EFFECTS OF AQUEOUS EXTRACT OF *ADANSONIA DIGITATA* LEAVES ON INDOMETHACIN INDUCED GASTRIC SECRETIONS IN WISTAR RATS

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ABSTRACT
Background: Parietal cells secrete hydrochloric acid at a concentration of approximately 160 mmol/L or pH 0.8. The principal stimulants of acid secretions are Histamine, released from enterochrommaffin like (ECL) cells, Gastrin, released from G cells and Acetylcholine (ACh), released from postganglionic enteric neurons. There are a number of uncommon conditions in which gastric acid secretion is abnormally high and ulcers develop. The aim of this study is to determine the gastro-protective effects of aqueous extract of Baobab leaves (*Adansonia digitata l.*) on indomethacin induced gastric secretion in Wistar Rats.
Methods: A total of 30 rats were randomly distributed into six groups of five Wistar rats each. Group 1 serve as control and receive distilled water only, Group 2 serve as ulcerated group and receive 50mg/kg of indomethacin (IND), Group 3 receive 100mg/kg cimetidine (CMD) for 14 days before ulcer induction, Group 4, 5 and 6 receive 100mg/kg, 150mg/kg and 200mg/kg aqueous extract *A. digitata l.* (A.D) for 14 days before IND administration respectively. IND (50mg/kg) was administered intraperitoneally to each group after 48 h of fasting to induce gastric secretion. At the end of the experiment, the pyloric portion of the stomach was ligated and after 5 h the stomachs were opened along the greater curvature and gastric juice was collected. Also gastric pH, total acidity, gastric juice volume and pepsin concentration were determined. Data were analysed using IBM SPSS version 22.
Results: Pre-treatment with CMD and aqueous extract of A.D leaves significantly increases the gastric pH ($P<0.05$) compared to controls. Total acidity and pepsin concentration were significantly reduced ($P<0.05$) in rats pre-treated with CMD and A.D extract groups compared to controls respectively. At low dose (100mg/kg AD) of the extract there was significant increase ($P<0.05$) in gastric juice volume compared to the controls.
Conclusion: Aqueous extract of A.D significantly decreases the effects of aggressive factors in gastric juice which conclude the gastro-protective effect of the extract.
Key words: *Adansonia digitata l.*, Indomethacin-induced gastric secretion, pyloric ligation, Wistar rats.

INTRODUCTION
Parietal cells are highly specialized epithelial cells, with distinctive morphologic features that support their acid-secreting function (Kopic *et al.*, 2010). In resting state, the apical plasma membrane presents small invaginations or canaliculi that project throughout the cell interior and interconnect (Arin *et al.*, 2017).
Cytoplasm contains abundant membrane structures called tubulovesicles rich in H^+/K^+-ATPase, the proton pump responsible for proton extrusion during acid secretion Duman *et al.* (2002).

The H^+/K^+-ATPase exchange an intracellular hydrogen ion for an exracellular potassium ion, consuming ATP in the process. Sustained proton extrusion requires two other ion transport processes to occur in the apical plasma membrane of parietal cells (Sidani *et al.,* 2007). One of them is chloride secretion, which is necessary to maintain electroneutrality during acid secretion (Xu *et al.,* 2008). The other one is potassium recycling, necessary to avoid luminal potassium depletion, which would impair H^+/K^+-ATPase activity (Heitzmann and Warth, 2007). Acid is thought to gain access to the lumen via channels in the mucus layer created by the relatively high intra-glandular hydrostatic pressures generated during secretion, approximately 17 mm Hg (Johansson *et al.,* 2001). Acid facilitates the digestion of protein and absorption of iron, calcium, and vitamin B-12 as well as prevents bacterial and enteric infection (Schubert and Shamburek, 1990). However, when levels of acid (and pepsin) overwhelm mucosal defense mechanisms, ulcers occur (Dufresne *et al.,* 2006). To prevent such damage, gastric acid must be precisely regulated which is accomplished by a highly coordinated interaction of neural pathway through the releasing of acetylcholine from postganglionic enteric neurons, hormonal by secretion of gastrin from G cells, and paracrine pathways by histamine released from enterochrommaffin like (ECL) cells (Yakabi *et al.,* 2006).

