Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 04, pp.14481-14485, April, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

GASTROPROTECTIVE EFFECTS OF *LORANTHUS MICRANTHUS* AND *ACALYPHA WILKESIANA* LEAVES EXTRACTS ON EXPERIMENTALLY INDUCED ULCER MODELS IN RATS

[1,*]Ijioma, S. N., [1]Nwankudu, O. N. and [2]Nwankwo, A. A.

[1]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria

[2]Department of Human Physiology, College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria

ARTICLE INFO

Article History:
Received 19th January, 2015
Received in revised form
25th February, 2015
Accepted 22nd March, 2015
Published online 28th April, 2015

Key words:

Acalypha wilkesiana,
Indomethacin,
Loranthus micranthus,
Anti-ulcer,
Rats,
Gastrointestinal mucosa.

ABSTRACT

The anti-ulcer activities of *Loranthus miranthus* leaf extract (LOME) and *Acalypha wilkesiana* leaf extract (AWLE) were investigated in rats in which ulcer was induced with indomethacin (40mg/kg). Results obtained indicate significant ($p<0.05$) reduction in ulcers indices in all animals treated with the extracts. LOME at 200 and 400mg/kg lowered ulcer index from 9.17±0.32 in the control group to 0.74±0.55 and 0.52±0.35, with % protections of 91.93% and 94.32% respectively, while same doses of AWLE yielded ulcer indices of 0.95±0.0.64 and 0.88±0.45, with percentage protections of 89.64 and 90.40 respectively. The observed effects of LOME and AWLE compared favourably with that of Cimetidine (32mg/kg), which lowered ulcer index to 6.06±0.09 and presented a percentage protection of 33.91%. LOME also inhibited charcoal transit in experimental rats in a manner similar to that of atropine (1mg/kg), while AWLE did not significantly affect charcoal transit when compared to the control. The results suggest that both LOME and AWLE may contain active agents with anti- ulcer and cytoprotective activities. The observed anticholinergic function of LOME also suggest that the extract may have achieved its effect by inhibiting parasympathetic induced gastric secretions in addition to local mucosa protective effect exhibited by both extracts. *Loranthus micranthus* and *Acalypha wilkesiana* leaves could be harnessed into safe and potent treatment agents for ulcer and could serve as templates for the development of new anti-ulcer drugs.

INTRODUCTION

Ulcers have become an endemic global problem with the developing countries experiencing the scourge in greater dimension. The growing use of non steroidal anti-inflammatory drugs (NSAIDs) has also not helped matters, as these agents increase acidity of the stomach content, erode the mucosa and generate oxygen free radicals that play important roles in the pathogenesis of gastrointestinal mucosal injury (Onwudiwe *et al.,* 2012). They also inhibit cyclo-oxygenases, which leads to reduced synthesis of prostaglandings – the chemical mediators that increase production of cytoprotective mucus in epithelial surfaces (Onwudiwe *et al.,* 2012). The sum effect is the breakdown of the body's mucosal defense leading to the development of ulcer. Peptic ulcer is a lesion of the gastric mucosa and duodenal mucosa occurring at a site where the mucosal epithelium is exposed to acid and pepsin, the imbalance between the gastric acid secretion and gastric mucosal integrity being the precipitating factor (Hemamalini *et al.,* 2012). Factors such as stress, smoking, nutritional deficiency, prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are among other causes of ulcer (Hemamalini *et al.,* 2012; Osim, 2002). Genetic factors have also been implicated (Musumba *et al*., 2009). Infection with *Helicobacter pylori*, a bacterium which disrupts the protective barriers of the gastric mucosa is yet another major cause of ulcer (Osim, 2002). Treatment is therefore aimed at inhibiting gastric acid secretion and enhancing mucosal resistance to acid in addition to eradicating *Helicobacter pylori.* In the face of many side effects associated with use of current orthodox anti-ulcer drugs, many medicinal plants have come under serious scientific scrutiny in a bid to develop new strategies for ulcer treatment. *Loranthus micranthus and Acalypha wilkesiana* are among plants that are traditionally being used for ulcer treatment. *Loranthus micranthus* is a semi parasitic shrub belonging to family *Loranthaceae.* The plant in Nigeria grows by obtaining nutrients and support from a host of trees including *Kola acuminata, Kola nitida, Azadirachta indica, Jatropha curcas and Persia spp*. The leaves extracts of *Loranthus micranthus* has been used traditionally to treat

