

Histomorphological Study of the Effect of Chronic Consumption of *Abelmoschus Esculentus* and *Piper Guineense* On The Gastric Mucosa Of Albino Wistar Rats

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Abstract

The effects of *Abelmoschus esculentus* (okra) and *Piper guineense* (black pepper) on the histomorphology of the gastric mucosa of the fundic stomach were investigated. Twenty adult wistar male rats weighing (123-207g), divided into four groups 1, II, III & IV, group 1 as control and groups II, III & IV as experimental groups. The rats in the control group were administered with distilled water, while rats in group II and III were administered with 500mg/kg of *Abelmoschus esculentus* and 20mg/kg of *piper guineense* respectively. Group IV received a combination of the two extracts. After 28 days of administration of extracts, animals were sacrificed stomach was extracted and processed to paraffin section, cut at 5micron, stained, and observed histopathologically under light microscope. Result showed marked significance of the effect of the single extract of *Abelmoschus esculentus*, *Piper guineense* and the combination of both extracts, while the histopathology findings revealed glandular erosion, mucosal erosion, inflammation, ulceration, degeneration of mucosal lining cells and epithelium, degeneration of chief and parietal cells and granulated eosinophilic cells as compared to control group 1. Statistical value in the weight of the body and stomach showed significant value ($p < 0.05$) compared to the control. In conclusion, it is observed that the usage of the two extract as single and combine posed a degree of pathological condition on the gastric mucosa.

Key Words: *Abelmoschus esculentus*, *Piper guineense*, gastric mucosa, Histopathology and wistar rat.

1.0 INTRODUCTION

For over some decades, there has been an high rise in the consumption of the fruit of *Abelmoschus esculentus* and *Piper guineense* in Nigeria. Furthermore, it has been reported that consumption of *Piper guineense* in medicinal amount of amount by mouth may include stomach upset or gastrointestinal adverse effect (Mc Namara, 2005) and that the antioxidant component of *Abelmoschus esculentus* is used in the treatment of gastrointestinal disorders. This study is significant because it addresses various symptoms of gastrointestinal disorder following the administration of *Abelmoschus esculentus* and *Piper guineense* along any point within the gaster, with a view to produce a therapeutic drug from plant origin. Hence, would be of importance to medical practitioners, pharmacist, nutritionist and the society at large.

The gastric mucosa is vulnerable to various disorders, some of which ranges from glandular atrophy, intestinal metaplasia, dysplasia, epithelial degeneration gastric ulcers, erosive gastritis and all other forms of gastritis (Leung, 2011). The pathophysiology of mucosal injury in gastritis, erosive ulcers, dysplasia, glandular atrophy, epithelial degeneration are thought to be an imbalance of aggressive factors (acid, pepsin and helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion prostaglandin, innate resistance of the mucosal cells) factor (Murra, 2002).

Abelmoschus esculentus (Okro) is a well known oriental herb used in traditional medicine which grows up to 3-6 feet or more in height. This annual dicotyledonous plant called okra is of West African origin and is cognate with "Okwuro" in the Igbo language, spoken in Nigeria (Mc Whorter, 2000).

Abelmoschus esculentus, popularly known among the Ibibio people of South-Southern Nigeria as “Etighi”, is used for the treatment of gastric problem on account of its mucilaginous content. It has also been reported that the fruit of this medicinal plant is considered an emollient, demulcent and diuretic with stimulant and vulnerary activities (James, 1993). This long green pod vegetable with a green furry outer skin, thin green flesh and small edible white seed is eaten in many different ways like; stir fried, cooked in soup, prickled, cooked in meat stew, with soy sauce, with sugar, with rice, deep fried, and even with fresh salads. The seeds are useful in the production of Okra oil (Mays, 2007).

Piper guineense, also known as African black pepper or hot leave is widely consumed in some part of West Africa especially Nigeria and Ghana on account of its nutritional and Medicinal properties (Negbenebor, 1999). *Piper guineense* also known as Kale, Guinea pepper, Sasema, leulaube, Sorowiso, Guinea cubeb, Masoro, false cubeb pepper, *Benin peper*. Like other members of the pepper family, *Piper guineense* is a climbing vine that can grow up to twenty meters in length, with glossy leaves which are about six inches long (Delziel, 1937). According to Irvine, they are native to the tropical regions of central and Western Africa and Semi-cultivated in countries such as Nigeria where the leaves known as “etinkene, odusa among the Efik/Ibibio and “Uziza” among the Igbo are used as flavouring for stew.

