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Anti-Ulcer Histomorphological Effect of Dr. Iguedo Goko Herbal Mixture, Esomeprazole and Ethanolic Extract of *Annona Muricata* (Soursop) Leaves, On Albino Rats Gastrointestinal Tract (GIT)

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ABSTRACT: The potentials in the use of plants and herbal remedies to provide newer drugs and as such are reservoirs of natural chemicals that can provide chemo protection against indomethacin (NSAIDS) induced ulcer or any form of inflammation in the GIT. This study aimed at investigating the role of Dr. Iguedo goko cleanser, esomeprazole and ethanol extract of Soursop [A. muricata leaves] on the histomophology of the GIT [stomach and colon] in indomethacin induced ulcer in albino wister rats. Dr. Iguedo goko cleanser was sourced from the distributor in OWERRI metropolis Imo State, esomeprazole [AstraZeneca brand] was also sourced from a pharmaceutical shop in OWERRI metropolis Imo State. Maceration ethanolic method was used for A.muricata leaves. Forty eight [48] apparently healthy albino rats of both sexes were classified into 6groups. Group 1 normal control, group 2 indomethacin, group 3, esomeprazole, group 4 Dr. Iguedo goko cleanser, group 5 A.muricata extract 1000mg/kg, group 6 A.muricata extract 2000mg/kg. Rats in group1were given only food and water no treatment. Rats in group 2 were induced with indomethacin 30mg/kg once. Rats in group 3 were treated with esomeprazole after indomethacin induction. Rats in group 4 were treated with Dr.Iguedo goko cleanser after ulcer induction. Rats in group 5were treated with 1000mg/kg of A.muricata leaves extract after ulcer induction. Rats in group 6 were treated with 2000mg/kg of the A. muricata leaves extract after ulcer induction. The treatment lasted for 2weeks. Comparative observations of the indomethacin induced GIT, clearly reveals induction of histopathology/cytology alterations as evidenced by emptied goblet cells, lacerations and suppressed mucosa, ulceration, destruction of surface epithelium which were not evidence in the control group .Treatment group exhibited marked repair and improvement in the histomophology of the GIT, which were better in the herbal mixture and the extract when compared with the reference drug. The result suggested that the herbal mixture and the extract of A.muricata have more promising therapeutic role against inflammation and ulceration induce in the albino rats.

KEYWORDS: anti-ulcer, dr. iguedo goko herbal mixture, esomeprazole, ethanolic, annona muricata (soursop) leaves, albino rats, gastrointestinal tract

INTRODUCTION

The utilization of traditional medicine (TMs) globally has a rich history as an accessible and affordable source of treatment. Traditional herbal medicine, with substantial historical use, coexists with modern medicine, often retaining popularity for historical and cultural reasons[1].The contemporary surge in using natural ingredients for disease treatment underscores the increasing importance of plants as sources of medicinal compounds. These compounds, responsible for plant activities against various diseases, can be identified through studies, highlighting the escalating use of natural products in disease treatment [2]

The global significance of herbal products arises from their minimal side effects, accessibility, and cost-effectiveness compared to orthodox drugs [3] Gastric ulceration, resulting from excess acid and aggressive pepsin activity on the stomach mucosal epithelium, remains a prevalent gastrointestinal disorder with significant mortality and complications [4]. Despite advancements in gastric ulcer management, gastrointestinal toxicity, particularly from nonsteroidal anti-inflammatory drugs (NSAIDs), poses challenges. Synthetic antiulcer drugs like cimetidine, misoprostol, ranitidine, omeprazole, and esomeprazole, while effective, carry side effects, necessitating exploration of non-toxic, accessible, and affordable alternatives [5]

Gastric ulcer, affecting approximately 10% of the global population, poses a significant health challenge, leading to complications such as hemorrhages and perforations if inadequately treated. Prolonged drug use for gastric ulcer management can result in adverse effects, emphasizing the need for alternative treatments with gastroprotective and mucosal healing properties [6]

The increasing global importance of herbal products, known

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for their favorable attributes, makes them a valuable consideration for gastric ulcer treatment. Traditional medicinal remedies, including herbal mixtures, are often indiscriminately consumed, especially by the younger population, lacking awareness of potential organ consequences. This study addresses the need to evaluate the effects of Dr. Iguedo Goko herbal mixture, esomeprazole, and ethanolic extract of A. muricata leaves on the gastrointestinal tract (GIT) of albino rats.