Corresponding author: Ijioma, S. N.,
Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria

diabetes mellitus, hypertension and schizophrenia (Bamidele *et al.,* 2011). The plant has also been reported to have antidiarrheal, antioxidant, hypoglycaemic, antiarthritic/ analgesic and immunological activities (Zorofchian *et al.*, 2013). Previous works done on phytochemical composition of the plant reveal the presence of terpenoids, steroids, oils, proteins, resins, flavonoids, tannins, saponins and glycosides (Zorofchian *et al*., 2013). *Acalypha wilkesiana* on the other hand is an evergreen shrub usually planted around homes for horticultural purposes. The plant may grow up to 3meters high with erect stems and many branches. The leaves may be coppery green and broad with white teeth around the edge. Previous scientific evaluation of *Acalypha wilkesiana* leaves revealed mycotic/antifungal activity (Oyelami *et al*., 2003). Olukunle *et al.,* (2014), conducted a toxicity study of the leaves extract and reported that liver toxicity was observed at doses up to 1600mg/kg after treatment for 28 days. This present study was designed to investigate the anti-ulcer activity of *Loranthus micranthus* and *Acalypha wilkesiana* leaves extract in animal experimental models to verify/substantiate traditional claims.

MATERIALS AND METHODS

Collection of plants materials and preparation of extracts

Fresh leaves of *Loranthus micranthus* were collected from a farmland in Umudike, Ikwuano Local Government Area while those of *Acalypha wilkesiana* were collected from a local settlement in Ozuitem, Bende Local Government Area both of Abia State, Nigeria. The leaves were air dried at room temperature for 7 days after which they were ground to coarse powder using a manual blender. 50g of each powdered material was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent. Extraction temperature was maintained at 70^0C for 48 hours. At the end of the period, the ethanol was evaporated at low temperature in an electric oven to obtain a crude extract which weighed 11.20g and represented a yield of 22.40% for *Loranthus micranthus* and 12.60g (25.20%) for *Acalypha wilkesiana.*

Animals

Fifty mice (20-25g) and 65 rats (90-140g) obtained from the Animal House unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for the study. They were housed under specific pathogen free (SPF) conditions with 13 H/11 H light/dark schedule and were provided standard feed and water *ad libitum*, but starved for 24 hours prior to commencement of experiments. All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised 1985), as expressed by Akah *et al.,* (2009). The study was carried out in the Physiology Laboratory of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Nigeria.

Acute toxicity test of Loranthus micranthus leaf extract (LOME) and Acalypha wilkesiana leaf extract (AWLE)

For *Loranthus micranthus,* Twenty five mice were divided into 5 groups of 5 mice each and were assigned graded oral doses of LOME in the order 1000, 2000, 3000, 4000 and 5000mg/kg body weight, while for *Acalypha wilkesiana*, 25 mice divided into 5 groups of 5 mice each were also administered graded doses of AWLE in the order1000, 2000, 3000, 4000 and 5000mg/kg . The mice were kept in aluminum cages after administration and allowed free access to feed and water. Observation was made for toxicity signs and number of deaths in each group after 24 hours. LD_{50} values were determined using Karber's formula expressed by Enegide *et al.,* (2013).