The medicinal value of this African species ranges from the treatment of respiratory diseases to correction of female infertility problems, control of weight uterine contraction for the expulsion of placenta and other remains from the womb (Udoh, 1999). The antiparasitic, antimicrobial and antifungal activities of the leaf and seeds of *Piper Guineense* have also been reported (Ekanem and Obiekezie, 2000; Ngane, 2003; Ekanem, 2010).

However, it has been reported that seed of *Abelmoschus esculentus* have antioxidant effect (Ansari, 2009). Antioxidant potential of the seeds of *Abelmoschus esculentus* and the traditional use of this plant in the treatment of gastrointestinal disorders has been the rationale for this study.

On the other hand, studies have revealed that piperine, found in *Piper guineense* could prevent gastrointestinal disorders developing from stress, hyperacidity and overgrowth of *Helicobacter Pylori* (Bai, 2000). However, possible side effects of taking *Piper guineense* in medicinal amount by mouth may include

stomach upset or other gastrointestinal adverse effects (Mc Namara, 2005), this signify the purpose in which is why this study is undertaken to study the chronic consumption of the plant when taking together to establish the histopathological effect .

2.0 Materials and Methods

2.1 Drugs and Chemicals

Sodium chloride, formaldehyde, sodium trioxocarbonate V, sodium bicarbonate, xylene, 70% alcohol, 90% alcohol, absolute alcohol, haematoxylin, eosin, egg albumin, distilled water, paraffin wax were all procured from BDH Chemicals, England. All other chemicals were of analytical grade.

2.2 Animals

20 wistar rats (123-207g) were obtained from the University of Uyo animal house. They were maintained on standard pellets (guinea feed) and water *ad libitum*. Permission and approval for animal studies were obtained from the college of health sciences animal ethnics committee, University of Uyo.

2.3 Sourcing of Plant material

Freshly fruit of *Abelmoschus esculentus* and *piper guineense* were obtained in July, 2012 from Itam market, Uyo, Akwa Ibom State, Nigeria. The plant was identified and authenticated by the Department of Botany, University of Uyo, Uyo, Nigeria.

2.4 Preparation of Extract

Abelmoschus esculentus was chopped and air dried. *Piper guineense* (seeds) was also air dried and after being dried they weighed 600g for *Abelmoschus esculentus* and 800g for *Piper guineense*. They were then macerated in 97% ethanol (SIGMA CO., UK) in a flat bottom flask and were kept for 72hrs at room temperature. At the end of 72hrs it was filtered. The filtrates were concentrated in water bath at 45 degree Celsius. The concentrated extract was preserved in refrigerator till commencement of research.

The weight of the extracts was 40.25g for *Abelmoschus esculentus* and 24g for *Piper guineense*.

2.5 Acute Toxicity testing.

The acute toxicity of *Abelmoschus esculentus* and *Piper guineense* on Wistar Albino rats were determined in two (2) stages for the two extracts.

For *Abelmoschus esculentus*, in stage one animals received 1000, 2000, 3000, 4000 and 5000mg/kg body weight while in stage two,

animals received 2300, 2400, 2500, 2600, 2700mg/kg body weight.

And in acute toxicity of *Piper guineense* the same two stages was observed, in stage one; animals received 10, 50, 100, 200, 300 mg/kg body weight. Stage 2; received 85, 90, 95, 100, 105 mg/kg body weight.

All experimental animals were observed for physical signs of toxicity such as writhing, gasping, palpitation, decreased respiratory rate, body limb and death within 24hours. The extract was administered intraperitoneally (i.p). The LD50 was found to be 2500mg/kg for *Albemoschus esculentus* and 100mg/kg for *Piper guineense*.

According to the modified lorke's method.500mg/kg and 20mg/kg per body weight were calculated respectively as middle doses for the *Albemoschus esculentus* and *Piper guineense*. Doses were considered as stock solution, they were calculated further using 20mls of distilled water for *Albemoschus esculentus* and 10 mls of distilled water for *Piper guineense* to obtain working solution.

