirin 400 mg/kg.b.wt to induce ulcer): There is ulceration of the surface epithelium, lamina propria and the muscularis mucosa. (x400), (stain: H and E)
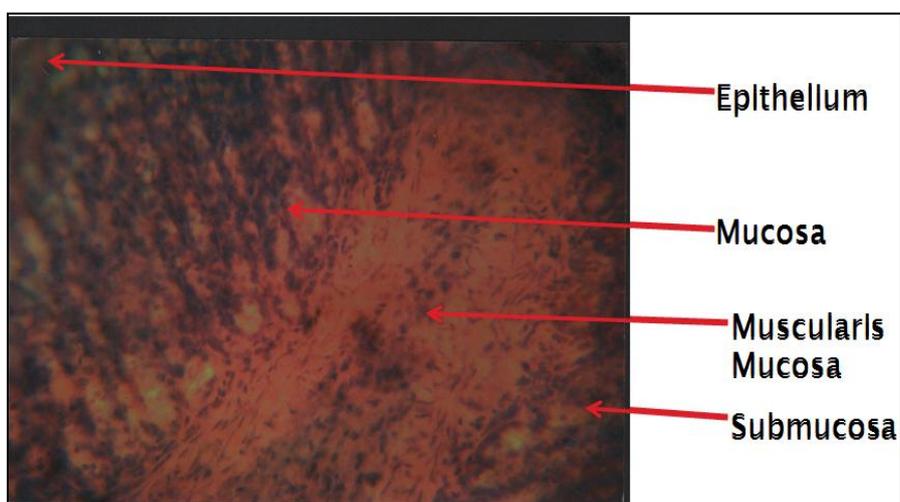

Plate 3. (Stomach of rat intoxicated with Aspirin 400 mg/kg.b.wt to induce ulcer + treatment with AQAUD 250 mg/kg.b.wt): There is regeneration of the gastric mucosa, submucosa and muscularis mucosa. (x400), (stain: H and E)

4. DISCUSSION

The present study evaluated the protective role and antioxidant potentials of Aqueous Anti-Ulcer Drug (AQAUD) on aspirin induced gastric ulcers in albino rats with the possible involvement of antioxidants. AQAUD is an anti-ulcer herbal medicamenet which is composed of juice from *Citrus aurantifolia* (lime), Gbogbonise, *Strophantus hispidus* (root) and *Hippocratea welwitschii* (root).

The qualitative phytochemical analysis showed that AQAUD had flavonoids, phenols, alkaloids, glycosides and tannins (Tables 1 and 2). These

compounds have been found to inhibit bacterial growth and are capable of protecting certain plants against bacterial infections [30,31]. Phenolic compounds and flavonoids are well known antioxidants and scavenging agents against free radical associated with oxidative damage and ulcers [32]. The presence of these compounds in AQAUD may give credence to its local usage in the treatment of gastric ulcers. Flavonoids are important secondary metabolites in plants, alleviating lipid peroxidation involved in artherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effect of flavonoids is correlating with their antioxidant activities [33].

Table 3. Nitric oxide radical scavenging effect of graded concentrations of quercetin and Aqueous Anti-Ulcer Drug (AQAUD)

Threshold Inhibitory Concentrations (IC)	% Nitric Oxide radical scavenging						
	5	10	20	40	50	70	80
AQAUD (µg/ml)	9.22± 0.46	17.46± 0.87	34.92± 1.75	80.91±4.05	114.66± 5.73	238.90 ±11.95	383.7± 19.19
Quercetin (µg/ml)	3.62± 0.18	6.57± 0.33	12.56 ± 0.63	27.64 ±1.38	38.44± 1.92	77.78± 3.89	124.3± 6.22

Table 4. Hydroxyl radical scavenging effect of graded concentrations of quercetin and Aqueous Anti-Ulcer Drug (AQAUD)

