HISTOMORPHOLOGICAL ALTERATION OF LUNG AND GIT FUNCTION AND POSSIBLE ALLEVIATIVE ROLE OF HERBAL MEDICINE (*ZINGIBER OFFICINALE, CURCUMA LONGA, GARCINIA KOLA)* ON WISTAR RATS

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ABSTRACT

Background: Herbal medicine is widely used globally for different purposes. It is used as a curative medicine, preventive medicine, food supplement and also serve economic purpose here in Nigeria.

Aim: This research item is aimed at evaluating the Histomorphological alteration of lung and GIT function and possible role of herbal medicine (Z*ingiber officinale, Curcuma longa, Garcinia kola)* on wistar rats.

Materials and Methods: A total of 40 rats were used for this research. The research was carried out in two phases. Stage 1, was divided into two groups (1 and 2). Group 1 with 6 rats was used as control while group 2 received 10mg /kg of cyclophosphamide made up of 34 rats. After 5 weeks, the animals were weighed, anaesthetized, 6 of the 34 rats were sacrificed alongside the control and organ of interest harvested and examined histologically. In the stag 2, the remaining 28 rats were further divided into seven groups, group A-G, with four rats in each group, A, B, C, D, E, F and G received water and rat pellet, 600 mg/kg weight of *Zingiber officinale*, *Curcuma* l*onga, Garcinia kola* respectively, 600mg / kg, 1200mg / kg and 2400mg / kg per b/w of combine extract respectively. After six weeks period, all animals were weighed, anaesthetized and sacrificed GIT and lungs harvested for histological examination.

Results: Significant weight loss, inflammation of the git and lung tissue in rats treated with cyclophosphamide, amelioration of the aforementioned degeneration was seen in pooled extract treated rats

Conclusion: The result shows that the plant extracts has reparative potential

Keywords: Herbal medicine, *Zingiber officinale*, *Curcuma longa*, Bitter kola

INTRODUCTION

Herbal has been in use since the days of our fore fathers and still being used today. It can use here in Nigeria for multiple purposes such as treatment, in preventive medicine, as food supplement and also serve economic purpose when sold.

In most parts of Africa and Asia, *Zingiber officinale* is utilized as an herbal medicine for inflammatory reactions, viral and bacterial infections. Various claims have been made about its ability to cure various ailments such as diabetes, cardiovascular illnesses, and GIT problems. *Garcinia kola* is widely used in Western Africa, as well as other parts of Africa and Asia. This plant is thought to be extremely powerful and capable of curing a wide range of ailments, including viral and bacterial infections, as well as serving as antidotes for poisoning (Ammon, *et al.*,1992).

The fruit is thought to work against anything foreign in the human body if used properly. It was extensively praised by researchers as a powerful herbal treatment in the fight against Ebola, and it is now also recommended for the emerging corona virus sickness (Covid-19) (Iyare *et al.*, 2022)

Cyclophosphamide is a medicine that belongs to the alkylating agent and nitrogen mustard families. It is thought to work by interfering with DNA duplication and RNA production. In the United States, cyclophosphamide was licensed for medicinal use in 1959. It is listed as an essential medicine by the World Health Organization (WHO, 2019)

The uncontrolled use of herbal medicine in Africa, specifically in Nigeria, the over the counter usage of orthodox medicine and the believe that herbal medicine can treat, heal or alleviate wide varieties of illnesses and even Sars Cov-2, the causative agent for covid 19 is a serious national issues ((Iyare, et al., 2022), Hence, this research item is aimed at evaluating the Histomorphological alteration of lung and Git function and possible role of herbal medicine (*Zingiber officinale, Curcuma longa, Garcinia kola)*in alleviating the effect on Wistar Rats

Materials and Method

Plant Preparation and Harvesting

Curcuma longa, Zingiber officinale, and Garcinia kola seeds were purchased and carefully washed at the New Benin Market in Benin city, Edo state. They were taken to the University of Benin, Department of Plant Biology and Biotechnology for identification and authentication, and a voucher number 11021 was assigned to them. The dried leaves were crushed into powder using a blending machine.

Analytical Phytochemistry

A qualitative phytochemical analysis was performed using a method outlined by Sheikh *et al,* (2013) to evaluate the phytonutrient contents in the blended powder of the seeds.