MATERIALS AND METHODS

Study Area

The study was carried out at Animal House of the department of Anatomy Faculty of health Sciences, Imo state University Owerri Imo state Nigeria. Imo state is located in the South Eastern part of Nigeria. It is located on longitude 7.035oE of the Greenwich meridian and latitude 5.485oN of the equator. It is approximately 100 square kilometers. Owerri is bordered by the Otamiri River to the East and the Nwaorie River to the South.

Collection and Preparation of Plant Materials

A. muricata leaves were sourced from a nursery in Owerri Imo State. The leaves were dried at room temperature for 2weeks, pulverized with an electric blender. 1000g of the leaf powder was weighed and ethanolic extraction carrried out with e using soxhlet extractor

Sources of Other Medicinal/Treatment Materials

i.Dr. Iguedo Goko herbal mixture: the herbal mixture is widely distributed in Nigeria and was purchased from a distributor in Owerri Imo State. It comes in liquid of 200ml per bottle.

ii.Esomeprazole: (Astral Zanica Product) 20mg/kg was purchased from an approved pharmaceutical shop in Owerri Imo State.

Experimental Animals

Healthy Albino wistar rats of either sex of 12weeks old were purchased from available sources. They were kept in the animal house of Anatomy department Imo State university Owerri. They were left to acclimatized for 1week.

Ulcer Induction

The rats were deprived of food for 24hrs., but have free access to water for 24hrs. 30mg/kg of indomethacin was administered with a single oral dose.

Experimental Design

Forty-eight albino rats of either sex with average weight of 150g rats were randomly assigned into (6) groups, I, to VI and maintained under standard laboratory conditions.

Experimental Rat Placed into Groups

Group of Rats	Treatment	No of rats
GROUP I	Normal Control (food &water)	Nine rats
GROUP II	Indomethacin group	Nine rats
GROUP III	Esomeprazole treatment group	Nine rats
GROUP IV	Dr. Iguedo Goko herbal mixture group	Nine rats
GROUP V	Low dose A. muricata group	Nine rats
GROUP VI	High dose A. muricata group	Nine rats

Treatment of the animals of all groups with reference drug and extract lasted for 2weeks and it was given once daily once daily prior to ulcer inducement.

a.Embedding

This was done using Tissue Tek embedding mould and Tissue Tek cassette. The tissue which were impregnated are placed in a mould, orientated and the mould was filled up with the molten paraffin wax. The wax was cooled, solidified and then removed from the mould. Embedding confers firmness and solid support to tissue to perform microtomy.

b.Microtomy

Material required during microtomy was: water bath, diamond pencil, glass slides, hot plate, staining rack, forceps glass and Carmel hair brush. Water bath, diamond pencil and glass slides, .With the aid of a Rotary microtome, tissue blocks of individual groups was sectioned, at (2-3) two – three micron. Ribbons of serial sectioning were produced. These sections were transferred to a slide on which 20% alcohol has been droped, before floating was done.

c.Floating

With the aid of a forcep and a camelhair brush, the serial ribbon colon sections were transferred to the water bath the temperature of the water bath stretches and flattens.

d.Picking/floating out

With the aid of a clean grease free side, fine sections are picked up, drain off excess water and place on hot plate.

e.Hot plate

Hot plate enhances the attachment of sections to side properly so that they may not be lifted during staining.

