Cymbopogon citratus reaction on aluminium nitrate induced stomach damage in adult female Wistar rat.

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Accepted 14th December, 2021.

Cymbopogon citratus (DC.) Stapf (Poaceae family) is grown around the world and has a century-long record of extensive therapeutic applications in traditional medicine in a number of countries. It is used in herbal medicine as antibacterial, antifungal and anti-carcinogenic agent due to its antioxidant properties. The aim of this study was to investigate the effect of aqueous extract of *C. citratus* leaf on the stomach of Wistar rats (*Rattus norvegicus*). A total of twenty female Wistar rats of average weight of 200g were randomly selected into five groups (A to E) with four rats in each group. Group A served as control group fed with rat feed and water *ad libitum*. Group B received 200mg/kg of aqueous extract of *C. citratus* for two weeks. Group C received 400mg/kg body weight of aqueous extract of *C. citratus* for two weeks. Group D received 100mg/kg body weight of aluminium nitrate orally while Group E received both 100mg/kg body weight of aluminium nitrate and 200mg/kg body weight of aqueous extract of *C. citratus* concurrently for two weeks. The animals were sacrificed using cervical dislocation. The animals were weighed on weekly basis and the results were analyzed using One Way Analysis of Variance (ANOVA). Histological findings revealed that administration of aluminium nitrate resulted in changes in cyto-architecture of the stomach. However, co-administration of aluminium nitrate and aqueous extract of *C. citratus* improved the stomach cytoarchitecture. The changes observed in the body weight were not significant at p value<0.05. This study showed that the aqueous extract of *C. citratus* has ameliorative effect on aluminium nitrate induced stomach damage.

Key words: Aqueous extract, *Cymbopogon citratus*, Aluminium nitrate, Wistar rat.

INTRODUCTION

Cymbopogon citratus (DC.) Stapf (Poaceae family) is broadly utilized in preparation of soups curries and teas. This herb contains calming character. Lemon grass (*C. citratus*) is a native of sweet-smelling tall sedge. It is a member of the family Poaceae. It grows in numerous parts of the tropical and sub-tropical South East Asia and Africa. *C. citratus* is a native grass to Pakistan, India and Sri Lanka (Manzoor *et al*., 2013).

C. citratus is an herb which is known throughout the world as lemon grass and is widely used as a source of medicines in tropical countries. Plants leaves are utilized as tea and are generally used in Brazil as analgesic, antipyretic, spasmolytic, tranquilizer, anti-inflammatory and diuretic. This plant contains 1 to 2% essential oil on a dry base. The chemical composition of the lemon grass brass oil (LGO) is broadly different due to its genetic diversity, habitat and the agronomic treatment of this plant (Tzortzakis and Economakis, 2007). Lemon grass essential oil is of high citral content (geranial and neutral

isomers), which is used as a source for the production of beta carotene and vitamin A etc.

Antimicrobial action of LGO is used for various pathogenic fungi (Vardar-Ünlü *et al*., 2003). Lemon grass is a native of tropical Asia and now-a-days it grows worldwide. Because of its slight citrus flavor, dried and fresh leaves of the lemongrass are common ingredients of the Asian cuisine in curries, teas and soups; it is also suitable for the poultry, seafood and fish. This herb is widely consumed as an aromatic herb in Latin and African countries. In addition, its aerial components are widely utilized in folk medicine for the treatment of digestive disorders, diabetes, nervous disorder, inflammation and fever (Amirdivani and Baba, 2011). Functional lemongrass components have been recognized in recent years. A strong contribution to the antioxidant and anti-inflammatory characteristics of an important oil-free lemongrass infusion has been recognized as mono-and polymeric flavonoid, such as apigenin glycosides, luteolin and and proantho cyanidins (Francisco *et al*., 2013).

Lemongrass is widely used in herbal teas and other non-alcoholic beverages in baked food, and also in confections. Essential oil from the lemongrass is commonly used as a fragrance in perfumes and cosmetics, such as creams and soaps. Citral, extracted from the oil of lemon grass, is used in flavoring of soft drinks, scenting detergents and soaps, as a fragrance in perfumes and cosmetics, and as a mask for dis-agreeable odors in various industrial products. Citral also is used in the formation of ionones used in perfumery. Lemon grass is a medicinal plant and has been considered as an insect repellent and carminative. West Indian lemongrass is reported to have strong antimicrobial action (Oladeji *et al*., 2019). Essential oils of West Indian lemongrass acts as a central nervous system depressant. The volatile oils also have some mutagenic and pesticidic action.

Aluminum is a very light metal with a specific weight of 2.7 g/cm3, about a third of that of steel. This cuts the costs of manufacturing with aluminum. Again, its use in vehicles, reduces dead-weight and energy Consumption while increasing load capacity. This also reduces noise and improves comfort levels. Its strength can be adapted to the application required by modifying the composition of its alloys. Aluminum-magnesium-manganese alloys are an optimum mix of formability with strength, while aluminum-magnesium-silicon alloys are ideal for automobile body sheets, which show good age-hardening when subjected to the bake-on painting process (Terken *et al*., 2003).

The stomach is a muscular, hollow organ in the gastrointestinal tract of humans and many other animals, including several invertebrates. The stomach has a dilated structure and functions as a vital digestive organ. In the digestive system, the stomach is involved in the second phase of digestion, following chewing. It performs a chemical breakdown by means of enzymes and hydrochloric acid. The stomach bed refers to the structures upon which the stomach rests in mammals (Habershon, 1909; Weber and Shearer, 1999). These include the pancreas, spleen, left kidney, left suprarenal gland, transverse colon and its mesocolon, and the diaphragm. The term was introduced around 1896 by Philip Polson of the Catholic University School of Medicine, Dublin.