2.6 Experimental Design/Study design

Matured 20 albino wistar male rats weighing between 123-207g were obtained from the faculty of Basic Medical Sciences Experimental Research Animal House of the University of Uyo, Uyo Nigeria. They were fed with standard laboratory diet and water ad libitum. Illumination was 12h light /dark cycle and room temperature was 25±2°C. The animals were divided into four groups, one control (I) and three experimental groups (II, III and IV), which consisted of 5 normal abino wistar rats per per group. The control group was given distilled water while the experimental group II, III and IV were exposed daily to 500mg/kg body weight of *Abelmoschus esculentus* alone, 20 mg/kg of body weight piper guineense alone and 500 mg/kg of *Abelmoschus esculentus* combined with 20 mg/kg of piper gineense respectively by oral administration for 28 days. In this study, all the animals' experimentations were carried out following the guidelines for the care and use of laboratory animals obtained from the institutional animal ethics committee.

3.0 Results

3.1 Statistical Analysis result.

Table 1: Showing the effect of extracts on initial and final body weights

Group s (n)	Drug Administered	Initial body weight(g)	Final body weight (g)
1	Control (no treatment)	137.90±6.16	169.50±6.53*
2	<i>Abelmochus esculentus</i> -500mg/kg-28 days	167.80±7.81	200.10±11.40**
3	<i>Piper guineense</i> -20mg/kg -28 days	181.10±8.56	198.10±12.22**
4	Combined <i>A.esculentus</i> + <i>P.guineense</i> -28days	151.78±7.86	205.44±12.73***

2.7 Sample collection and Histopathological analysis.

Twenty four hours after last exposure, the animal were anesthetized with chloroform vapour and dissected. The harvested stomach were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly The sections were designated "vertical sections". Serial sections of 5 µm thick were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides, stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope

2.8 Gross morphometrical analysis

The initial and final weight of the rats and the weight of the kidney in each group were taken using the weighing balance. The values of all the morphometric analysis were compared statistically using SPSS 17 Software.

2.9 Photomicrography Records of the Histological and histochemical results were obtained by photomicrography using digital photomicrographic microscope at the Gross Anatomy Research Laboratory, Department of Human Anatomy, College of Health sciences, University of Uyo,Uyo,Akwa- Ibom, Nigeria as illustrated in Plate 1 to 5.

Mean \pm SEM, $*=P<0.05$, $** = p<0.01$, $***= p<0.001$ compared to final body weight, n=10 no of animal in a group

Table 2: Showing the effect of extracts on percentage terminal stomach weights

Groups (n)	Extracts Administered	Stomach Weight(g)	% stomach Weight(g)
1	Control(no treatment)	1.30 \pm 0.06	0.76
2	<i>Abelmochus esculentus</i> -500mg/kg-28 days	1.60 \pm 0.07*	0.79
3	<i>Piper guineense</i> -20mg/kg -28 days	1.67 \pm 0.08*	0.84
4	Combined <i>A.esculentus</i> + <i>P.guineense</i> -28days	1.43 \pm 0.08***	0.69

Mean \pm SEM, $*=P<0.05$, $** = p<0.01$, $***= p<0.001$ compared to initial weight of stomach, n=10 no of animal in a group

Note All result were analyzed using one way ANOVA and using SPSS version 17.P-values of less than 0.05 were considered statistically significant

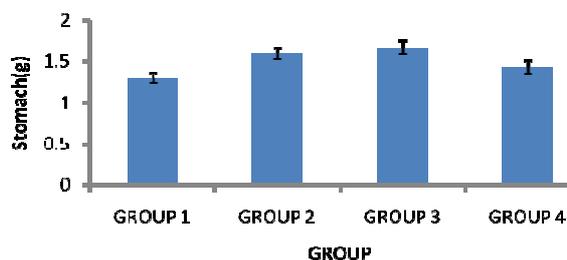


Figure 1: showing mean stomach weight.