Ethanol Extraction

Five hundred gram (500 g) each of *Zingiber officinale, Curcuma longa, Garcinia kola* and pooled extract powder was dissolved in 5000 ml of 70% ethanol, sealed in a bottle, and left for four days with constant stirring and shaking at intervals. In addition, 150 g powder extracts *(Zingiber officinale, Curcuma longa, and Garcinia kola*) were dissolved in 1500 mL of ethanol and macerated for four (4) days. The solutions were then filtered to remove unwanted debris and the liquid was evaporated to dryness using a rotary evaporator.

Animal Sources

A total of 40 albino rats of both sexes ranging in age from 2 to 3 months were used in the study. The Wistar rat stain of Albino rats were purchased from the Department of Animal and Environmental Science, Faculty of Life Sciences, University of Benin animal house and they were housed for a period of two weeks to allow them acclimatize. They were fed with rat pellet and allowed access to water. The female rats were kept in separate cages from the male rats.

Ethical Consideration

Policies outlined in the Guide for the Care, Handling, and use of Laboratory Animals were strictly adhered to in the course of this work (NRC, 2012).

Acute Toxicity Study

Acute toxicity testing in this study was carried out following method conducted and described by Lorke's (Lorke, 1983).

The median lethal dose (LD_{50}) was calculated as follows:

LD_{50} = (minimal lethal dose) x (maximum tolerated dose)he dosage thereafter was informed by following the LD_{50} minimal dose an animal can be exposed to accidentally or at a time.

Experimental Design

In this study, 40 wistar strain of Albino rats of both sexes with an average weight of 200 g were used for the purpose of this research. The animals were thereafter weighed and recorded at the start of the study. The study was divided in two stages (1 and 2).The stage 1 of the research was divided into two Group A and B:

Group A which comprise of 6 rats were administered water and rat pellet and serve as control.

Group B: This rats in this group which were randomly distributed into 5 separate cages received 10 mg/kg body weight of cyclophosphamide across board for five (5) weeks period. After the 5th week, six (6) rats out of total of 34 rats were randomly selected and separated from the 5 cages in the group B. The six (6) randomly selected rats from group B and all the rats in group A were weighed and kept in a closed container before they were anesthetized with chloroform inhalation. This method was also applied to the control. The gastro-intestinal tract and lung tissues harvested for examination.

In the second stage of the work, Group B was further divided into seven groups (n=4, in each group)

Group A were administered pelleted rat feed and water only, serving as the control, group B were administered 600 mg/kg body weight of *Zingiber officinale*, water and pelleted rat feed, Group C were administered 600 mg/kg body weight of *Curcuma longa* extract, rat feed and water, Group D were administered 600 mg/kg body weight of *Garcinia kola* extract, rat feed and water only, Group E were administered 600 mg/kg body weight of equal proportion of combined extract (*Zingiber officinale*, *Curcuma longa* extract and *Garcinia kola* extract in ratio 1:1:1), water and rat feed only, group F were administered 1200 mg/kg body weight of equal proportion of combined extract (*Zingiber officinale*, *Curcuma longa* extract and *Garcinia kola* extract in ratio 1:1:1), water and rat feed only, group G were administered 2400 mg/kg body weight of combined extract (*Zingiber officinale*, *Curcuma longa* extract and *Garcinia kola* extract in equal proportion), water and rat pellet only.

After six weeks period, all animals in Groups A–G were weighed, anaesthetized with chloroform, and the GIT and lungs harvested for histological examination

Fixation of Tissue and Processing

After processing, the tissues were stained according to paraffin wax embedding technique and stained with freshly prepared haematoxylin and Eosin technique (Omorodion, *et al*., 2021).

Staining Procedures

Haematoxylin and Eosin staining

where taken and processed using the automatic tissue processor (Tissue TEK). The schedule is as follows:

0% alcohol-3hrs, 90% alcohol -3hrs, Absolute alcohol I--1hr, Absolute alcohol II--1hr, Absolute alcohol III - 2hrs, Absolute alcohol I--2hrs, Xylene I--1 ½ hrs, Xylene II--2 ½ hrs, Wax bath I--3hrs, Wax bath II --3hrs

After processing, the tissue were stained according to paraffin wax embedding technique and stained with freshly prepared haematoxylin and Eosin technique.