Staining

This was done by Harris Haematoxylin and Eosin Method

Slides were mounted with DPX and a clean grease free cover slip. All stained sections were cover slipped with (DPX) Dibutyl phthalate polystyrene xylene a synthetic non-aqueous mounting medium for microscopy. DPX impacts to the

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refractive index of the slide during microscopy.

Microscopy

The sections of all the experimental rats were viewed using leica ICC50 HD light microscope.

RESULTS

PHYSICAL OBSERVATIONS

Fur appearance at the onset of the experiment before the administration of the products, the fur of all the animals were smooth in all the animal groups. As the ulcer was induced in the treatment groups prior to starvation for 24hrs. with only water given, the fur appearance changed. The fur appearance became rough but during the period of administration, the fur appearance began to improve in its smoothness appearance compared to the control group that was not induced with the ulcer.

Food and water intake: the rate of consumption of food and water was decreased in the treatment groups compared with the control group. It was also observed that the treatment group was passing urine more frequently than the control group, though the rate of stool passage and its consistency was observed to be normal with the control.

Morphologic changes observed on the gross is swelling of the abdomen in the treatment groups on the first 2 days of the ulcer induction, which was observed returning to normal as the treatment continues.

Sign of weakness was noticed after the induction and first 2 days on commencement of the treatment.

MEASURABLE DATA

Table 4.1: Mean Weight (g) of experimental animals

Time(weeks)	Group 1	Group II	Group III	Group IV	Group V	Group VI
Before administration	150±2	151±2	150±1	151+2	150+3	154+1
1st Week	190±1	190±2	192±2	192+1	189+1	190+2
2nd Week	200±3	-	180±3	208 +2	200+2	210+3

Mean Body Temperature (0C) of Experimental Animals

Time (Weeks)	Group I	Group II	Group III	Group IV	Group V	Group VI
Before Administration	37.0±2	36.6±2	37.9±2	38.0±1	38.0 ±1	37.0+2
1st Week	37.7±1	-	37.7 ±2	37.0±2	37.7±2	37.0+2
2nd Week	38.0±1	-	37.0±1	37.0±2	38.0+1	37.0+2

Histological observations

Haematoxylin and eosin technique

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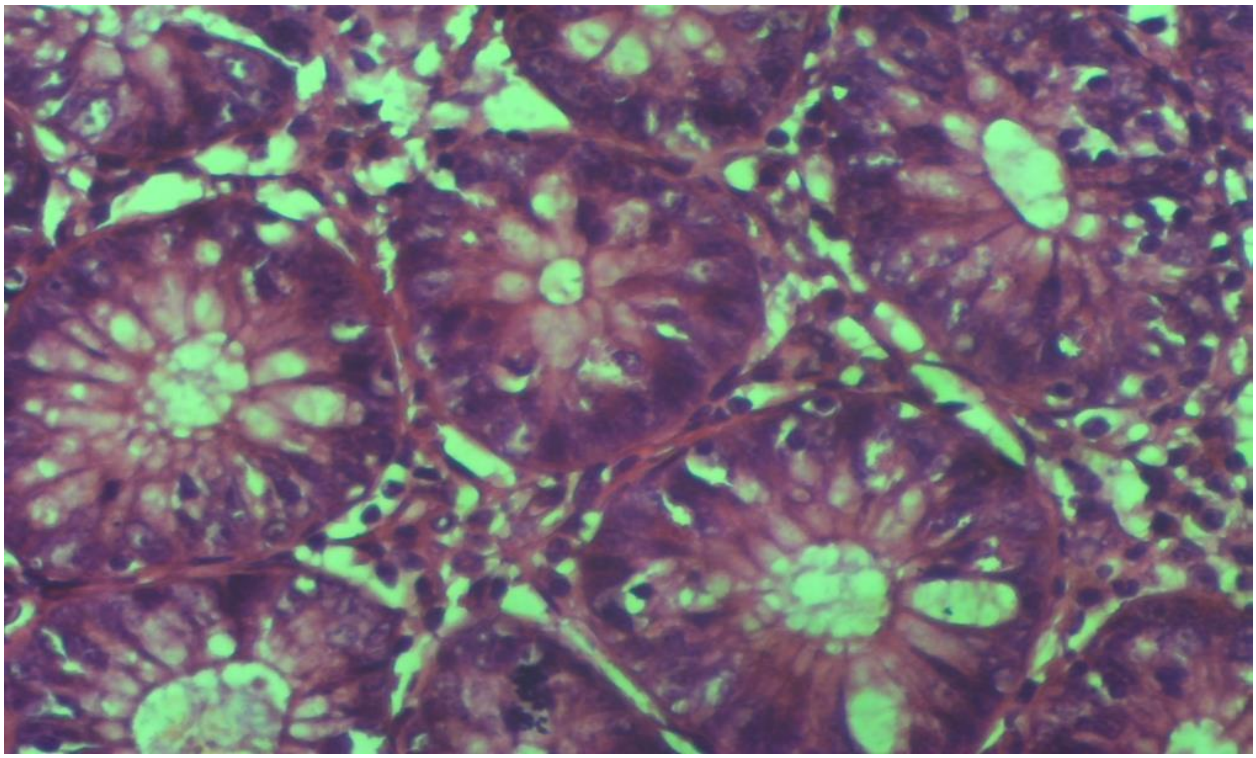