A series of radiographs can be used to examine the stomach for various disorders. This will often include the use of a barium swallow. Another method of examination of the stomach is the use of an endoscope. A gastric emptying scan is considered the gold standard to assess gastric emptying rate (Masaoka and Tack, 2009). A large number of studies have indicated that most cases of peptic ulcers, and gastritis, in humans are caused by Helicobacter pylori infection, and an association has been seen with the development of stomach cancer (Brown, 2000). Hence, this study aims to evaluate some reaction of aqueous extract of *C. Citratus* on aluminum nitrate induced stomach damage using female Wistar rat.

MATERIALS AND METHODS

List of materials and instruments

The materials and instrument used for this study includes; dried leaves of *C. citratus,* rat feeds, feeding plates, aluminium nitrate salt

(nonhydrate crystals), drinking water, water bowls, plastic cages with iron nettings, mortar and pestle, saw dust, twenty adult female Wistar rats, syringe, cannula and needles, weighing scale, sample bottle, distilled water, measuring cylinder, pail, dissecting blades and dissecting boards, bouin's fluid, 40% formaldehyde, tissue cassete, sensitive weighing balance**,** graded alcohol (50, 70, 90% and absolute alcohol)**,** cotton wool, embedding mould and pot, DPX mountant, haematoxylin and eosin stain, light microscope, camera, rotatory microtome, glass slide, slide cover-slips, slide holders, freezer, hot plate and water bath.

Study design

All experimental rats were fed with standard rat feeds and drinking water ad libitum. The LD_{50} of Aluminium nitrate was reported to vary between 200mg/kg to 4500mg.bw. LD_{50} of aluminium nitrate was reported to be 542mg Al/kg (BW) (National Research Council, 1981), 261mg Al/Kg (BW) (Llobet *et al*., 1987); and 3671mg Al/kg (BW). LD_{50} by oral route is 65mg.kg. The oral median lethal dose (LD_{50}) of aluminium nitrate, chloride and sulfate in mice and rats ranges from 200 to 1000 mg of aluminium per kilogram of body weight. The LD_{50} of aqueous extract of *C. citratus* was reported to be 400mg/kg.

Collection and identification of material

C. citratus leaves ware collected in Ogbomosho, Oyo State. The leaf was identified at Department of Biology, Faculty of Pure and Applied Science, Ladoke Akintola University of Technology to *C. citratus* family of Poaceae.

Aluminum Nitrate Crystal (non-anydrate) was procured from a reputable supplier at Denis Chemical Nigeria Company, Ilorin, Kwara State, Nigeria.

Preparation of *Cymbopogon citratus* extract

C. citratus leaves were air-dried at room temperature for 14 days without exposing it to the sun. The leaves were dried for 14 days and did not lose its green colour. The dried leaves were then pulverized into powder form by making use of a mortar, pestle and a sieve. About 500g of powdered leaves was dissolved in 5 liters of distilled water inside a closed container for 72 h. After 72 h, the mixture was sieved using a muslin bag and the filtrate was then taken to the Department of Food and Science Technology at Lautech where the filtrate was heated in a reflux apparatus at a temperature of 50°C which extracted the *C. citratus* concentrate.

Experimental animals

Twenty female Wistar rats (mean weight of 186.5g) were procured from Seminary, Taki area in Ogbomosho, Oyo State. They were kept in plastic cages in the animal house which was well kept and ventilated. The animals were allowed to acclimatize for 7 days with feed and water al libitum. Animals were weighed weekly during the acclimatization and administration with care taken in their handling in accordance to the rules of Institutional Animal Care and Use Committee (IACUC). Administration of aluminium nitrate solution and aqueous solution of *C. citratus* were carried out through orally whilst making use of syringe and cannula. Some animals however were used as control and received only water and feed while some animals received doses of aqueous extract of *C. citratus* for 14 days. Some received doses of aluminium nitrate for 14 days and the last batch of animals received doses of aqueous extract of *C. citratus* and Aluminium Nitrate for 14 days.

Experimental design and grouping

After two week of acclimatization, a total of 20 female albino rats with an average weight of about 200g were grouped into five; A, B, C, D and E of four rats each where Group A served as the control group, consisting of four Wistar rats which were fed with rat feeds and water *ad libitum* for 2 weeks concurrently, Group B received aqueous extract of *C. citratus* at a dosage of 200mg/kg body weight for two weeks concurrently, Group C received aqueous extract of *C. citratus* at a dosage of 400mg/kg body weight concurrently for two weeks, Group D received aluminium nitrate at a dosage of 100mg/kg body weight for two weeks, Group E received a100mg/kg body weight of aluminium nitrate and 200mg/kg of aqueous extract of *C. citratus* at 200mg/kg body weight concurrently for two weeks. Table 1 show the experimental

Table 1. Experimental design and grouping.

Groups	Number of Animals	Dose of aqueous extract of Cymbopogon citratus	Dose of Aluminium Nitrate	Feed and Water (Ad Libitum)
A	4	.....	……	
B	4	1800mg/kg	.....	
C	4	3850mg/kg	.....	
D	4	…….	1700mg/kg	
E	4	3850mg/kg	1700mg/kg	

design and grouping.
LD50 of aluminium nitrate = 542mg/kg.
LD50 of *C. citratus* is reported to be 3500mg/kg.

Tissue processing
Tissue processing is a procedure of removing water from cells and replacing it with a medium which solidifies allowing thin sections to be cut on a microtome. Most histological samples need preparation before microscopic observation; these methods depend on the specimen and method of observation.