Staining procedures

Dewax and hydrate section in water, Stain section in Cole's Hematoxylin (10mins), Wash section thoroughly in running tap water, Differentiate section in 1 acid alcohol briefly, Blue in Scot's water for 5mins, Counter stain section in eosin for 3mins, Wash section in running tap water until excess eosin has been removed., Dehydrate section in ascending grade of alcohol (70%, 90% and absolute), Clear section in xylene, mount in DPX. (Avwioro, 2002; Baker *et al*., 2001; Omorodion, *et al.*, 2021).

Microscopy and Photomicrography

All slides were examined and photomicrographs taken from selected slides and presented as plates.

Immunohistochemistry

Immunohistochemical was carried out using Ki67in the evaluationsof thetissueswith ulcerations and inflammatory changes. Ki67 evaluation was considered more necessary and was done on representative tissue blocks using the avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques described by Hsu *et al*, (2017).

Statistical Analysis

Data were collected and analyzed by one way analysis of variant (ANOVA) using statistical package for social science (SPSS) software for windows and post hoc test was performed for experimental group comparison against the control. All data were expressed as mean $\pm$ SD. $P < 0.05$ were consider significant.

RESULTS

Table 1: Phytochemical component of *Zingiber officinale, Curcuma longa*, *Garcinia kola* and Combine extract (ratio 1:1:1)

S/N	Phytochemical *component*	*Zingiber officinale*	*Curcuma longa*	*Garcinia kola*	Combined Extract
1	Tannin	+	+	+	+
2.	Alkaloids	+	+	+	+
3	Phenols	+	+	+	+
4	Steroids		++		
5	Flavinoids	+	+	+	+
6	Phlobatannins	+			+
7	Saponins	+	+	+	+
8	Terpenoids	+	+		+
9	Cardiaglycosides	+	+	+	+
10	Cyanogenic glycosides			+	+