Effect of *Loranthus micranthus leaf extract (LOME) and Acalypha wilkesiana leaf extract (AWLE)* on indomethacin induced gastric ulcer in rats

Thirty fve rats divided into 7 groups of 5 animals each were used. Groups 1 (normal control) and 2 (negative control) were given 0.2ml normal saline. Group 3 (positive control) received 32mg/kg cimetidine. Groups 4 and 5 received LOME at the doses 200 and 400mg/kg body weight respectively, while groups 6 and 7 were treated with 200 and 400mg/kg doses of AWLE respectively. Thirty minutes after treatment, all animals in groups 2 to 7 were given 40mg/kg Indomethacin to induce ulcer. All administrations were done via the oral route. Two hours after Indomethacin administration, all animals were sacrificed by cervical dislocation. The stomach of each was carefully isolated and incised along the greater adventure and examined for ulcers. Ulcer index was scored and percentage protection calculated using the methods of Hemamalini *et al.,* (2012).

Scoring for ulcer

Normal Stomach = 0
Red Colouration = 0.5
Spot ulcers = 1
Haemorrhagic streaks = 1.5
Ulcers > 3mm < 5mm = 2
Ulcers > 5mm = 3

$$\text{Ulcers index} = \frac{UA+US+UP}{10}$$

Where:

UA = Average number of ulcers per animal
US = Ulcer severity score
UP = Percentage of animals with ulcers

$$UP = \frac{\text{Total ulcers in a group}}{\text{Total number of Animals}} \times \frac{100}{1}$$

Percentage inhibition of ulcer was calculated using the method of Hemamalini *et al.,* (2012) expressed as:

$$\text{Percentage inhibition} = \frac{UIC - UIT}{UIC} \times \frac{100}{1}$$

Where:

UIC = Ulcers index of control group
UIT = Ulcer index of test group

In vivo effect of LOME and AWLE on charcoal meal transit in rats

Thirty rats of both sexes divided into 6 groups of 5 rats each were used. Group 1 was administered 0.2ml normal saline orally. Group 2 received 1mg/kg Atropine (i.p). Groups 3 and 4 received oral administrations of LOME at doses 200 and 400mg/kg respectively, while groups 5 and 6 were treated with AWLE at doses 200 and 400mg/kg body weight respectively. Thirty minutes later, 0.2ml of charcoal meal was given orally to all the rats. The animals were all sacrificed in a further 30 minutes by suffocation in a chloroform chamber. Each animal was opened and the full length of the small intestine isolated and was measured. The distance travelled by the charcoal meal was also measured and expressed as a percentage of the length of the intestine using the formula:

$$\text{Percentage distance moved by charcoal meal} = \frac{\text{Distance moved by charcoal}}{\text{Full length of intestine}} \times 100$$

Percentage Inhibition for the *in vivo* study was evaluated using the expression

$$\text{Percentage Inhibition} = \frac{A-B}{B} \times 100$$

Where A = % distance moved by charcoal in control
B = % distance moved by charcoal in test

Statistical Analysis

Data were analyzed statistically by application of student's t-test using SPSS version 17 software and presented as means ± standard error of mean (SEM). Values of $p<0.05$ were considered to be statistically significant.

RESULTS

Acute Toxicities of LOME and AWLE

For LOME no death was recorded at the end of the 24 hours of acute toxicity study, even at the highest dose administered (5000 mg/kg). The mice instead had normal disposition both physically and mechanically indicating high safety margin for the leaf extract of *Loranthus micranthus.* However, for AWLE toxicity signs and deaths were recorded in some groups, particularly for groups that received up to 2000mg/kg oral dose of AWLE as1000, 2000, 3000, 4000 and 5000mg/kg produced 0, 1, 2, 3 and 5 deaths respectively. LD_{50} value stood at 3300mg/kg as was evaluated using kerbar's formula.