Plate 1: Histological section of colon tissue shows intact goblet cells, muscularis and epithelium no ulceration, nor colonic mucosa aberration (H&E stain total magnification X 400 X40). Group 1 Normal Control; Colon

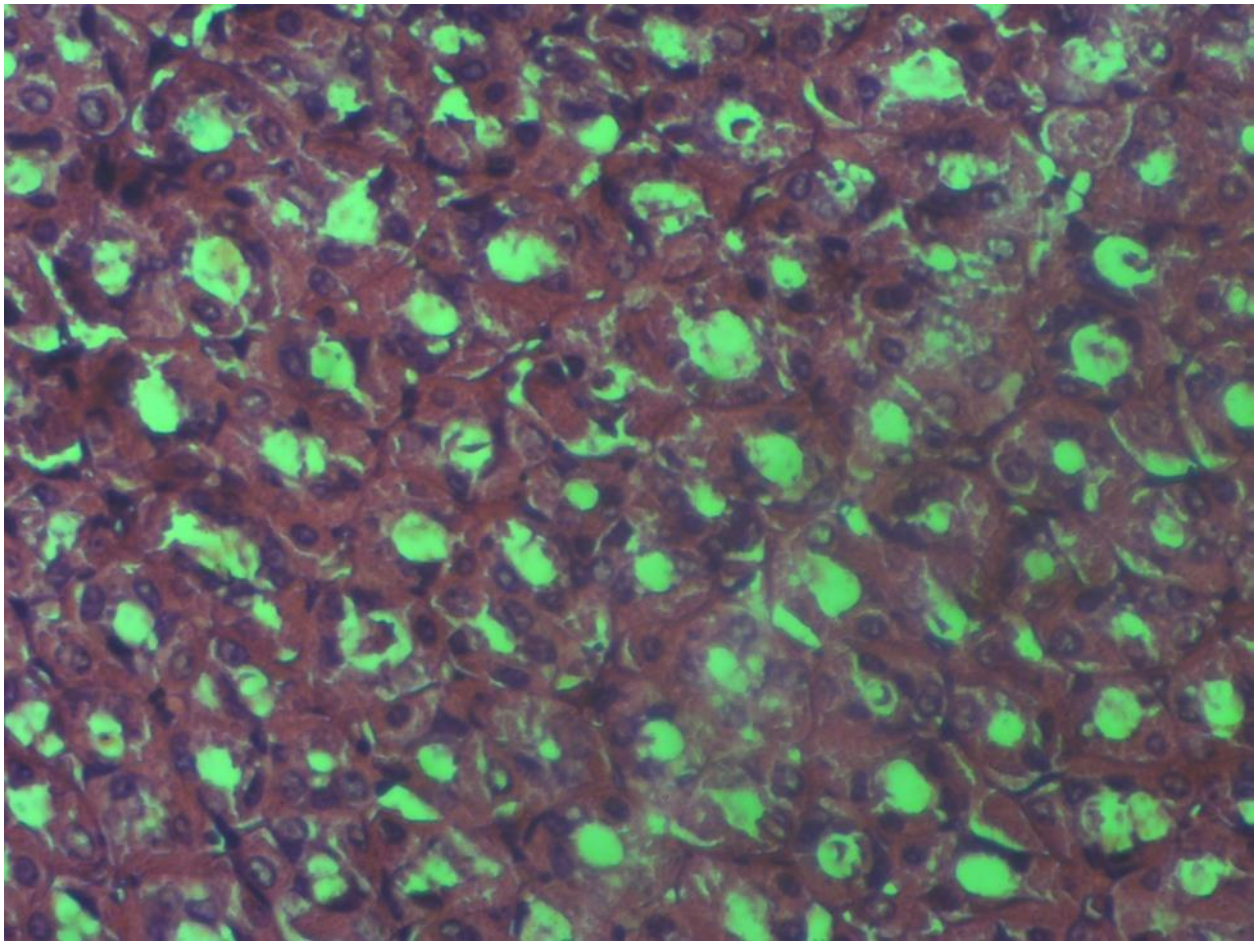