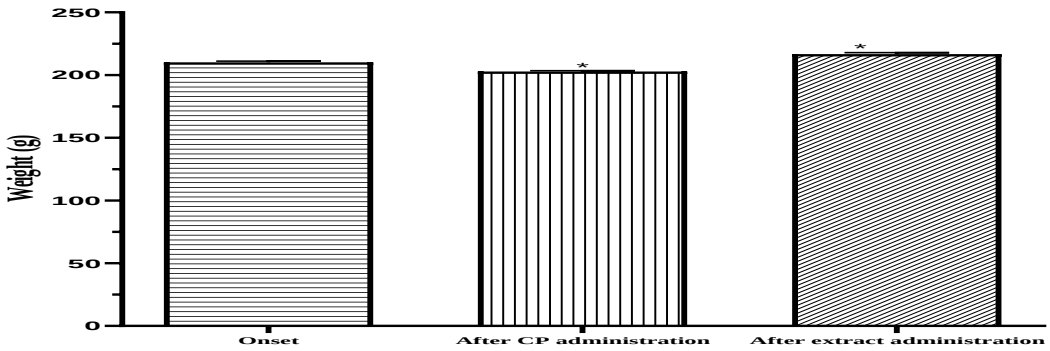

Figure 1: Weight before and after cyclophosphamide administration

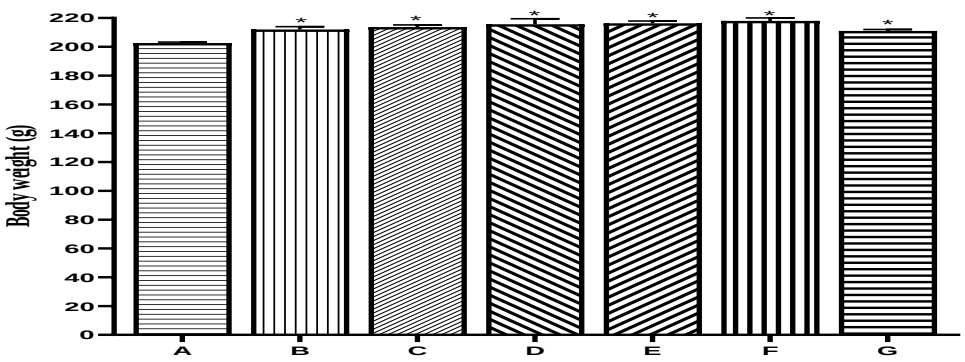

Figure 2: A chart of control against various test groups (Plant extract

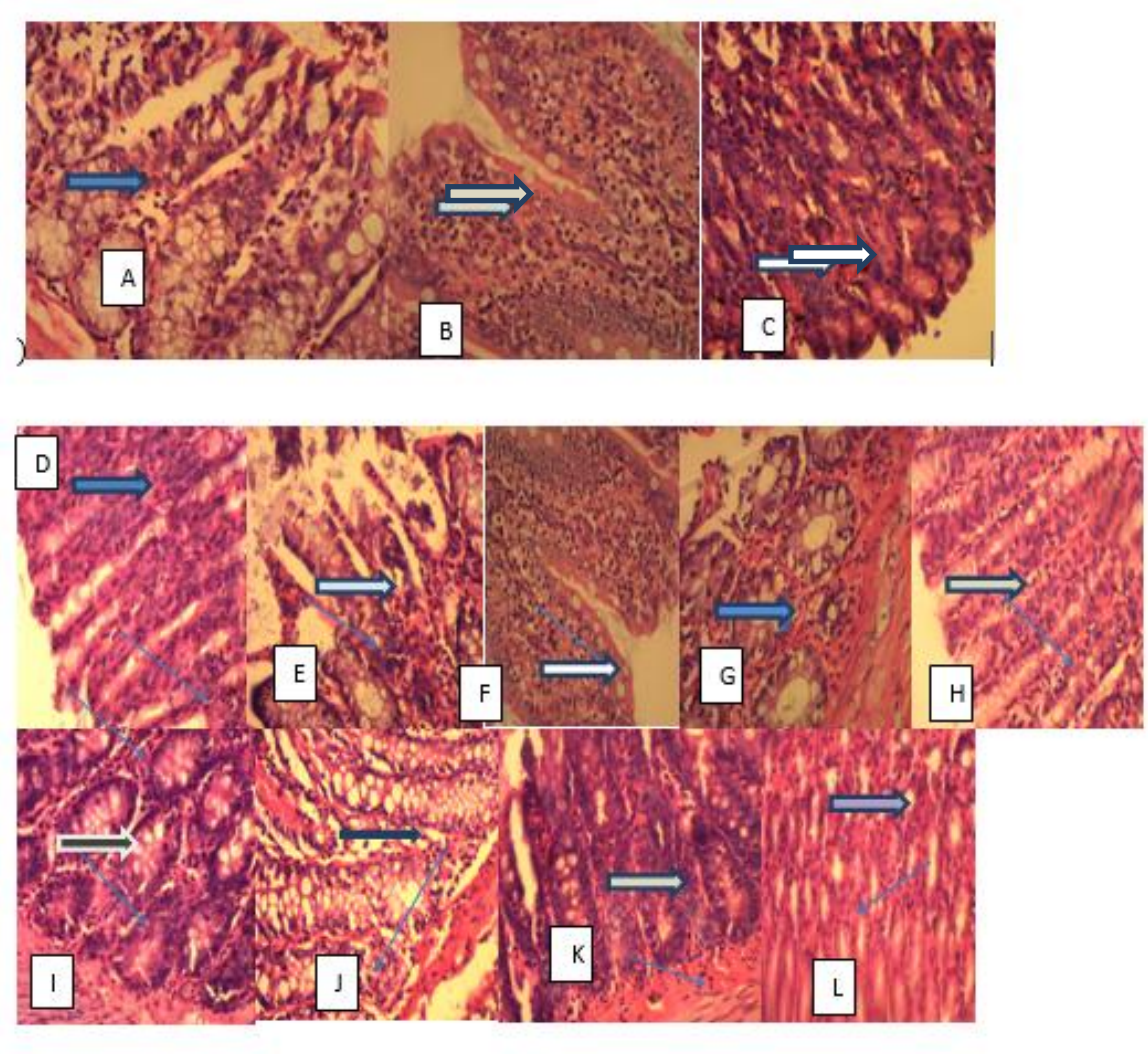

Plate 1: Section tissue labeled A, is a photomicrograph of rat large intestine (Control Group). Note the mucosa with normal epithelial lining as seen in the thick blue arrow, section B, small intestine (Control Group). Showing the mucosa with normal epithelial cells as seen in the tan arrow, section C is photomicrograph of rat stomach (Control Group) revealing the gastric mucosa with normal epithelial lining as seen in the white arrow, section (D) of rat large intestine treated with 10mg/kg cyclophosphamideextract. Note the mucosa with erosion of epithelial lining (dark blue arrow), section (E) of rat small intestine treated with 10mg/kg cyclophosphamide. Note the erosion of surface epithelium as seen in the tan arrow, section (F) of rat stomach treated with 10mg/kg cyclophosphamide. Note the gastric mucosa with normal epithelial lining (white thick arrow).Section (G) of rat large intestine treated with 600mg/kg *Zingiber officinale* extract. The intestinal mucosa shows normal globlet cells as seen in the dark blue arrow, section (H) of rat small intestine treated with 600mg/kg of *Zingiber officinale* extract. The mucosa shows normal Epithelial and globlet cells as seen in the tan arrow, section (I) of rat stomach treated with 600mg/kg *Zingiber officinale* extract. Note the gastric mucosa with normal gastric pits (dark tan arrow). Section (J) of rat large intestine treated with 600mg/kg of *Curcuma longa* extract. Note the mucosa with normal globlet cells as seen in the dark purple arrow, section (K) of rat small intestine treated with 600mg/kg *Curcuma longa* extract. .Note the mucosa with normal epithelial cells as seen in the tan arrow, section of rat stomach treated with 600mg/kg *curcuma longa* extract. Note the gastric mucosa with normal epithelial lining (purple arrow).