Effect of *Loranthus micranthus leaf extract (LOME) and Acalypha wilkesiana leaf extract (AWLE)* on indomethacin induced gastric ulcer in rats

All doses of LOME and AWLE used significantly ($p<0.05$) inhibited the development of ulcers in the treated rats with 200mg/kg body weight lowering ulcer indices from 9.17±0.32 in ulcerated control group to 0.74 ±0.55 and 0.0.95±0.64 producing a percentage protection of 91.93% and 89.64% respectively. The protective effects of LOME and AWLE were better than that offered by Cimetidine which produced an inhibition of 33.91%. The mucosal epithelia of all rats treated with LOME and AWLE were not significantly different from those of the normal control rats (Table 1).

Table 1. Effects of LOME and AWLE on indomethacin induced ulcer in rats

Group	Treatment	Ulcer Index	% Protection
1.	Normal control	0.00±0.00	100
2.	Ulcerated control	9.17±0.32	-
3.	Cimetidine, 32mg/kg	6.06±0.09	33.91
4.	LOME, 200mg/kg	0.74±0.55*	91.93
5.	LOME, 400mg/kg	0.52±0.35*	94.32
6.	AWLE, 200mg/kg	0.95±0.64*	89.64
7.	AWLE, 400mg/kg	0.88±0.45*	90.40

*= $P<0.05$ for test versus ulcerated control

Effects of LOME and AWLE on charcoal meal transit in rats

All doses of LOME significantly ($P<0.05$) reduced the distance moved by charcoal meal in the rat's gastrointestinal tract when compared to control as 200 and 400mg/kg of LOME inhibited charcoal transit by 28.87 ± 4.67 and 33.89±2.40% respectively and compared favorably with the effect of atropine (1mg/kg) which yielded a mean 41.28±4.15% inhibition. AWLE on the other hand did not significantly inhibit charcoal transit in the treated rats (Table 2).

Table 2. Effects of LOME and AWLE on charcoal transit in rats

Group	Treatment	Mean % distance moved by charcoal	Mean% inhibition of charcoal movement
1	0.2 normal saline	92.77 ± 6.68	
2	Atropine, 1mg/kg	60.98 ± 7.32*	41.28 ± 4.15
3	LOME, 200mg/kg	65.98 ± 4.45*	28.87 ± 4.67
4	LOME, 400mg/kg	61.33 ± 7.35*	33.89 ± 2.40
5	AWLE, 200mg/kg	88.40 ± 9.28	4.71 ± 5.76
6	AWLE, 400mg/kg	85.62 ± 7.88	7.71± 6.80

*=$P<0.05$ for test versus normal control

DISCUSSION

Indomethacin, a non steroidal anti-inflammatory, analgesic and antipyretic agent became the first drug to produce ulcers in the experimental animals by gastric damage via inhibition of the release of protective factors like cyclo-oxygenase enzyme, prostaglandins, bicarbonate and mucus and increasing aggressive factors like acid, oxidant parameters with corresponding decrease in antioxidant parameters. These physiological processes have indeed being implicated in the induction of ulcer by non steroidal anti-inflammatory drugs (NSAIDs) like indomethacin (Suleiman *et al.,* 2010; Musumba *et al*., 2009). Morsy and Fouad, (2008) had reported that increase of gastric mucosal lesions, gastric acid output, increased pepsin activity and rise in nitric oxide and free radical levels are part of mechanisms responsible for indomethacin induced ulcers. Prostaglandins have been reported to be key factors in mucosal cytoprotection and achieve this by exerting positive influence on mucus and bicarbonate secretion on surface epithelial cells, mucosal circulation, prevention of hemorrhagic lesion and accelerating platelets aggregation.