Fixation: the liver was fixed in formal saline solution so as to preserve the cells and tissues and to protect it from autolysis *(*Ross, *et al*., 2016).

Dehydration: This step has to do with removing the water from the specimen. This is done by padding the liver through a serious of alcohol solution specifically (70, 90 and 100%) *(*Bancroft and Stevens, 1982).

Clearing: This is done by making use of xylene to remove the alcohol from the tissue. To ensure that the alcohol is gone, it is important to replace the xylene with fresh one until there is no trace of alcohol *(*Bancroft and Stevens, 1982).

Infiltration: The liver is removed from xylene and infiltrated in paraffin wax. The wax is liquid at room temperature and must be allowed to cool to 20ºC (Bancroft and Stevens, 1982).

Embedding: This is also referred to blocking out. The liver must be thoroughly infiltrated in the paraffin wax and then formed into a block so as to allow sections to be cut from it (Ross *et al*., 2016).

Sectioning: For light microscopy, a knife mounted in a microtome is used to cut tissue sections (between 5-15 micrometers thick) which are mounted on a glass microscope slide (Ross *et al*., 2016). Stainining comes after sectioning. The procedure includes; dewaxing in xylene for 5 min, taking the sample through ascending grades of alcohol, rinsing in distilled water, Stain in hematoxylin solution for 15 min KO, differentiating in acid alcohol for 2 seconds, staining in eosin for 3 min, washing in tap water carefully to remove excess stain, dehydrating in ascending grade of alcohol, clearing in z changes of xylene 1 and 2, mounting in DPX (Dibutyrl Pthalate in Xylene) and covering with cover slip.

Method of statistical analysis
The statistical analysis for the respective weights of the animals was done using one-way analysis of variance (ANOVA) using Statistical Analysis System, version 5.1. The weights of the animals were compared against that of the control group. The level of significance was set at $p<0.05$. The mean + S.D (standard deviation) was used to present the results.

RESULTS
Table 2 shows the result of the effect of *C. citratus* on the body weight of the experimental animals. The result showed the weekly mean body weight of the animals in each group. There is statistical increase ($P<0.05$) in the body weight of group A, C, D and E when compared with the initial weight of the animals. Group A shows an increase body weights in week 1 and week 2 respectively. After the administration of 200mg/kg body weight of *C. citratus* extract,

Table 2. Mean ± S.E.M of the body weights of Wistar rats before and during administration.

GROUPS	WEIGHT (g) week 1	WEIGHT (g) week 2	WEIGHT (g) week 3
A	200 ± 0	210 ± 10	210 ± 10
B	180 ± 4.082	182.5 ± 8.539	180 ± 9.129
C	192.5 ± 6.292	200 ± 16.33	212.5 ± 14.93
D	170 ± 7.071	182.5 ± 8.539	200 ± 14.72
E	190 ± 4.082	197.5 ± 8.539	202.5 ± 14.93

Significance P < 0.05, value greater than 0.05 are considered insignificant while values less than 0.05 are considered significant (*) values are expressed as mean ± standard error of mean.

animals in group B are seen to increase in weight in week 1 and subsequently regain weight in week 2. Administration of 400 mg/kg body weight of aqueous extract of *C. citratus* to animals in Group C reveals increase in mean weight in week 1 and week 2. Administration of 100mg/kg body weight of aluminium nitrate to animals in Group D reveals increase in mean weight of animals in week 1 and 2 respectively. Subsequently, administration of 100mg/kg body weight of $AlNO_3$ and administration of 200mg/kg body weight of the extract to animals in group E reveals increase in body weight in week 1 and week 2 respectively.

The initial and final weights of experimental animal in each groups is revealed in Table 3. There were increases in the final weight of experimental animals in control Group A, C, D and E while Group B recorded no change in weight.

Table 3. Showing the initial and final weights of experimental animals in each groups.

Groups	Initial	Final
A	200 ± 0	210 ± 10
B	180 ± 4.082	180 ± 9.129
C	192.5 ± 6.292	212.5 ± 14.93
D	170 ± 7.071	200 ± 14.72
E	190 ± 4.082	202.5 ± 14.93

Significance: P < 0.05, value greater than 0.05 were considered insignificant while values less than 0.05 were considered significant (*). Values were expressed as mean ± Standard error of mean.

The standard error of the mean weight of the animals in the experimental Group A, B, C, D and E is recorded in Table 4 with their respective standard deviation. There were significant differences in the S.E.M in all the groups except group A which is the control.

Table 4. Showing the mean ± S.E.M of the stomach weight of Wistar rats.

Groups	Weight(g) of stomach
A	2.998 ± 0.06183
B	2.393 ± 0.6659**
C	2.47 ± 0.4424*
D	3.268 ± 0.7025**
E	1.67 ± 0.04041***

Significance: P < 0.05, value greater than 0.05 were considered insignificant while values less than 0.05 were considered significant (*). Values were expressed as mean ± Standard error of mean.

HISTOLOGICAL FINDINGS AND DISCUSSION

Plates 1 to 5 respectively show the histological findings of the study. The movement and the flow of chemicals into the stomach are controlled by both the autonomic nervous system and by various digestive hormones of the digestive system. The hormone gastrin causes an increase in the secretion of HCl from the parietal cells, and pepsinogen from chief cells in the stomach. It also causes increased motility in the stomach.

Gastrin is released by G cells in the stomach in response to distension of the antrum, and digestive products (especially large quantities of incompletely digested proteins). It is inhibited by a pH normally less than 4 (high acid), as well as the hormone somatostatin. Aluminium exposure causes changes in the gastric cells in the stomach (Zulfa *et al*., 2016).