Threshold Inhibitory Concentrations (IC)	% Hydroxyl radical scavenging					
	5	10	20	50	70	80
AQAUD (µg/ml)	74.92± 3.75	75.92± 3.80	146.28± 7.31	ND	ND	ND
Quercetin (ug/ml)	49.57± 2.48	99.23± 4.96	236.36±11.82	1017.62±50.88	2218.37±110.92	3525.66±176.28

Table 5. Effect of "AQAUD" administration on the ulcer index, % protection, free and total acidity of the gastric content in aspirin induced gastric ulcers in albino rats

	NC	ASP	AQAUD 250 mg/kg b.wt	AQAUD 500 mg/kg b.wt	OMEP 20 mg/kg b.wt
Ulcer index	0[a]	8.50[c]	0.83[b]	2.50[a]	1.33[ab]
%Protection	0±0	0±0	90.20±11.57[a]	70.59±12.34[b]	84.31±17.71[a]
Free Acidity (mEq/L)	8.80±2.39[a]	13.60±2.30[b]	12.00±1.83[ab]	8.75±1.50[a]	11.00±1.22[ab]
Total Acidity (mEq/L)	18.80±4.60[a]	27.60±2.30[b]	23.50±2.65[ab]	21.50±3.42[ab]	21.60±4.62[ab]

Legend: NC = normal control, ASP = aspirin group, OMEP = omeprazole group

Table 6. Effect of "AQAUD" administration on SOD and CAT activity, MDA, GSH, nitrite concentration, and total protein concentration of the stomach homogenates in aspirin induced gastric ulcers in albino rats

Parameters	NC	ASP	AQAUD 250 mg/kg b.wt	AQAUD 500 mg/kg b.wt	OMEP (20 mg/kg b.wt)
SOD (UX10^-2/mg Protein)	37.24±5.38	23.64±2.91	32.31±2.34	34.88±5.90	30.40±4.56
CAT (IU*100^-2/mg Protein)	63.38±1.42	35.63±1.90	43.56±6.47	63.25±5.88	61.60±5.58
MDA (nmol/mg Protein)	7.53±3.37	1.01±4.90	9.41±5.80	8.73±3.25	6.92±0.00
GSH (ug/ml)	51.08±6.83	49.05±8.26	54.39±7.33	52.03±2.44	49.32±8.64
Protein (g/l)	7.20±0.91[c]	5.29±0.83[a]	5.77±0.29[ab]	6.10±0.17[bc]	7.13±0.69[c]
Nitrite	0.05±0.01[a]	0.11±0.01	0.08±0.01	0.06±0.01	0.07±0.01

Legend: NC = normal control, ASP = aspirin group, OMEP = omeprazole group

Table 7. Determination of the LD$_{50}$ of the Aqueous Anti-Ulcer Drug (AQAUD)

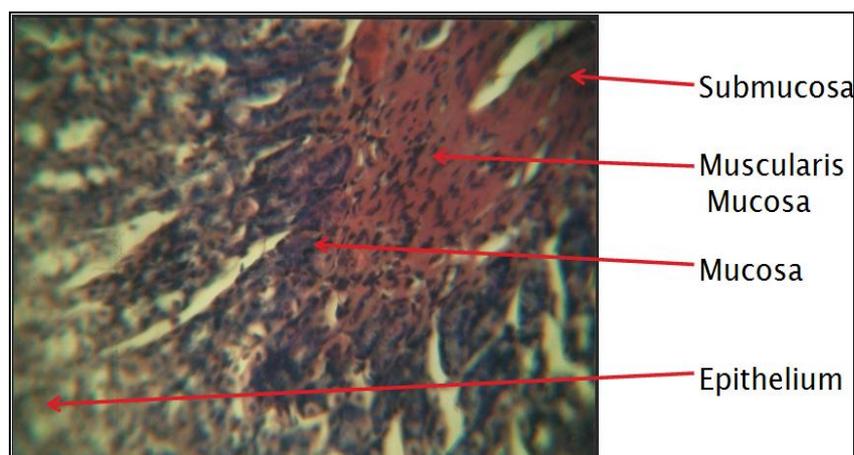| Group of rats	Dose of AQAUD (mg/kg b.wt.)	No. of death recorded
Phase1		
1 (Control)	---	0/3
2	10	0/3
3	100	0/3
4	1000	0/3
Phase 2		
1	1600	0/2
2	2900	0/2
3	5000	0/2