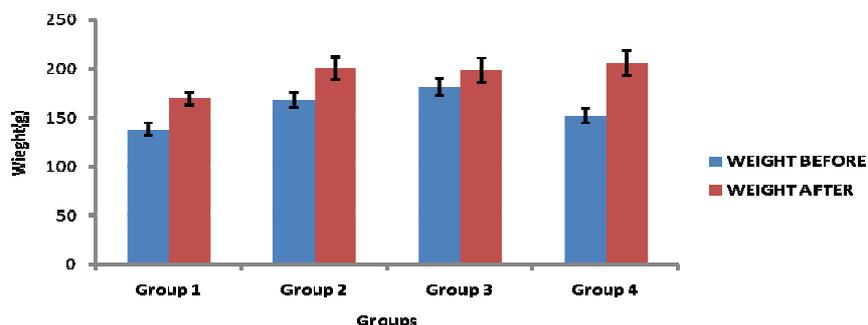


Figure 2: showing comparison between initial and final body weight.

3.2 Histopathological findings.

Plate 1 Control group of Stomach showed normal mucosa, submucosa, musculari, serosa, gastric gland, Glandular mucosa, Lymphatic nodules, mucus neck cells, parietal cells, chief cells.

Plate 2 Stomach treated with *Abelmochus esculentus*-500mg/kg for 28 days showed severe abnormalities area of gastric mucosal lining cells, lamina propria, submucosa, musculari mucosa and externa, the area of gastric mucosa revealed degenerating chief and parietal cells and erosion of gastric gland and mucous neck cells and inflammation as compared to control group.

Plate 3 Stomach treated with *Piper guineense*-20mg/kg for 28 days showed severe

abnormalities area of gastric mucosal lining cells, lamina propria, submucosa, musculari mucosa and externa, the area of gastric mucosa revealed degenerating chief and parietal cells and erosion of gastric gland and mucous neck cells and inflammation as compared to control group.

Plate 4 Stomach treated with a Combination of *Abelmochus esculentus* and *Piper guineense* for 28days showed severe abnormalities area of gastric mucosal lining cells, lamina propria, submucosa, musculari mucosa and externa, the area of gastric mucosa revealed degenerating chief and parietal cells and erosion of gastric gland and mucous neck cells and inflammation with granulated eosinophilic cells as compared to control group.

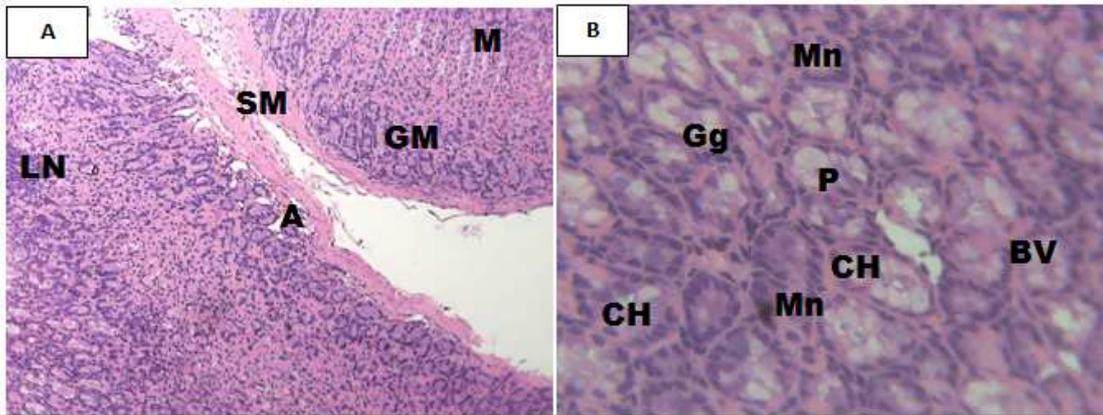


Plate 1. Control fundic stomach at magnification A(x100) & B(x400) stained with H & E technique. Note: *M*-mucosa, *SM*-Submucosa, *Gg*-gastric gland, *GM*-Glandular mucosa, *LN*-Lymphatic nodules, *Mn*- mucus neck cells, *P*-parietal cells, *CH*- chief cells.