The plant also contained flavonoids which were phenolic compounds that serve as flavoring ingredients of plant leaves, higher contents of tannins, flavonoids and phenolics were observed than alkaloids, saponins and glycosides in lemongrass (*C.citratus*), Isoorientin, Caffeic acid, Quercetin and Chlorogenic acid (Uraku *et al*., 2015). Lemongrass was found in folk remedy for

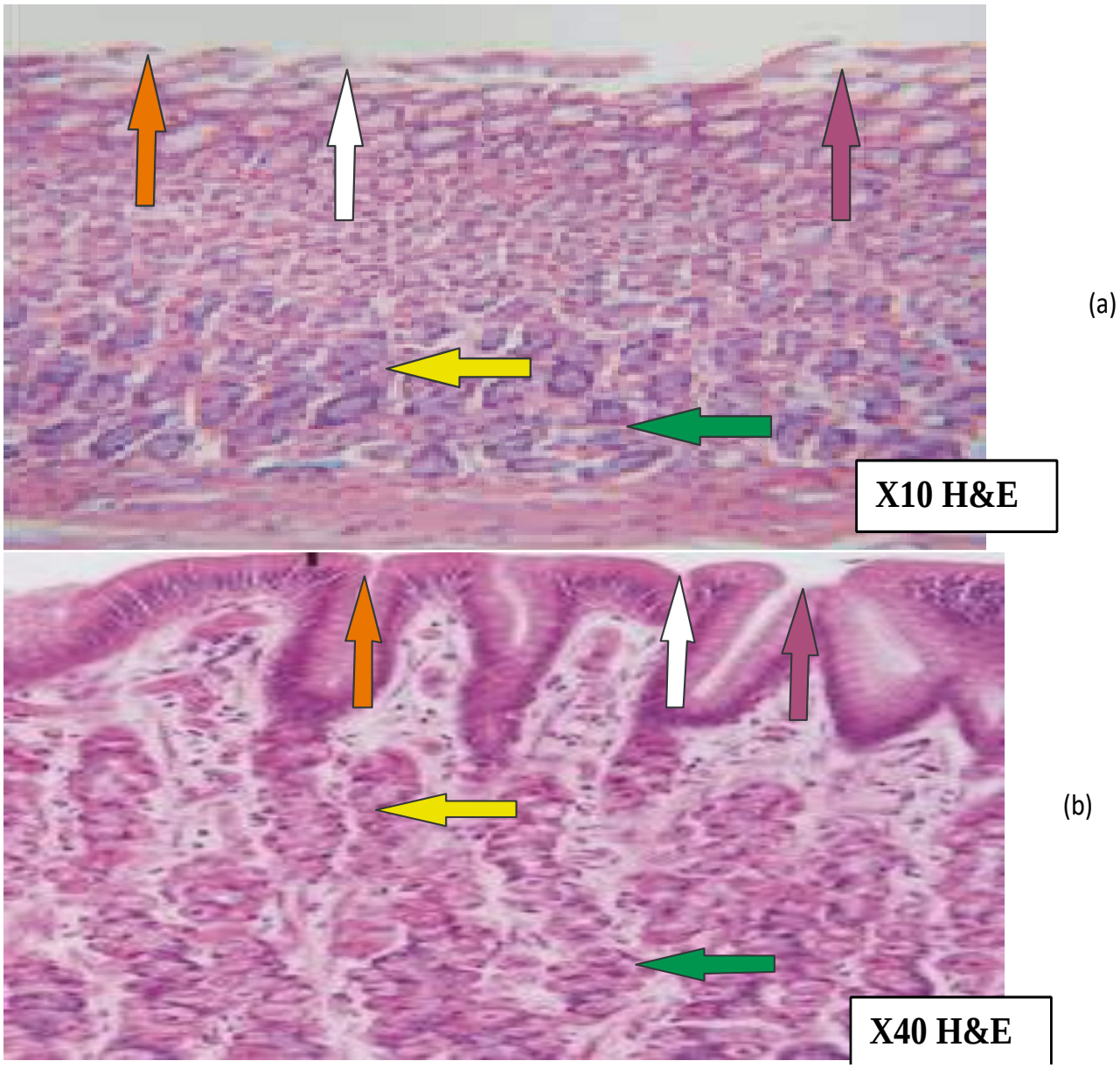


Plate 1 (a and b). Photomicrographs showing sections of Wistar rat stomach with normal histologic appearance. The inner wall of the stomach has a normal appearance and is lined with simple columnar epithelium (White arrow). There were normal invaginations of the gastric pit (Purple arrow) throughout the stomach lining, which also has normal secretory sheath (Orange arrow). There were numerous gastric cells, including Parietal cells (yellow arrow) and Chief cells (green arrow).

coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorders, researchers have found that lemongrass holds antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties (Naik *et al*., 2010). *C. citratus* had antibacterial, antifungal, antitumoral, anticancer and insecticide activities. The antimicrobial activity of lemongrass against a series of microorganisms was due to the abundance of citral and essential oil components Geranial, Myrcene, which led to suggestion that *C. citratus* may have antimicrobial activities against *Bacillus cereus*, *Escherichia coli O157:H7, Klebsiella pneumonia*, *Staphylococcu aureus and Candida albicans* (Zulfa *et al*., 2016).

Statistical results showed that the final weights of all animals increased in Group A, C, D, and E and decreased in Group B respectively. Experimental animals in group A showed an increase in week 2 with the same value in week 3.

Experimental animals in group B administered with aqueous extract of *C. citratus* at 200mg/kg body weight showed an increase week 2 and a decrease in weight in week 3.