Plate 2: Histological sections of stomach tissue shows normal appearing chief cells, muscularis (gastric glands) and mucosa. (H&E stain total magnification X 400 X40) Group 1 Normal Control; Stomach

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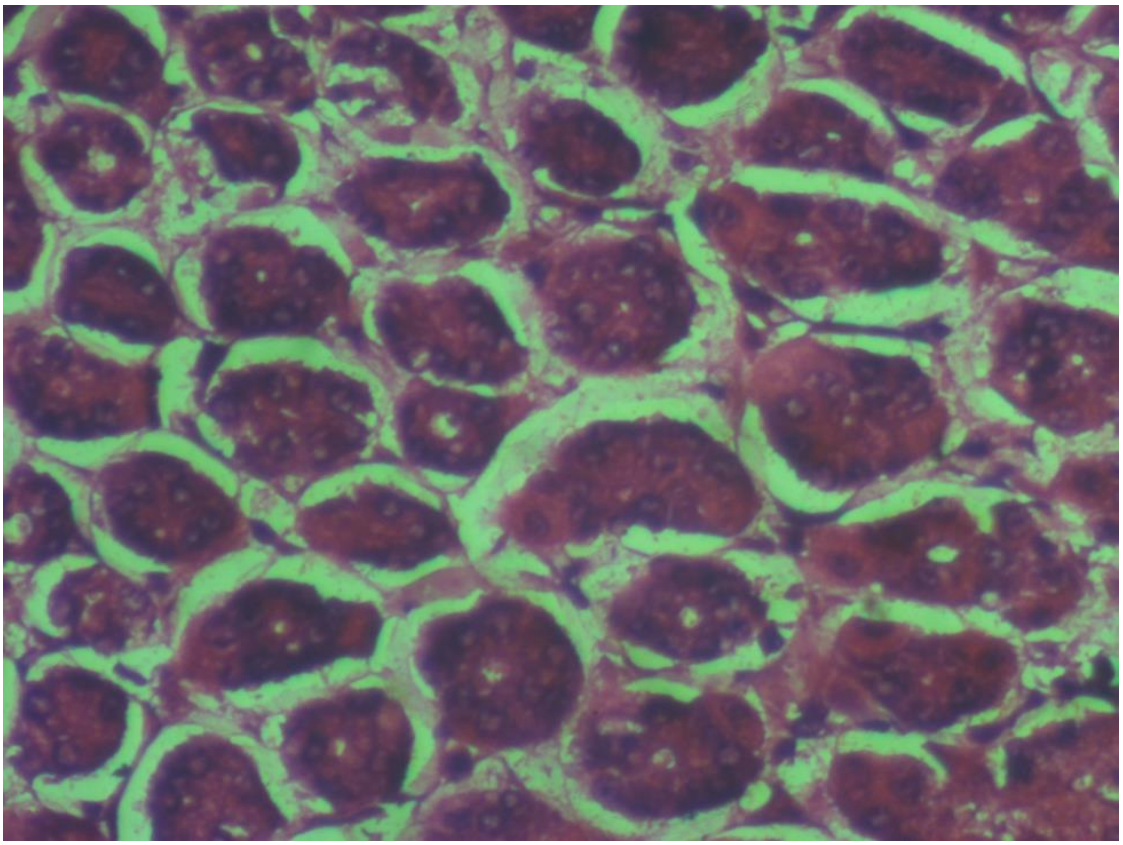