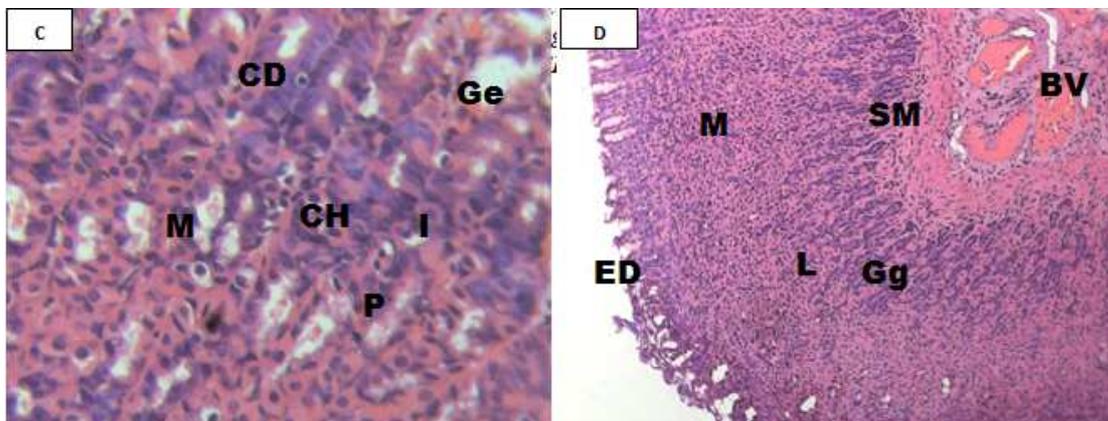


Plate 2 Fundic stomach treated with *ABELMOSCHUS ESCULENTUS* (500mg/kg) at magnification C(x100) & D(x400) stained with H & E technique. Note: *M*-mucosa, *SM*-Submucosa, *Gg*-gastric gland, *I*-inflammation, *CD*-Cellular degeneration, *Ge*-gastric erosion, *P*-parietal cells, *CH*-chief cells, *BV*-Blood vessel, *L*-lamina propria, *ED*-Epithelial degeneration.

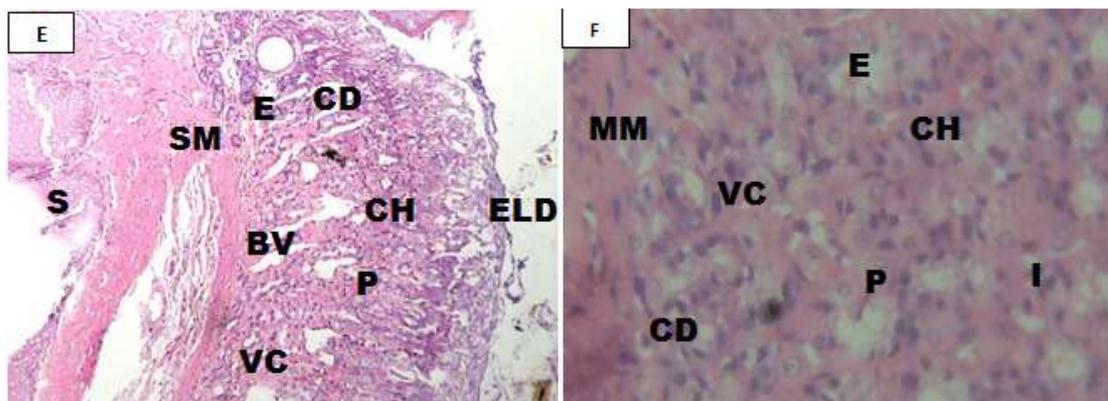


Plate 3 Fundic stomach treated with *PIPER GUINEENSE* (20mg/kg) at magnification E(x100) & F(x400) stained with H & E technique. Note: *CD*-Cellular degeneration, *I*-inflammation, *M*-mucosa, *ELD*-Epithelial lining cells, *Gp*-gastric pit, *CH*-Chief cells, *P*-Parietal cells, *Mn*-Mucus neck cells, *E*-Erosion.