Plate 4. (Stomach of rat intoxicated with Aspirin 400 mg/kg.b.wt to induce ulcer + treatment with AQAUD 500 mg/kg.b.wt.): There is ulceration of the gastric mucosa, submucosa and muscularis mucosa. (x400), (stain: H and E)
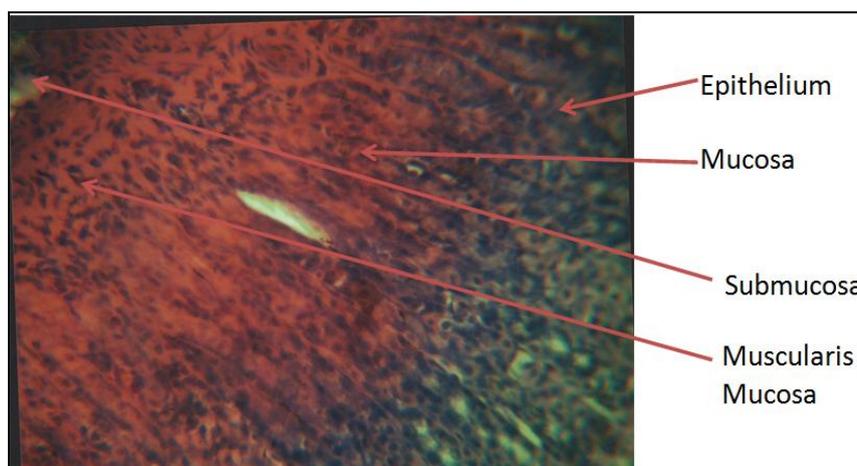

Plate 5. (Stomach of rat intoxicated with Aspirin to induce ulcer + treatment with Standard drug, Omeprazole 20 mg/kg.b.wt): There is regeneration of the gastric mucosa, submucosa and muscularis mucosa but little ulceration was still evident. (x400), (stain: H and E)

Nitric oxide is a key signaling molecule that plays a crucial role in the pathogenensis of various diseases associated with inflammation [34]. It is a free radical generated from sodium nitroprusside in aqueous solution at physiological pH and reacts with oxygen to form oxides of nitrogen. Nitrite is one of the oxides of nitrogen which was significantly inhibited by plant extracts

through direct competition with oxygen in the reaction medium [19]. The scavenging activity of AQAUD against nitric oxide formation was comparable to that of the standard drug (quercetin) used in this study. The IC_{50} and IC_{80} for the AQAUD and quercetin were 114.66±5.73 µg/ml and 38.44±1.92 µg/ml and 383.73±19.19 µg/ml and 124.31±6.22 µg/ml respectively (Table 3). However, Quercetin effectively scavenged nitric oxide than AQAUD. It can be inferred that the presence and the quantity of antioxidant compounds in AQAUD could justify the observed results and thus may give support to its traditional use in the treatment of diseases caused by inflammation and cellular damage like ulcers. The mechanism of action of flavonoids and phenolic compounds are through scavenging process [35]. Nitric oxide scavenging effect of AQAUD may be due to the phenolic and flavonoid compounds present in it.

Hydroxyl radical has been implicated in the oxidative damage of DNA, proteins and lipids [36]. The hydroxyl radicals generated by Fe^{2+}-ascorbic acid and EDTA-H_2O_2 system (Fenton's reaction) was scavenged by the AQAUD and quercetin in a concentration dependent manner. The IC_{20} for the both were 146.28±7.31 µg/mL and 236.36±11.82 µg/mL respectively (Table 4). This observation showed that quercetin is a better hydroxyl radical scavenger. However, AQAUD can be used as a remedy to combat the oxidative activity of hydroxyl radical in the absence of quercetin.