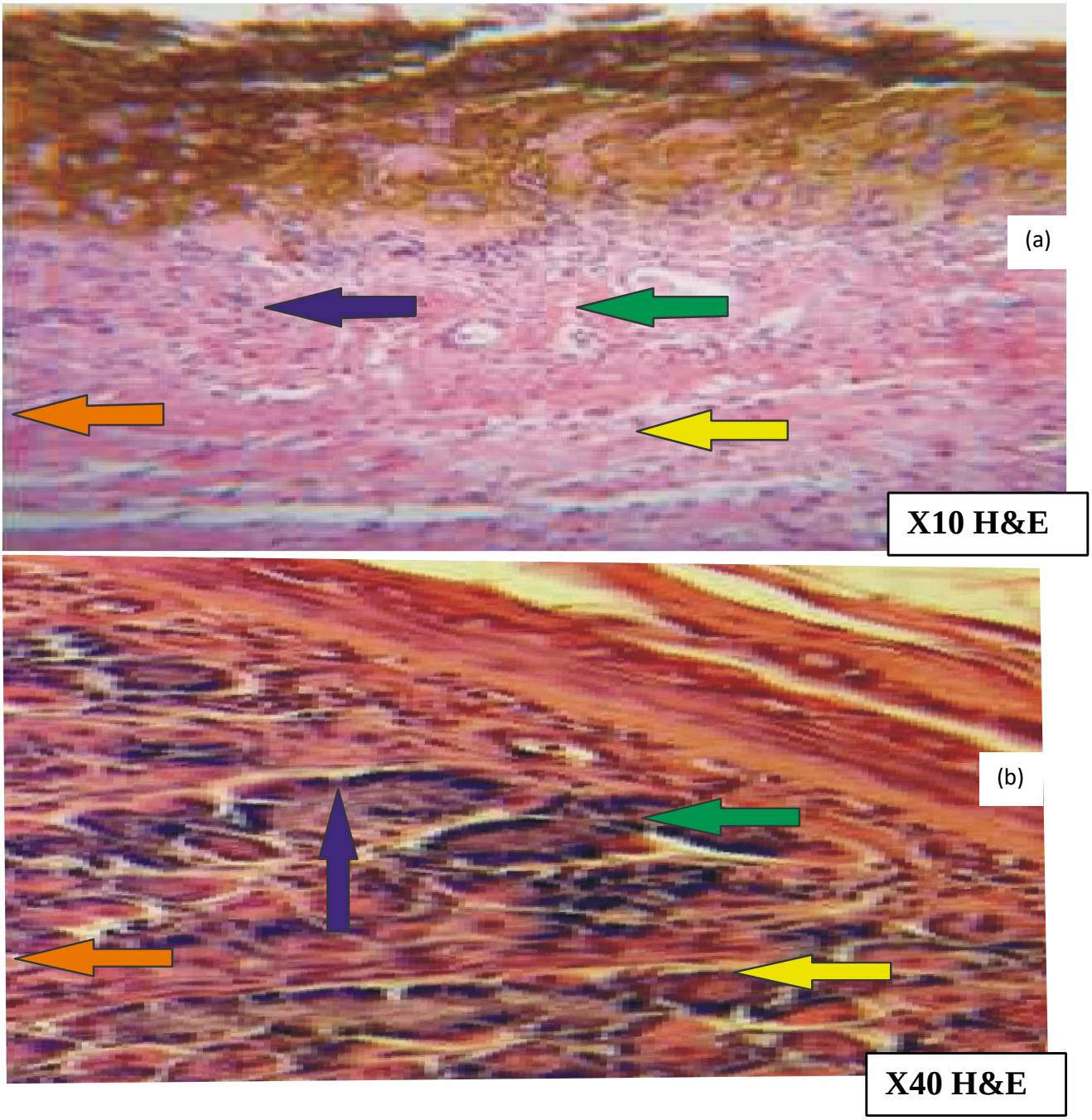
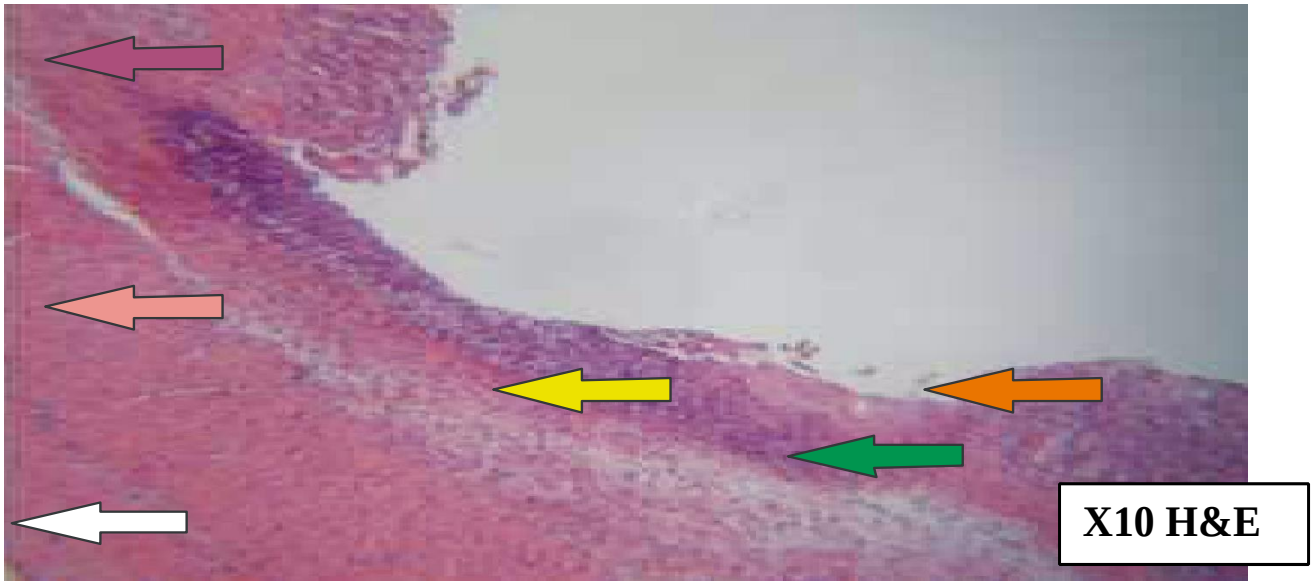