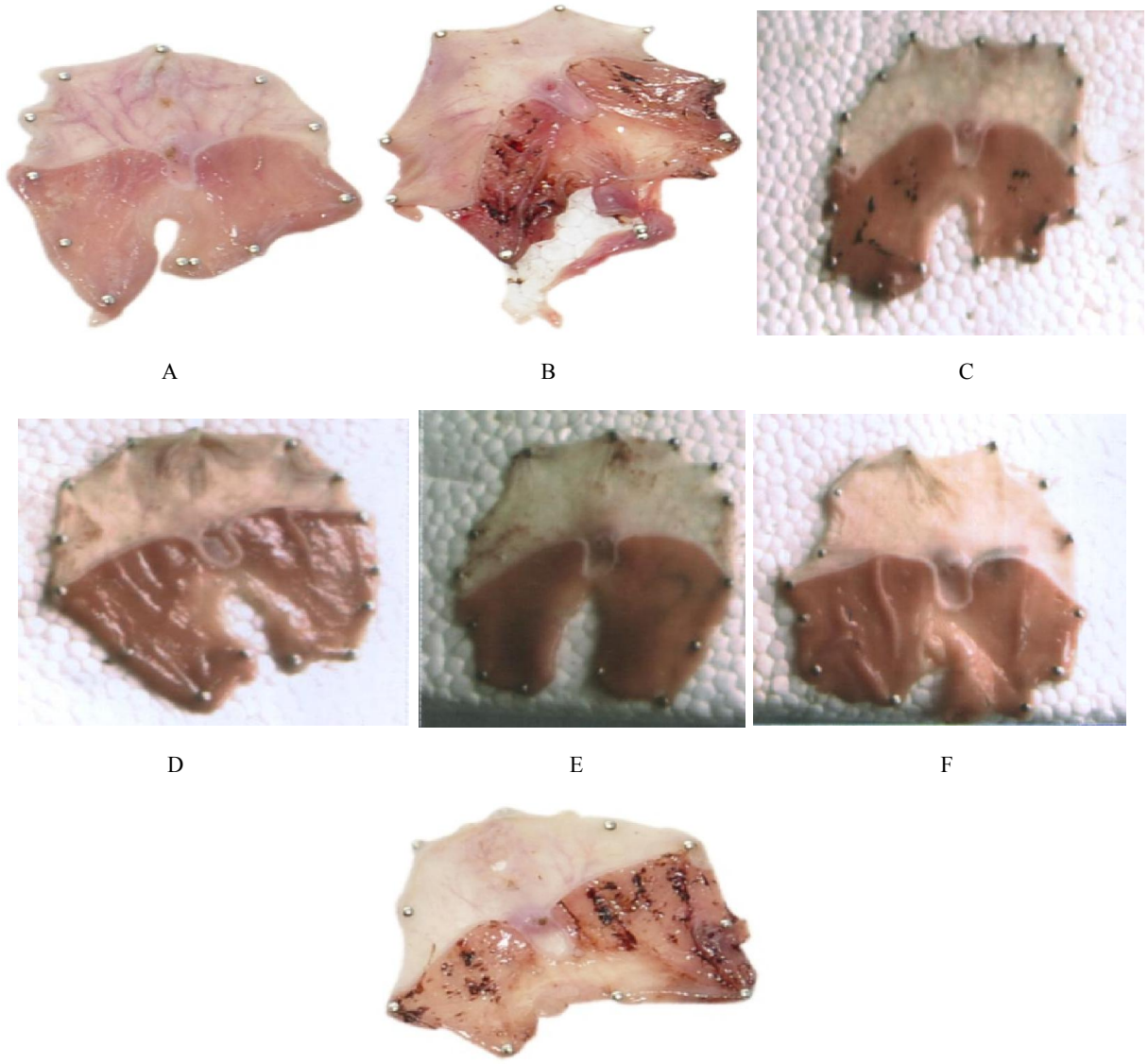

Figure A-F. Anti-ulcer activity of LOME and AWLE in experimental rats
A: Stomach mucosa of rats in which no ulcer was induced (normal control)
B: Ulcerated control rats with marked lacerations of the stomach mucosa
C: Protective effect of LOME (200mg/kg)
D: Protective effect of LOME (400mg/kg)
E: Protective effect of AWLE (200mg/kg)
F: Protective effect of AWLE (400mg/kg)
G: Protective effect of Cimetidine (32mk/kg)

By these effects, prostaglandin protects the mucosa from ulceration (Ananya *et al.,* 2012). Moreover, free carboxyl group present in all NSAIDs form a strong electrostatic bond with positively charged head of Zwitterionic phospholipids of mucus layer, such that the resulting increase in the solubility of the phospholipids neutralises its surface activity and topically act on tissue to disrupt the hydrophobic protective lining of the mucus gel layer leading to ulcerations (Al-Harbi *et al.,* 1995). These may be the mechanism behind the induction of ulcers in the current experiment (Fig. B). Treatment with LOME and AWLE at all doses used significantly inhibited indomethacin induced ulcers in the treated rats and offered significant protection to the stomach mucosa.