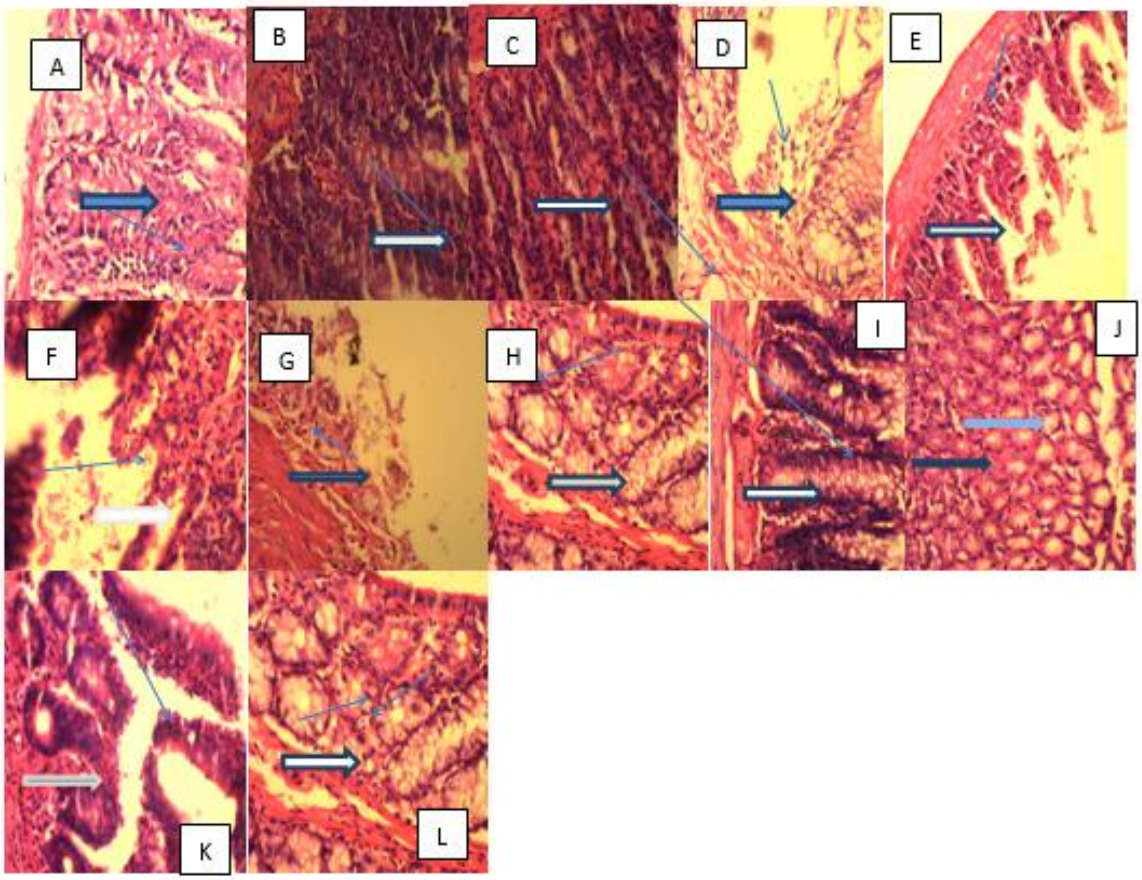

Plate 2: Section (A) of rat large intestine treated with 600mg/kg *Garcinia kola* extract. Note the mucosa with normal epithelial lining as indicated by the thick dark blue arrow, section (B) of rat small intestine treated with 600mg/kg *Garcinia kola* extract. Note the mucosa with normal epithelial cells (tan arrow), section (C) of rat stomach treated with 600mg/kg *Garcinia kola* extract. Note the gastric mucosa with normal epithelial as indicated by the thick white arrow. Section (D) of rat large intestine treated with 600mg/kg *pooled* extract. Note the intestinal mucosa with ulceration of surface epithelia (dark blue arrow), section (E) of rat small intestine treated with 600mg/kg *pooled* extract. Note the villi with normal surface epithelium (thick tanarrow), section (F) of rat stomach treated with 600mg/kg *pooled* extract. Note the ulceration of surface epithelium as seen in the white arrow.Section (G) of rat stomach treated with 1200mg/kg *pooled* extract. Note the gastric ulceration of surface epithelium indicated by the thick blue arrow, section (H) of rat large intestine treated with 1200mg/kg *pooled* extract. Note the intestinal mucosa shows normal epithelial lining (thick tanarrow), section (I) of rat small intestine treated with 1200 mg / kg combined extract. Note the mucosa with normal epithelial cells (white arrow). Section (J) of rat stomach treated with 2400mg/kg pooled extract. Note the gastric mucosa with normal gastric pits as indicated by the tick bluish arrow, section (K) of rat small int323estine treated with 2400mg/kg pooled extract. Note the mucosa lined with normal epithelial cells as indicated by the tan arrow, section (L) of rat large intestine treated with 2400mg/kg pooled extract. The intestinal mucosa shows normal epithelial lining as indicated by the thick white arrow.