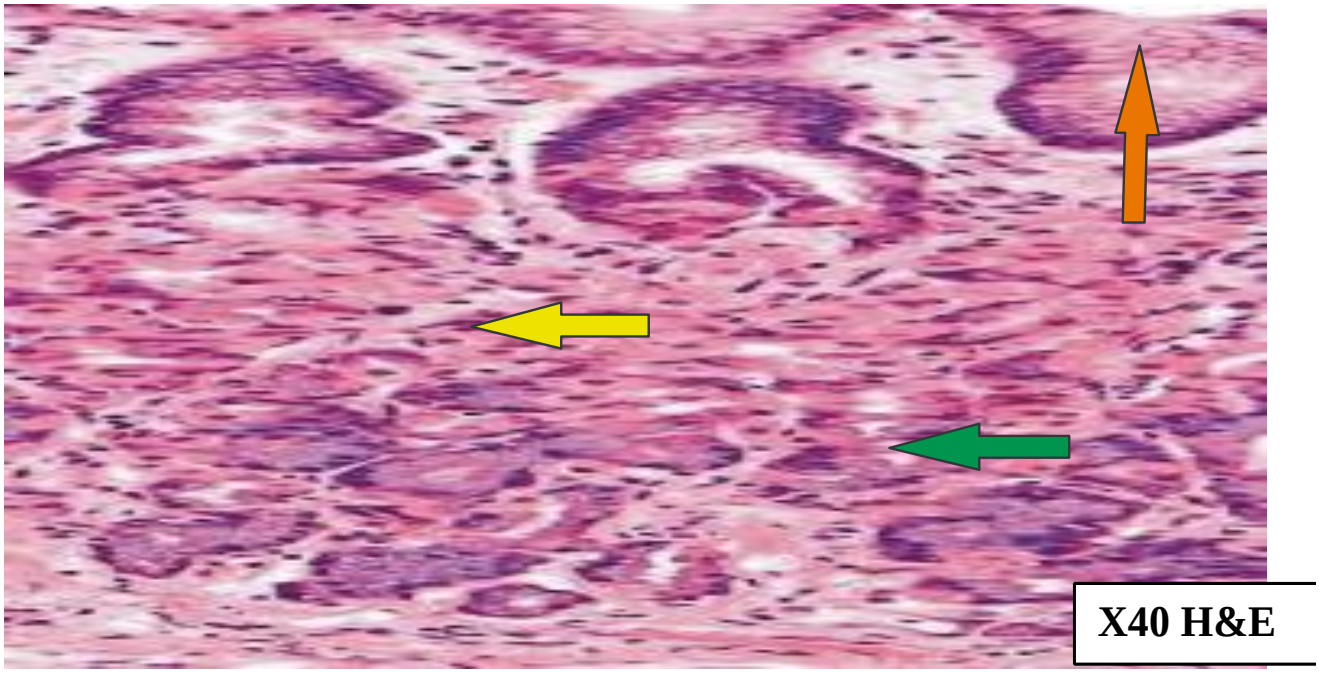
Plate 2 (a and b). Photomicrographs showing sections of Wistar rat stomach with a fairly normal histologic appearance, which however shows evidence of inflammation and change in the cell staining intensity. The gastric pit (orange arrow), containing fairly normal gastric glands (Orange arrow) appears normal. There were numerous gastric cells, including Parietal cells (yellow arrow) and Chief cells (green arrow).

Group C showed an increase in weight in week 2 and week 3 at a dosage of 400mg/kg body weight. Administration of aluminium nitrate in group D revealed an increase in the weights of the animal in week 2 and week 3 respectively. Group E received extract and aluminium nitrate and revealed an increase in the animal weight in week 2 and 3 respectively.

There was no significant difference ($P>0.05$) in the stomach weights of the rats in group C exposed to 400mg/kg body weight of *C. citratus* compared with the control group A while in Group D there was significant difference ($P<0.05$) in the stomach weights of the rats exposed to 100mg/kg body weight aluminum nitrate when compared to the control group. Group E exposed to 100mg/kg aluminum nitrate and 200mg/kg of *C. citratus* also showed



(a)



(b)