Plate 3: Histological sections of colon tissue shows aberration of the colonic mucosa, severe ulceration and distortion of the goblet cells and muscularis. (H&E stain total magnification X 400 X40).
Group2 (Indomethacin); colon

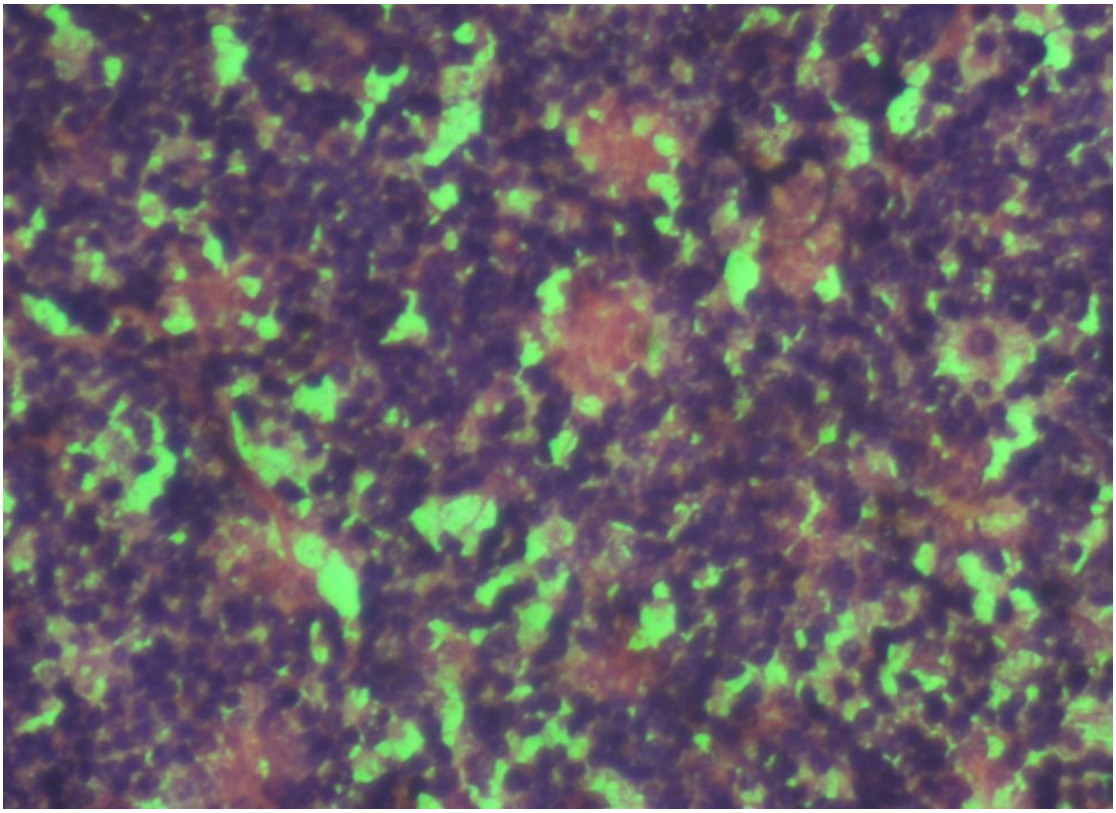

Plate 4: Histological sections of colon tissue shows severe aberration of the colonic mucosa, severe ulceration, damage of the goblet cells and muscularis with inflammations. (H&E stain total magnification X 400 X40).
Group2colon (Indomethacin)

DISCUSSION

The current study was to determine whether Dr. Iguedo Goko mixture and extract of A. muricata leaves could decrease the risk of indomethacin induced ulceration(gastritis) rate over the reference drug esomeprazole. According to the result obtained, ulceration of different degree was observed with few inflamed cells. This result corresponds to report of [5] in which indomethacin exposures of laboratory animals caused tumors and various degrees of ulceration.

In all the experimental groups it was observed that there no decrease in the body weight of the animals after induction however, in the A. muricata groups, fluctuation in weight was recorded after treatment in the group, where after there was increase. This could be as a result of the nutritional contents in the extract recorded by [6]

From the physical observation, the rate of consumption of food by the treatment groups, decreased when compared to that of control group, this may be due to the ulceration effect. The frequent passage of urine by the treatment groups when compared to the control could be as a result of the high water intake by the treatment groups.

The temperature of the animals in all the groups though there are variations, still fall within the normal range of laboratory animal temperature (39.0+ 2oC) in all the groups before and during the period of administration. Thus there were no obvious pyrexia.

Fur appearance at the onset of the experiment before the administration of the products, the fur of all the animals were smooth in all the animal groups. As the ulcer was induced in the treatment groups prior to starvation for 24hrs. with only water given, the fur appearance changed [7].