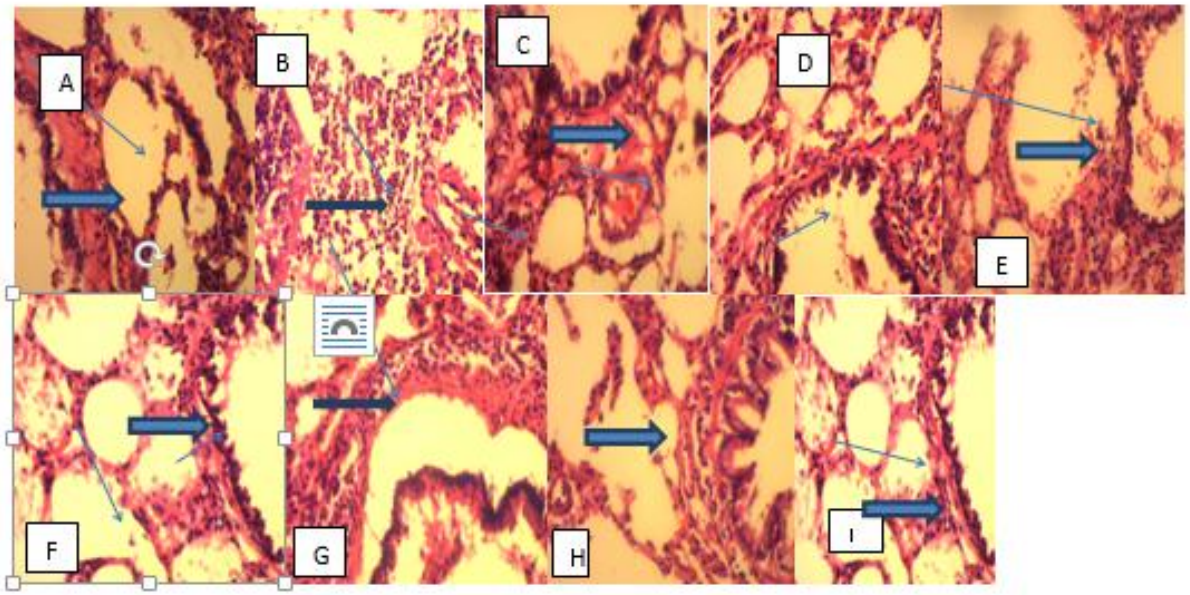

Plate 3: Section (A) of rat lungs (control Group). Note the normal bronchiole (thick arrow) & normal alveoli space (thin arrow, section of rat lungs treated with 10mg/kg cyclophosphamide. Note the neutrophil infiltration and inflammation of the bronchiole. Features revealing bronchitis and bronchiole pneumonia section of rat lungs treated with 600mg/kg *zingiberofficinale*extract. Note the normal bronchiole (thick arrow) and normal alveoli space (thin arrow), section of rat lungs treated with 600mg/kg *curcuma longa* extract. Note the mild desquamation into bronchiole (thick arrow) & normal alveoli space (thin arrow) section of rat lungs treated with 600mg/kg *garciniakola* extract. Note the mild desquamation and hemorrhage into bronchiole (thick arrow) and mild desquamation with hemorrhage into alveoli space (thin arrow). H and E: X400.Section of rat lungs treated with 600mg/kg pooled extract. Note the normal bronchiole (thick arrow) and mild desquamation and hemorrhage into alveoli space (thin arrow), section of rat lungs treated with 1200mg/kg *pooled* extract. Note the normal aveoli space (thin arrow) and debris in the bronchiole (thick arrow), section of rat lungs treated with 2400mg/kg pooledextract Note the normal bronchiole (thick arrow) and normal alveoli space (thin arrow).

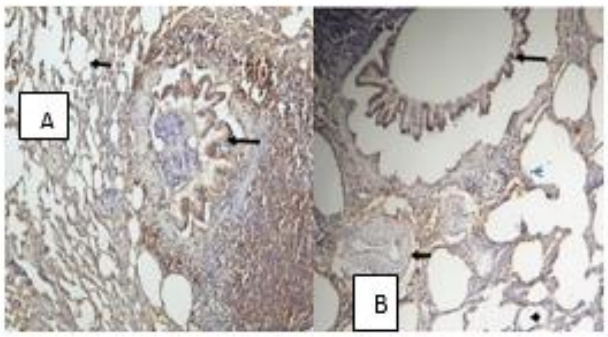

Plate 4: Lung tissue (A) immunohistochemistry which received cyclophosphamide and rat pellet, the second slide received combine extract respectively, reveal thickened bronchiole with high ki67 expression in cells surrounding it (long arrow). The alveoli cells also reveal low ki67 expression (short arrow), Lung immunohistochemistry (B) reveal Bbronchi with low ki67 expression in cells surrounding it (long arrow) and vessel (medium arrow). The alveoli cells also reveal low ki67 expression (short arrow). **IHC Ki67, x400**

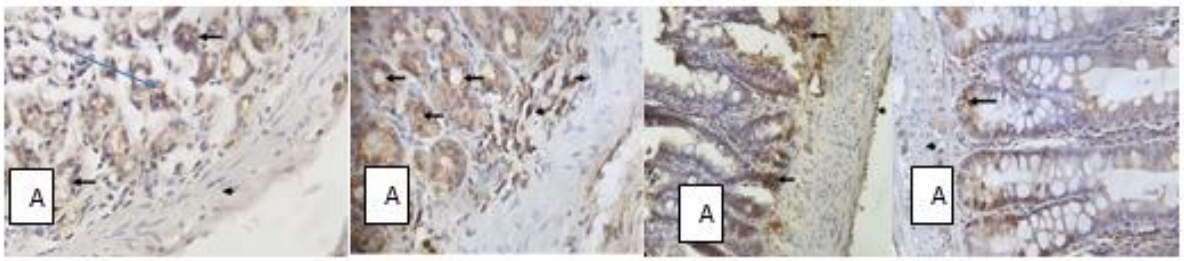

Plate 5: Stomach (A) immunohistochemistry, which received the combine extract reveals low positive ki67 expression in the gastric glands (long arrow) embedded in the mucosa with negative expression in the muscularis mucosa (short arrow), stomach (B) immunohistochemistry, which received cyclophosphamide reveals high positive ki67 expression in the gastric glands (long arrow) embedded in the mucosa with high positive expression in the muscularis mucosa (short arrow). Small intestine immunohistochemistry, which received cyclophoshamide reveals high positive ki67 expression in the intestinal crypts and villi (long arrow) embedded in the mucosa and high positive expression in the adventitia (short arrow), small intestine immunohistochemistry, which received cyclophosphamide reveals high positive ki67 expression in the intestinal crypts and low positive expression in the villi (long arrow) and negative expression in the submucosa (short arrow). **IHC Ki67: x400**