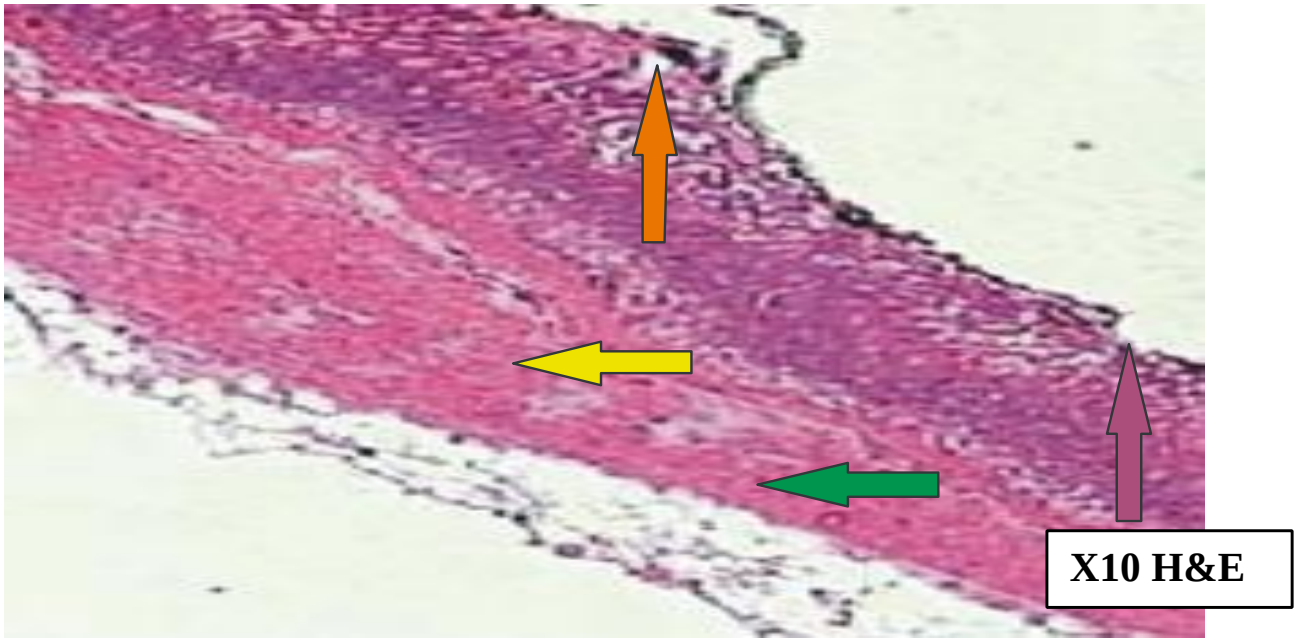
Plate 3 (a and b). Photomicrographs showing sections of Wistar rat stomach with disorganization in the mucosa (Purple arrow), atrophy in the gastric pit (Orange arrow), and degenerative changes in the submucosa (Pink arrow). There are presence of degenerative changes in the gastric cells, including Parietal cells (yellow arrow) and Chief cells (green arrow). The muscularis externa (white arrow) appears fairly normal.

significant difference. This study supports the previous work done by Salome *et al*. (2012).

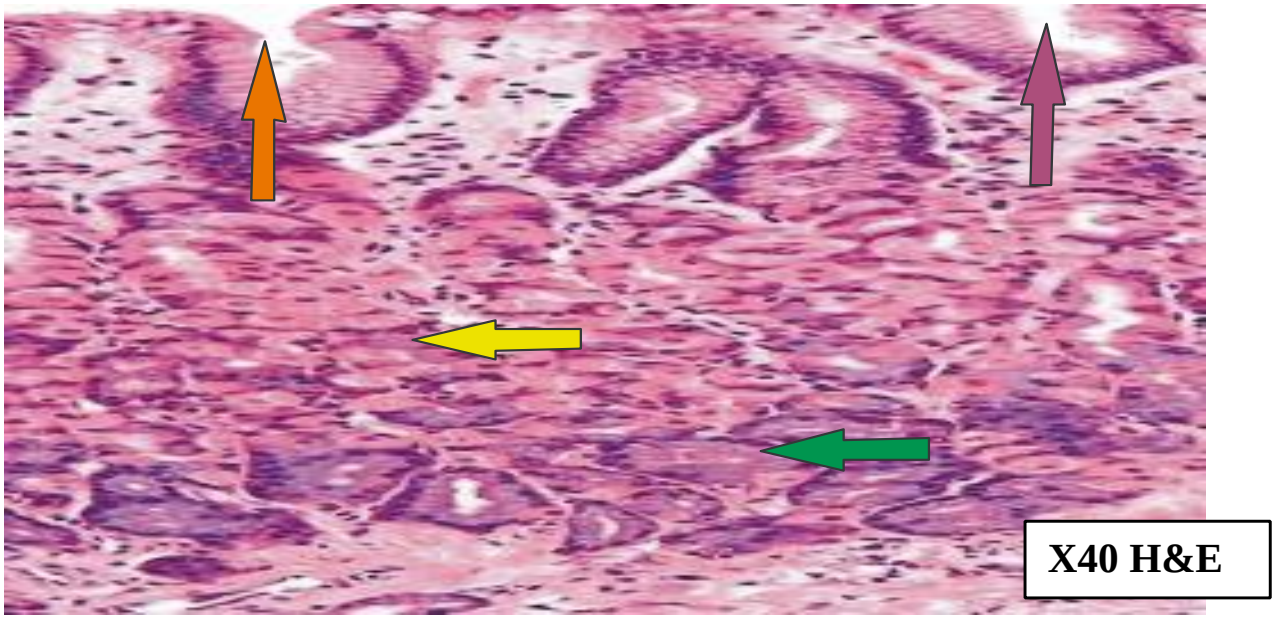
Extract of *C. citratus* showed fairly normal gastric gland with degenerative changes in the mucosa and submucosa. This study also supports the previous work done by Salome *et al*., 2012.

Conclusion

The result revealed that the administration of aluminium nitrate caused some distortion in the cyto-architecture of the stomach and co-administration of aqueous extract of *C. citratus* revealed an improved stomach cyto-architecture. Therefore, it is evidence that *C. citratus* has a



(a)



(b)

Plate 4 (a and b). Photomicrographs showing sections of Wistar rat stomach with marked degenerative changes in the histologic appearance. There were distortions in the epithelial lining. There were disruptive changes in the delineation of the invaginations of the gastric pit (Purple arrow) throughout the stomach lining, which has degenerating secretory sheath (Orange arrow), as well as some degrees of degeneration of the gastric cells, including Parietal cells (Yellow arrow) and Chief cells (green arrow).