In gastric secretion parameters, aspirin significantly increased ($P<0.05$) the free and total acidity of the gastric contents. AQAUD 250 mg/kg b.wt treatment did not show a significant difference in free acidity, while AQAUD 500 mg/kgb.wt significantly decreased ($P<0.05$) the free acidity. Also, AQAUD 250 mg/kg b.wt. and 500 mg/kg b.wt did not show a significant difference in total acidity of the gastric content (Table 5). Aspirin induces gastric ulcer through erosion of the gastric mucosa. In ulcer index, aspirin also significantly increased ($P<0.05$) the ulcer index of the gastric mucosa. AQAUD significantly reduced the ulcer index of the gastric mucosa with attendant regeneration of gastric mucosa. These results could be explained by the fact that prostaglandins normally protect the gastrointestinal mucosa from damage by maintaining blood flow and increasing mucosal secretion of mucous and bicarbonate [37]. NSAID's like aspirin cause mucosal damage by interfering with prostaglandins synthesis,

increasing acid secretion and block the diffusion of H^+ [38]. Aspirin blockade of cyclooxygenase-1 (COX-1) and (COX II) results in reduction of prostaglandins synthesis. This interruption of prostaglandins synthesis results in impairment of mucosal damage repair, thus facilitating mucosal injury [39].

Free radicals are involved in the development of gastric ulcers. If the generation of free radicals exceeds the ability of these free radical scavenging enzymes like Superoxide dismutase (SOD) and Catalase (CAT), gastric mucosa may be injured by the free radicals resulting to the development of gastric ulcer (Vanisree, et al. 1996). From the experimental results, aspirin administration significantly decreased the SOD and CAT activities in the stomach homogenates, which is likely to be due to free radicals generation. AQAUD 250 mg/kg b.wt. and 500 mg/kg b.wt significantly raised the decreased SOD and CAT activities to almost normal. These observations may be attributed to the flavonoid and phenolic contents of AQAUD which are known antioxidants and scavenging agents against free radicals associated oxidative damage and ulcers [32].

Reduced glutathione (GSH) is a tripeptide and superoxide radical scavenger. It protects thiol protein groups required for maintaining the integrity of cells against oxidation [40,41]. GSH is present in the stomach at high concentration and plays an important role in maintaining the integrity of the gastric mucosa [42]. From the results, aspirin did not reduce GSH concentration, when compared to the control (Table 6). This may be as a result of other compensatory mechanisms. AQAUD 250 mg/kg b.wt significantly ($P<0.05$) increased the GSH concentration when compared to the control. This could be linked to the flavonoid and phenolic content of the AQAUD as well as its radical scavenging ability. It is possible that flavonoid and phenolic compounds had a sparing effect on the GSH concentration or administration increased the GSH biosynthetic capability of the cells from the cystein amino acids present in the body. AQAUD 500 mg/kg b.wt. and omeprazole increased GSH concentration but not significant when compared to the control (Table 6).

Malondialdehyde (MDA) concentration can be used to assess lipid peroxidation used as an index to quantify the damage that occurs in membranes of tissues as a result of free radical generation [43,44]. Gastric mucosal lipid

peroxidation has been reported to increase in incidence of experimental ulcers [45]. From the results, oral administration of aspirin significantly (P<0.05) increased the MDA concentration in the stomach homogenates (Table 6). This significant elevation of MDA concentration in aspirin treated rats is possibly due to the generation of free radicals through metal ion or superoxide catalyzed peroxidation process.

Also from the results, AQAUD 250 mg/kg b.wt and 500 mg/kg b.wt reduced the elevated MDA concentration but not significantly when compared to the control (Table 6). This reduction may be linked to the flavonoids and phenolic compound content of AQAUD which function as antioxidants that scavenged free radicals. It has been shown that both lime juice and peels (*Citrus aurantifolia*) present in AQAUD demonstrated antioxidant properties [46]. AQAUD 500 mg/kg b.wt. was comparable to omeprazole (the standard antiulcer drug) in reducing MDA concentration.