The Treatment Plan for all Rats in all experimental groups as shown in Treatment Table showed that after induction with indomethacin, animal in the group displayed dull looks and reduced physical activity. The GIT harvested from this group revealed histopathology report of severe inflammation and ulceration. After treatment, rats in the various experimental test group showed different signs of reduced activity rate, standing, paw licking, and reduced feeding rate. Ultimately, very few of the rats died. But as the treatment progresses, the rats that survived displayed an improved healthy appearance until the end of the experiment. Moreso, the histopathology report after treatment with different extracts revealed a marked improvement. The influence of A. murucata leaves extract and Dr Iguedo goko herbal mixture restored the damage done by indomethacin to the rat GIT better when compared to the reference esomeprazole drug [8].

GIT (stomach and colon) of rats treated with Dr. Iguedo goko herbal misture, ethanolic extract of A muricata leaves continue to display a close resemblance to the GIT in the normal control group. Hence this work is suggestive of the fact that the products possesses anti-ulcer and inflammatory therapeutic property.

It has been shown that the extract reduces ulceration by activating prostaglandin synthesis as a gastro protector and suppressed aggressive factors of gastric mucosa [9] Another mechanism of its antiulcer activity is the upregulations of Hsp70 and downregulation of Bax, which takes part in gastric injury suppressions [10]

CONCLUSION

Indomethacin induced ulceration/ toxicity in the present research which was ameliorated by the A. muricata leaves extract given was more and Dr. Iguedo Goko herbal mixture was more potent than the reference drug esomeprazole 20mg/kg. This is an indication that A. muricata leaves extract and Dr. Iguedo Goko herbal mixture possesses high restorative, anti-inflammatory and therapeutic potential than the reference drug.. Though the herbal products may contain the above mentioned properties, but should be taken with caution pending when an establish dosage was approved by a regulatory body.

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