These results suggest that LOME and AWLE contain principles with anti-ulcer and mucosa protective activities. The presence of flavonoids in leaf extract of *Loranthus micranthus* has been reported (Lewis *et al*., 1999). Rajesh *et al.* (2013) also reported the presence of flavonoids in leaf extract of *Acalypha wilkesiana*. Flavonoids have indeed been implicated in wound healing, cellular regeneration and cytoprotection (Lewis *et al.,* 1999; Kumar *et al*., 2013). In addition to its flavonoid content, AWLE was also found to possess antioxidant and anti-acid property, which plays major role in stress alleviation and may be part of the its anti- ulcer mechanisms. The anti- cholinergic activity exhibited by LOME suggests that the extract could inhibit gastric acid

secretion by providing sufficient block to gastrointestinal parasympathetic activity which may have contributed to the its observed mucosa protective effect. Osadebe *et al.,* (2012), had reported the antimotility effect of *Loranthus micranthus* on the gastrointestinal tract. It is also on record that agents with anti-secretory/antimotility effects on the gastrointestinal tract can be used to limit the development of ulcer (Rajesh *et al.,* 2013). The reported antibacterial property of *Acalypha wilkesiana* and *Loranthus micranthus* leaves extracts may yet be another advantage (Rajesh *et al.,* 2013; Osadebe, 2006), since this could inhibit the growth of *Helicobacter pylori*- a major player in ulcer development. The extracts may also have achieved this cytoprotective effect by enhancement of the release of protective factors like cyclo-oxygenase, prostaglandin, bicarbonate, mucus, and increasing mucin concentration and increasing gastric mucosal blood flow. These mechanisms have also been implicated in ulcer healing and prevention/control (Hemamalini *et al.,* 2012; Ananya *et al., 2012;* Adewoye and Salami, 2012). To conclude, this study has shown that leaf extracts of *Loranthus micranthus* and *Acalypha wilkesiana* contain principles with anti-ulcer activity and that oral administration of the extracts may be valuable in the prevention/treatment of ulcer and may eliminate the numerous side effects associated with the use of orthodox antiulcer agents in addition to providing templates for the development of new anti-ulcer drugs.

REFERENCES

Adewoye, E.O. and Salami, A.T. 2012. Anti-ulcerogenic mechanism of magnesium in Indomethacin induced gastric ulcer in rats. *Niger J. Physiol Sci*., 28, 193-199.

Akah, J.A., Lemji, J.A., Salawu, O.A., Okoye, T.C. and Offiah, N.V. 2009. Effects of *Vernonia amygdalina* on biochemical and haematological parameters in diabetic rats. *Asian Journal of Medical Sciences* 1(3): 108 – 113.

Al-Harbi, M.M. Islam, M.W., Al-Shabanah, O.A. and Al-Gharably, N.M. 1995. Effects of acute administration of fish oil (Omega-3 marine triglycende) on gastric ulceration and secretion induced by various ulcerogenic and neurotizing agents in rats. *Fed. Chem. Toxic, 33(7)*, 555-558.

Ananya, C., Sirshendu, C., Smita, D., Arpita, S., Sulivata, C. and Sandip, K.B. 2012. Ellagic acid facilitates Indomethacin-induced gastric ulcer healing via COX-2 up regulation. *Acta Biochim Biophys. Sin*, 44 (7): 565-576.