In this study also, significant elevation of nitrite concentration in gastric mucosa was observed in aspirin induced gastric ulceration. AQAUD 250 mg/kg b. wt and 500 mg/kg b. wt significantly (P<0.05) reduced the elevated nitrite level in the gastric mucosa (Table 6). Nitric oxide is a mediator of gastro-intestinal mucosal defense but paradoxically, it also contributes to mucosal damage. The synthesis of nitric oxide is mediated by the enzyme nitric oxide synthase (NOS), which is present in gastric mucosa in two constitutive (cNOS) isoforms, namely endothelial (eNOS) and neuronal (nNOS) [47]. The inducible (iNOS) enzyme is found in macrophages and neutrophils (Wallace and Miller, 2000). In the digestive system, nitric oxide produced by cNOS is cytoprotective and nitric oxide produced by iNOS is cytotoxic [48]. Nitric oxide at low concentration (from cNOS) plays a role in protecting the integrity of epithelial tissues by improving the mucosal blood flow in the GIT system [49]. This protective effect of nitric oxide is because of the inhibition of activation, adhesion and migration of leucocytes in the inflammatory reaction [50].

NSAIDs have been reported to decrease tissue cNOS-derived nitric oxide [2,48]. Souza, et al. [51] used genetic inducible nitric oxide synthase (iNOS) deficient mice to prove that iNOS-generated nitric oxide is involved in gastric damage. Nitric oxide produced from activation of iNOS reacts directly with superoxide to form peroxynitrite, a potent autotoxic oxidant that causes gastric damage [52]. This could explain the increase in nitrite concentration (as an indicator of nitric oxide production) in gastric mucosa of aspirin treated rats compared with control which may be due to increased production of iNOS in gastric tissues.

The result of the acute toxicity test showed that AQAUD is generally safe at a concentration of 5000 mg/kg b.wt since no death was observed up to the concentration of 5000 mg/kg b.wt in mice (Table 7). This means that AQAUD is safe for consumption up to the concentration 5000 mg/kg b.wt.

Histological results showed that aspirin caused severe ulcerations of the surface epithelium, Lamina proporia and Muscularis mucosa of the gastric mucosa (Plate 2). This observation is in line with the biochemical parameters evaluated and could be as a result of free radicals generated and excess production of gastric acid that eroded the gastric mucosa. The significant regeneration of cells of the gastric mucosa and healing of the ulcerations observed with AQAUD 250 mg/kg b.wt. (Plate 3) could be due to efficient scavenging of the free radicals generated as a result of aspirin administration as well as its adequate antioxidant property. AQAUD 500 mg/kg b.wt. showed significant regeneration of cells of the gastric mucosa but still had ulceration (Plate 4). It is possible that the anti-ulcer effect of AQAUD may be due to other mechanisms not well understood within the scope of study. This is because AQAUD 250 mg/kg b.wt. was more protective than AQAUD 500 mg/kg b.wt., yet AQAUD 500 mg/kg b.wt. had a better antioxidant capacity. AQAUD may be combining other mechanisms with antioxidative, blocking of Acid secretion and interference with cyclooxygenase activity. Omeprazole 20 mg/kg b.wt. also showed regeneration of the cells of the gastric mucosa with little ulceration still evident (Plate 5). These observations showed that AQAUD 250mg/kg b.wt. might be the most appropriate dosage or concentration to be taken.

5. CONCLUSION

Aqueous Anti-Ulcer Drug (AQAUD) offered some protection against aspirin-induced gastric mucosal damage. The antioxidant compounds present in AQAUD played protective roles against the production of free radicals. The present study revealed that AQAUD