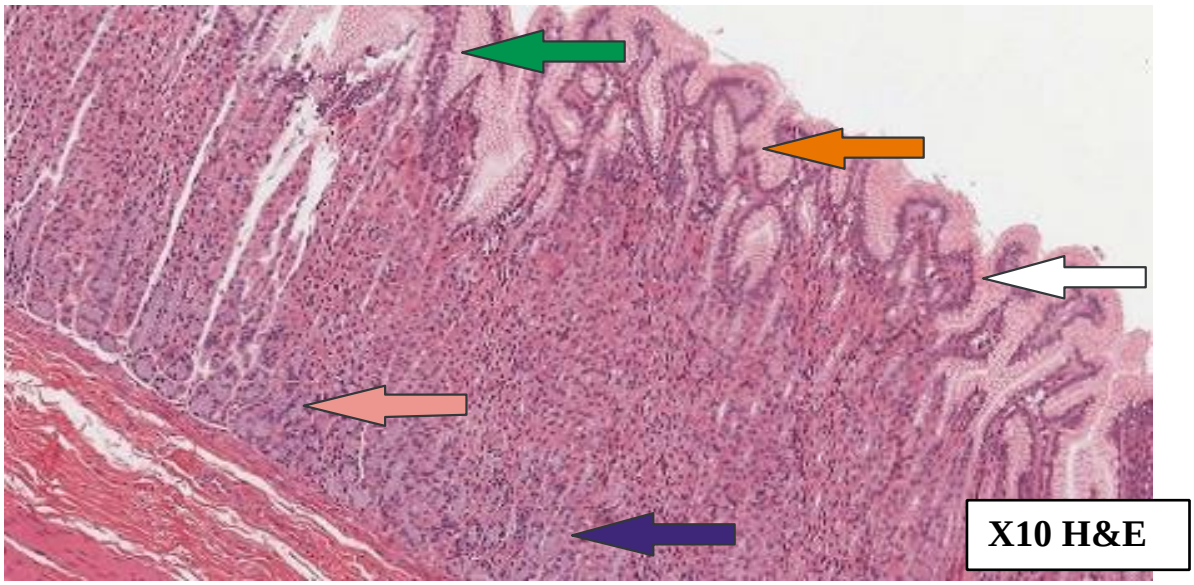
dose dependent ameliorative effect as it restores the histologic appearance of the stomach.

RECOMMENDATION

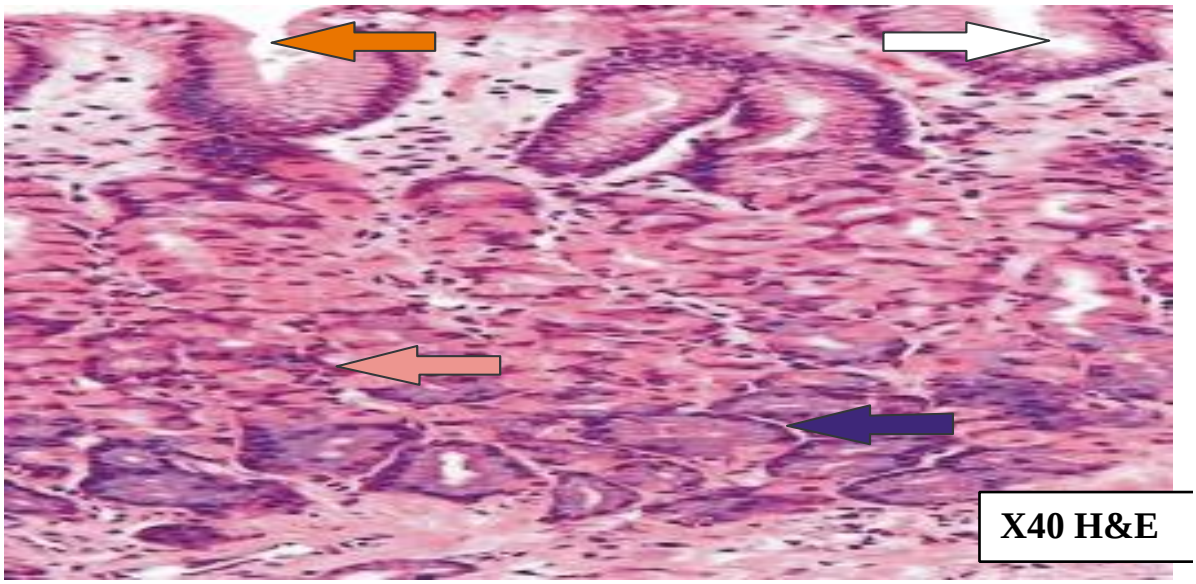
Further studies should be carried out on the effects of *C. citratus* on the stomach and other organs to discover further ameliorative effects of *C. citratus.*

CONFLICTS OF INTERESTS

The authors have not declared any conflicts of interests.



(a)



(b)

Plate 5 (a and b). Photomicrographs showing sections of Wistar rat stomach with fairly normal histologic appearance. The inner wall of the stomach has a normal appearance and is lined with simple columnar epithelium (Orange arrow). The gastric pit (White arrow) has a fairly normal appearance throughout the stomach lining, which also has fairly normal secretory sheath (Green arrow). There were numerous gastric cells, including Parietal cells (Pink arrow) and Chief cells (Blue arrow).

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