DISCUSSION

The phytochemical content of the combine extract showed metabolites of various uses. The phytochemicalconstituents which includes: tannin, terpenoidscardia, saponins, phlobatannins, cardiac glycosides, flavonoids, steroids, alkaloids and phenol. Some of these phytonutrients such as flavonoids, alkaloids, tannins, cardiac glycosides have been found by researchers to have properties such as playing a defensive role, antioxidant potentials perform pharmaceutical roles, ant-malarial properties, anti-bacterial properties, improving dietary intake rich in vitamins, treat congestive heart failures (Uniyal, et al., 2001**;** Mahato andSen, 1997; Asase, et al., 2010).

Phenol is known to alleviate disease conditions by improving the dietary intake of nutrients with its antioxidant properties, such as vitamic C, vitamin E, carotene and carotenoids (Haslam, 1998)

The reduction in the body weight of rats after cyclophosphamide could be attributed to the body to break down the drugs or the drug may have affected the anterior pituitary gland of the animal. This effect on growth was reported by Poupon, *et al.(*1993) wherein, they revealed a single dose administration of a of Cyclophosphamide (300 mg/ kg, about 8 mg/mouse) was able to lead to drastic reduction in the number of nucleated spleen cells which followed by a regeneration and it eventually reverted to normal. The increase in body weight of the animal could have resulted from the rich phyto-nutrients composition of the plant. Though a slight unexplainable decline was noticed in the combine group with the highest dosage (2400mg/kg/ body weight), which could be attributed to the dosage intake or the duration of the high dosage regimen.

From the plates presented, there were evidence of tissue desquamation, erosion, ulceration and inflammatory changes in parts of the GIT and the lungs at lower concentrations of individual herbs and the pooled extract. Pooled extract at 2400mg/ kg body weight shows great healing properties as there were no tissue abnormalities or alteration or degeneration. These further prove the high medicinal impact of the combined herbs in remedying the organ and tissue architectural alterationsimposed by cyclophosphamide (Nworu, *et al*., (2007).

The curative effect of *Zingiber officinale, Curcuma Longa* and *Garcinia kola* when used separately is not as impactful compared to the results of the combined herbs. The ability of the individual herbs to cure wide range of infections and diseases was minimal when compared to their combined effect of the herbal remedy.

There where ulceration (inflammation) of the epithelial lining of the gastric wall of the stomach after treatment with cyclophosphamide. This ulceration of thestomach gastric mucosa was alleviated with extract from the three plant extract which is probably due to some of the active ingredients such as flavonoid and alkaloid.

The lung function was altered by the administration of 10 mg/ kg body weight of cyclophosphamide which resulted in bronchitis (inflammation of the bronchus) and bronchiole pneumonia. This alteration was repaired by 600mg/kg body weight of *Zingiber officinale* and 2400mg / kg body weight of the combine extract. Six hundred milligram per kilogram (600mg /kg) body weight of *Curcuma longa*, *Garcinia kola* and combine extract caused mild desquamation and hemorrhage into bronchiole (thick arrow) and mild desquamation with hemorrhage into alveoli space, which shows that the 600mg / kg body weight of ginger and 2400 mg /kg body weight of combine extract were perfect dosage for lung function. The Ki67 expression of immunohistochemistry was positive in groups administered with cyclophosphamide but was negative in combine extract in both lungs and GIT, this is an indication that the extract aid lung function and ameliorate GIT damage.

CONCLUSION

In conclusion, our current findings have shown that some tissue damage can be repair or ameliorated using plants that are easily accessible, affordable and potent. Mechanism of actions may not be ascertain as at the time of this research but the phytochemistry evaluation have revealed so many viable phyto-nutrients, that has help to explain the remedial properties of the pooled extract.

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