administered at 250 mg/kg b.wt. has promising potentials for the development of alternative treatment against gastric ulcers.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anderson JW, Smith BM, Gustafson NJ. Health benefits and practical aspects of high-fiber diets. American Journal of Clinical Nutrition. 1994;59(5):1242-1247.
2. Airaodion AI, Olayeri IM, Ewa AO, Ogbuagu EO, Ogbuagu U, Akinmolayan JD, Agunbiade AP, Oloruntoba AP, Airaodion EO, Adeniji AR, Obajimi OO, Awosanya OO. Evaluation of *Moringa oleifera* leaf potential in the prevention of peptic ulcer in wistar rats. International Journal of Research. 2019;6(2):579-584.
3. Guyton AC, Hall JE. Textbook of medical physiology, physiology of gastrointestinal disorder. 11th Edn., Elsevier Saunders. 2006;Chapter 66:891-826.
   [ISBN: 0-7216-0240-1]
4. Airaodion AI, Ogbuagu U, Ogbuagu EO, Airaodion EO, Agunbiade AP, Oloruntoba AP, Mokelu IP, Ekeh SC. Investigation of aqueous extract of *Zingiber officinale* root potential in the prevention of peptic ulcer in albino rats. International Journal of Research and Innovation in Applied Science. 2019;4(2):64-67.
5. Graziani G, D'Argenio G, Tuccillo C, Loguercio C, Ritieni A. Apple polyphenol extracts prevent damage to human gastric epithelial cells *In vitro* and to rat gastric mucosa *In vivo*. Gut. 2005;54:193-200
6. Oliveira CP, Kassab P, Lopasso FP, Souza HP, Janiszewski M. Protective effect of ascorbic acid in experimental gastric cancer: Reduction of oxidative stress. World Journal of Gastroenterology. 2003;9:446-448.
7. Airaodion AI, Olatoyinbo PO, Ogbuagu U,Ogbuagu EO, Akinmolayan JD, Adekale OA, Awosanya OO, Agunbiade AP, Oloruntoba AP, Obajimi OO, Adeniji AR, Airaodion EO. Comparative assessment of phytochemical content and antioxidant potential of *Azadirachta indica* and *Parquetina nigrescens* leaves. Asian Plant Research Journal. 2019;2(3):1-14.
8. Airaodion AI, Ibrahim AH, Ogbuagu U, Ogbuagu EO, Awosanya OO, Akinmolayan JD, Njoku OC, Obajimi OO, Adeniji AR, Adekale OA. Evaluation of phytochemical content and antioxidant potential of *Ocimum gratissimum* and *Telfairia occidentalis* leaves. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2019;7(1):1-11.
9. Tierney LM, Mephee SJ, Papadakis MA. Current medical diagnosis and treatment. Pub. Mc Craw Hill, New York. 2001;77–91.
10. Airaodion AI, Obajimi OO, Ezebuiro CN, Ogbuagu U, Agunbiade AP, Oloruntoba AP, Akinmolayan JD, Adeniji AR, Airaodion EO. Prophylactic efficacy of aqueous extract of *Curcuma longa* leaf against indomethacin-induced ulcer. International Journal of Research. 2019;6(1):87-91.
11. Airaodion AI, Adekale OA, Airaodion EO, Ogbuagu EO, Uloaku Ogbuagu U, Osemwowa EU. Efficacy of combined crude extract of *Curcuma longa* and *Moringa oleifera* in the prevention of peptic ulcer in albino rats. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2019;7(2):1-8.
12. Ishola IO, Awodele O, Oreagba IA, Murtala AA, Chijioke MC. Antinociceptive, anti-inflammatory and anti-ulcerogenic activities of ethanol root extract of strophantus hispidus DC (Apocynaceae). The Journal of Basic and Clinical Physiology. 2013;24 (4):277-286.
13. Taylor EF, Francis KW. A dictionary of true etymologies. Adman Room. 1986;101.
14. Hatherill RJ. Eat to beat cancer. Tropical medicine and International Health. Neurosignals. 2014;9(3-4):137-159.
15. Adeleye IA, Ayolabi CI, Onubogu CC, Isawunmi OA, Nshiogu ME, Sobande O. Screening of crude extracts of twelve medicinal plants and "wonder-cure" concoction used in Nigeria unorthodox medicine for activity against *Mycobacterium tuberculosis* isolated from tuberculosis patients' sputum. African Journal of Biotechnology. 2008;7(8):3182-3187.
16. AOAC. Official methods of analysis of the Association of Official Analytical Chemists.15th edition. Washington, DC.