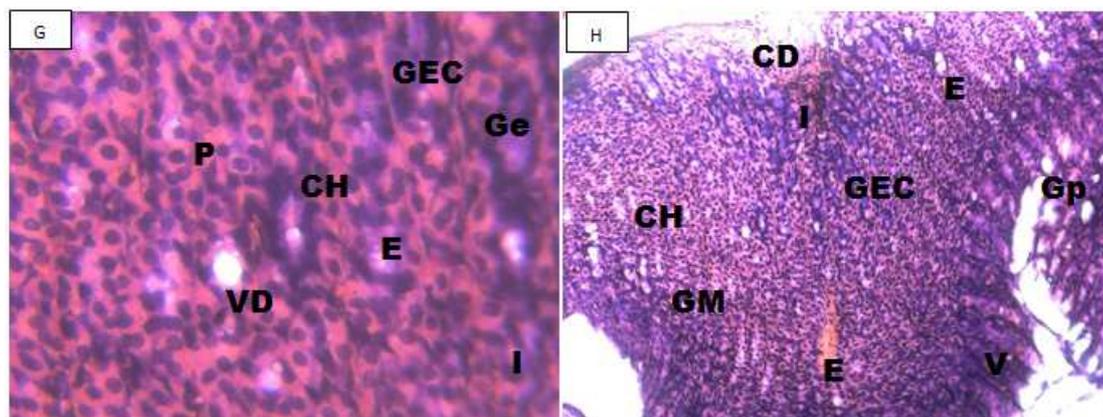


PLATE 4 Fundic stomach treated with ABELMOSCHUS ESCULENTUS (500mg/kg) and PIPER GUINEENSE (20mg/kg) at magnification G(x100) & H(x400) stained with H & E technique.

Note: *E*-Erosion, *CD*-Cellular degeneration, *S*-Serosa, *CH*-chief cells, *BV*-Blood vessel, *LN*-Lymphatic nodules, *ELD*-Epithelial lining cells, *VC*-vascular congestion, *P*-Parietal cells, *Mn*-mucous neck cells, *GM*-glandular mucosa, *I*-Inflammation, *Ge*-gastric erosion, *IO*-Interstitial oedema.

4.0 Discussion

This study investigated the Histopathological effect of chronic consumption of combination Abelmoschus esculentus and piper guineense on the fundic stomach in rat model. In assessing the gastric effects of these extracts, the gross morphology of the stomach were examined, study showed a significant increase in weight of animals and a significant decrease in organ weight in each group. The decrease in the weight of the stomach of the experimental groups may have occurred as a result of gastric necrosis and erosion and cellular degeneration of fundic stomach. Significant distortions in the architectural integrity of the mucosal, lamina propria and submucosa were observed. Specifically, Inflammation of the gastric mucosa and epithelial lining degeneration, infiltration of polymorphonuclear cells into the pits and degeneration of the parietal and chief cells are also noted. The observations made from this study indicated a condition of gastric cellular abnormalities and correlated the previous report on the gastric ulceration effects of cayenne pepper, another specie of pepper which contains capsaicin a known active ingredient in piper guineense, (Mbongue *et al.*, 2005). The results of this study therefore provide a clear indication that Abelmoschus esculentus and piper guineense contain some chemical substances with that is capable of inducing Glandular atrophy, intestinal metaplasia, superficial gastritis, gastric erosion, erosive gastritis and gastric ulcer. The specific chemical constituents and mechanisms responsible for these effect reported in this study are not clear. It may be assumed that the reactive metabolites of Abelmoschus esculentus and piper guineense constituents

could have interacted with the gastric mucosa and intestinal glands to cause derangements ingastric structures and functions.

The combination of the two extracts showed severe ulcerative effect of the gastric mucosa, indicating that in combination their effect to the fundic stomach potentiates each other. The interaction of these metabolites with the fundic stomach may be responsible for cellular injury and subsequent damage to the tissues. The functionality of the stomach may be compromised due to damage of the gastrointestinal tract.

5.0 Conclusion

Results obtained in this study show that chronic exposure to Abelmoschus esculentus and piper guineense induced adverse and detrimental effects on the gastric mucosa function in rat model. These observations indicated that exposure to Abelmoschus esculentus at doses as high as 500mg/kg body weight and piper guineense at doses of 20mg/kg body weight and above is a risk factor for gastric function impairment and the associated disorders. This work showed Glandular atrophy, intestinal metaplasia, superficial gastritis, gastric erosion, erosive gastritis and gastric ulcer in all experimental groups. Further study is recommended with isolated components of these extracts and with lower concentration of extract of Abelmoschus esculentus and piper guineense to confirm the underlying mechanism and active constituents responsible for the observed activity documented by the results of this study.

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