Bamidele, A.I., Sedoten, A.H., Nwoke, S., Olabisi, O. and Philip, A.I. 2011. Evaluation of the possible mechanisms of antihypertensive activity of *Loranthus micranthus*: An African mistletoe. *Biochemistry Research International*, 2011, Article I.D: 159439

Enegide, C., David, A. and Fidelis, S.A. 2013. A new method for determining acute toxicity in animal models. *Toxicology International*, 20(3): 224 – 226

Hemamalini, K., Lavanya, C.H., Anurag, B. and Uma, V. 2012. Anti-ulcer Activity of Methanolic Extracts of *Wattakaka volubilis* and *Tabebula rosea* in rats. *Asian Journal of Pharmaceutical and Clinical Research.* 5(3); 242-246.

Kumar, M., Manish, K.G., Anit, S. and Goel, R.J. 2013. Healing effects of Musa sapientum var. Paradisiacal in diabetic rats with co-occuring gastric ulcer, cytokines and growth factor by PCR amplification. *BML Complementary and Alternative Medicone*, 13, 305.

Lewis, D., Field, W., Shaw, G. 1999. A natural flavonoid present in unripe plankain banana pulp protects the gastric mucosa from aspirin-Induced erosion. *Journal of Ethnorpharmacology*, 65(3): 283-6.

Morsy, M.A. and Fouad, A.A. 2008. Mechanism of gastroprotective effects of Eugenol in Indomethacin induced ulcer in rats. *Phytother. Res.*, 22 (10): 1361-6.

Musumba, C., Pritchard, D.M. and Pirmohamed, M. 2009. Cellular and molecular mechanism of NSAIDs- induced peptic ulcers. *Aliment. Pharmacol. Ther* 30, 517-531.

Olukunle, J.O., Jacobs, E.B., Ajayi, O.K., Biobaku, K.T., Abatan, M.O 2014. Toxicological evaluation of the aqueous extract of *Acalypha wilkesiana* in Wista albino rats. *Journal of Complementary and Integrative Medicine,* Vol.8

Onwudiwe, T.C., Ughachukwu, P.O., Unekwe, P.C. and Ogamba, J.O. 2012. Evaluation of Antiulcer properties of ethanolic and hot aqueous Stem Extracts of Synclisia scabrida on Experimentally induced ulcer models in Albino Mice. *Annals of Medical Science Research,* 2(2): 134-139.

Osadebe, P.O 2006. Antimicrobial activity of *Loranthus micranthus* harvested from Kola nut tree. *Fitoterapia*, 77(1): 54-56.

Osadebe, P.O., Abba, C.C., Agbo, M.O 2012. Antimotility effects of the extracts and fractions of Eastern Nigerian Mistletoe (*Loranthus micranthus* Linn.). Asian Pacific Journal of Tropical Medicine, 5(7): 556-560.

Osim, E.E. 2002. Elements of Gastrointestinal Tract Physiology. Helimo Associates Publishers, Calabar, Nigeria. pp87-88.

Oyelami, O.A., Onayemi, O., Oladimeji, A., Onawunmi, O. 2003. Clinical evaluation of Acalypha ointment in the treatment of superficial fungal skin diseases. *Phytotherapy Research*, 17: 555-557

Rajesh, K., Prakash, G. and Manjuryatha, H. 2013. Invitro antioxidant and H^+, K^+-ATPase inhibition of activities of Acalypha wilkesiana foliage extract. *Journal of Pharm Bioallied Sciences*, 5(3): 214-223

Suleiman, H.I. Albayrak A., Bilici M., Cadirci E., Halici, Z. 2010. Different mechanisms in formation and prevention of Indomethacin induced gastric ulcers. *Inflammation*, 33 (4), 224-34.

Zorofchian, M.S., Hairezae, A.H., Zandi, k. 2013. *Loranthus micranthus* Linn: Biological activity and phytochemistry. *Evid Based Complement of Alternative Medicine,* 2013: 273712

****