Association of official Analytical Chemists; 1984.

17. Wettasinghe M, Shahidi F. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of bonage and evening primrose meals. Food chemistry. 2000;70 (10):17-26.

18. Marcocci L, Packer L, Droy-Lefiax MT, Sekaki A, Gardes-Albert M. Antioxidant action of *Ginkgo biloba* extract EGb 761. Methods in Enzymology. 1994;234:462-475.

19. Alisi CS, Onyeze GOC. Nitric oxide scavenging ability of ethyl acetate fraction of methanol leaf extracts of *Chromolaena odorata* (Linn). African. Journal of Biochemistry Research. 2008;2:45-50.

20. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: A simple test tube assay for determination of rate constant for reactions of hydroxyl radicals. Analytical Biochemistry. 1987;165:215-219.

21. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983;54(4):275-287.

22. Kulkarni AR, Kulkarni VH, Shastry CS, Sateesh B, Kukkeri VI, Marihal CS. Screening of gulva leaves extracts for analgesic, anti-inflammatory and anti-ulcer activities in albino rats. Indian Drug. 1999;177(6):363-367.

23. Suzuki Y, Hayashi M, Ito M, Yamagami I. Antiulcer effects of 40-(2-carboxyethyl) phenyl trans-4-aminomethyl cyclohexane carboxylate hydrochloride (Cetraxate) on various experimental gastric ulcers in rats. The Japanese Journal of Pharmacology. 1976;26:471-480.

24. Gornall AG, Bardawill CT, David MM. Determination of serum protein by means of the biuret reaction. Journal of Biological Chemistry. 1949;177(2):75-76.

25. Green LC, Wanger DA, Glogowski J, Skipper PL, Wishnok JS, Jamenbaum SR. Analysis of nitrate, nitrite and (^{15}N) nitrate in biological fluids. Analytical Biochemistry. 1982;126:131-138.

26. Kumar P, Kumar A. Prolonged pretreatment with carvedilol prevents 3-nitropropionic acid induced behavioral alteration and oxidative stress in rats. Pharmacological Reports. 2008;60:706-715.

27. Sinha AK. Colorimetric assay of catalase. Analytical Biochemistry. 1972;47:389-394.

28. Vijayalakshmia HR, Kiran-Kumar Y. Evaluation of goitrogenic and antithyroidal effect of the *fern Adiantum capillus-veneri*. The Brazilian Journal of Pharmacognosy. 2013;23(20):802-810.

29. Culling CF. Handbook of histopathological and histochemical techniques. Butterworth and co, London. 1974;37.

30. Clark MS. Antimicrobial activities of phenolic constituents of Manoliagradiflora. Journal of Pharmaceutical Science. 1981; 10:951-952.

31. Okwu DE. Phytochemical and vitamin content of indigenous spices of south eastern Nigeria. Journal of Sustainable Agriculture and the Environment. 2004;6: 30-34.

32. Fergusion LR, Philpott M, KarunaSinghe N. Oxidative DNA damage and repair: Significance and biomarkers. Journal of Nutrition. 2006;136(10):2687-89.

33. Shi J, Yu J, Pohorly J, Young C, Bryan M, Wu Y. Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. Journal of Food, Agriculture and Environment. 2006;1:42-47.

34. Gates PE, Strain WD, Strain WD. Human enthothelial function and microvascular aging. Experimental Physiology. 2008;94: 311-316.

35. Cook NC, Samman S. Flavonoid chemistry, metabolism, cardio-protective effects and dietary sources. Nutritional Biochemistry. 1996;7:66-76.

36. Spencer JPE, Jemer A, Aruoma IO, Evans PJ, Kaur H. Intense oxidative DNA damage promoted by L-Dopa and its metabolites, implications for neuro-degenerative disease. Federation of European Biochemical Societies Letters. 1994;353:246-250.

37. Voutilainen M, Martynen T, Farkkils M, Juhola M, Syponene P. Impact of non-steroidal anti-inflammatory drug and aspirin use on prevalence of dyspepsiaand uncomplicated peptic ulcer. Scandinavian Journal of Gastroenterology. 2001;36(8): 817-21.

38. Roa CV, Maiti RN, Goel RK. Effect of mild irritation on gastric mucosal offensive and defensive factors. Indian Journal of Physiology and Pharmacology. 1999;44: 185-191.

39. Burke A, Smyth E, Fhzgerald GA. Analgesic-antipyretic agents, pharmaco-therapy of gout. In: Brunton LL, Lazo JS,

Parker KL. (Eds.), Goodmen and Gilman pharmacological bases of therapeutics. 11th Edn., McGraw Co. Inco., New York. 2006;671-715.

40. Airaodion AI, Ogbuagu EO, Ogbuagu U, Adeniji AR, Agunbiade AP, Airaodion EO. Hepatoprotective effect of *Parkia biglobosa* on acute ethanol-induced oxidative stress in Wistar rats. International Research Journal of Gastroenterology and Hepatology. 2019;2(1):1-11.

41. Airaodion AI, Ogbuagu EO, Ekenjoku JA, Ogbuagu U, Airaodion EO. Therapeutic effect of methanolic extract of *Telfairia occidentalis* leaves against acute ethanol-induced oxidative stress in wistar rats. International Journal of Bio-Science and Bio-Technology. 2019;11(7):179-189.

42. Altinkaynak K, Suleyman H, Akcay F. Effect of nimesulide, refecoxib and celecoxib on gastric tissue glutathione level in rats with indomethacin-induced gastric ulcerations. Polish Journal of Pharmacology. 2003;55(4):645-648.

43. Airaodion AI, Ogbuagu U, Ekenjoku JA, Ogbuagu EO, Airaodion EO, Okoroukwu VN. Hepato-protective efficiency of ethanol leaf extract of *Moringa oleifera* against hydrocarbon exposure. International Journal of advances in Herbal and Alternative Medicine. 2019;03(01): 32-41.

44. Ogbuagu EO, Airaodion AI, Ogbuagu U, Airaodion EO. Prophylactic propensity of methanolic extract of *Vernonia amygdalina* leaves against acute ethanol-induced oxidative stress in wistar rats. International Journal of Bio-Science and Bio-Technology. 2019;11(7):37-46.

45. Sairam K, Rao CV, Babu MD, Goel RK. Prophylactic and curative effects of *Bacopa monniera* in gastric ulcer models. Phytomedicine. 2001;86:423–30.

46. Maryan B, Jamal M. Antioxidant effects of *Citrus aurantifolia* juice and peel extract in LDL oxidation. Journal Research in Medical Sciences. 2011;6(7):951-955.

47. Cho CH. Current roles of nitric oxide in gastrointestinal disorders. Journal of Physiology- Paris. 2001;95:253-6.

48. Motawi TK, Abd EHM, Shahin NN. Modulation of indomethacin-induced gastric injury by permine and taurine in rats. Journal of Biochemical and Molecular Toxicology. 2007;21:280-288.

49. Wittle BJ. Nitric oxide in gastrointestinal physiology and pathology. In: Johnson IR. (ed). The physiology of gastrointestinal tract. Raven. New York. 1994;267-94.

50. Banick PD, Chen Q, Xu YA, Thum SR. Nitric oxide inhibits neutrophil beta 2 integrin function by inhibiting membrane-associated cyclic GMP synthesis. Journal of Cell Physiology. 1997;172:12-24.

51. Souza HM, Lemois HP, Oliveira RB, Cunha FQ. Gastric damage and granulocyte infiltration induced by indo-methacin in tumor necrosis factor receptor 1(TNF-R1) or inducible nitric oxide synthase (iNOS) deficient mice. Gut. 2004; 53:791-796.

52. Lanas A. Role of nitric oxide in gastrointestinal tract. Arthritis Research and Therapy. 2008;10:1-6.