Baobab (*Adansonia digitata* Linn.), a tree plant belonging to the Malvaceae family, widespread throughout the hot, drier regions of tropical Africa (De Caluwe *et al.,* 2010). Powdered leaves are used as anti-asthmatic agent and known to have antihistamine (De Caluwe *et al.,* 2010). Leaves, bark, roots, pulp and seeds are used for multiple medicinal purposes in many parts of Africa and were found to show interesting medicinal properties including antioxidant, pre-biotic-like activity, anti-inflammatory, analgesic, antipyretic activity, anti-diarrhea, anti-dysentery activity (Sanchez *et al.,* 2011). Several studies have been conducted regarding the development of a wide spectrum of anti-ulcer drugs, however, most of these drugs have adverse drug reactions like arrhythmias, gynaecomastia, haemopoetic changes and are considerably expensive (Bech *et al.,* 2000). Hence, the need to search for alternative and effective agents with fewer side effects, greater availability, affordability, efficacy and safety (Antonisamy *et al.,* 2014), therefore, discovering natural agents, which are believed to be safe, effective and affordable is still very essential for ulcer therapy (Bhattacharya *et al.,* 2007). The aim of this study is to determine the gastro-protective effect of aqueous extract of *A.digitata linn.* leaves against indomethacin induced gastric secretion in Wistar rats

MATERIALS AND METHODS

Drugs and chemicals used in the study include the following:

1-Cimetidine (Greenfield Pharmaceutical Company Limited, Jiangsu, China) tablet was dissolved in distilled water and given in a dose of 100mg/kg orally.

2-Distilled water was used to dissolve the indomethacin capsule and aqueous extract of *Adansonia digitata linn.* leaves.

3-Sodium hydroxide (NaOH) (BDH laboratory supplies poole England) pellet was used for the preparation of 0.01N NaOH.

4-Phenolphthalein (BDH laboratory supplies poole England) powder was used to prepare phenolphthalein indicator.

5-Casein (BDH Chemicals Ltd poole England) powder was used for the preparation of 3% casein solution.

6-Trichloroacetic acid (Molychem Mumbai India) pellet was used for the preparation of 6% trichloroacetic acid solution.

7-Indomethacin (DEVA Holding A.S Kucukcekmece/ Istanbul) capsule was dissolved in distilled water and given in a dose of 50mg/kg intraperitoneally.

8-Ketamine hydrochloride (ROTEXMEDICA Germany) injection was used as an anaesthesia.

Reagents

1. Casein substrate solution 3% (w/v): Three gram of casein powder was dissolved in 100ml of 0.1N HCl, then boiled for 15 minutes and the volume was readjusted to 100ml by 0.1N HCl.
2. Trichloroacetic Acid solution 6% (w/v): This was prepared by dissolving 6 gram of trichloroacetic acid crystals in 100ml of distilled water.

Leaves Collection, Authentication and Processing

The leaves of the *Adansonia digitata linn.* tree were obtained from the farm land located in Chiranchi Tudu, under Chiranchi ward, Kumbotso Local Government Area, Kano State and were submitted to taxonomist Dr. Yusuf Nuhu for authentication at the Department of Plant Biology, Faculty of Life Sciences, Bayero University Kano. The plant was given a herbarium accession number as BUKHAN 0036, and a voucher of the sample was deposited in the Department, then 25 grams of the leaves were washed and cleaned to remove impurities present on them. They were dried under shade for 5 days.

Preparation of the extracts.

The dried leaves were made into fine powder using mortar and pestle then sieved using a muslin cloth and stored in air tight containers for future use as described by Mukhtar and Tukur (2000).

Ten (10) grams of the powdered sample was soaked with 1L of distilled water using a rotary shaker. The sample was filtered with Whatman's filter paper (size 1) and filtrate was evaporated to dryness at 50 ºC under reduced pressure with Buchi Rotavapor for 6 hours. The residue obtained was then used as the aqueous extract (Singh *et al*., 2014). This extraction was carried out at the Department of Pharmacognosy laboratory, Faculty of Pharmaceutical Sciences, Bayero University, Kano. The extract was dissolved in distilled water at the various desired concentrations (100mg/kg, 150mg/kg and 200mg/kg) before use.

Phytochemical Analysis

The phytochemical screening of aqueous extract of *A. digitata l.* leaves were tested by the simple and standard qualitative methods described by Evans (2002), and Sofowora (2008).

Animals and Environmental Conditions

A total of 42 male Wistar rats 7-8 weeks' old weighed 160-200g were purchased from the animal house of Pharmacology Department, Faculty of Pharmaceutical Sciences, Bayero University Kano. The animals were housed in large cages in a well ventilated animal House under normal room temperature with free access to rodent chow and tap water was supplied *ad libitum*. The animals were randomly distributed into different experimental groups. The control and experimental groups consisted of five rats each.

Animals were handled in accordance with the guidelines of the National Institute of Health (NIH) for laboratory animal care and use.

Acute toxicity study

The method of Lorke (1983), was used to determine the median lethal dose (LD50) of the extract.

The median LD_{50} is calculated using the formular below:

$LD_{50} = \sqrt{D_{100} \times D_0}$ (Adefisayo *et al.*, 2017).

Where D_{100}= least dose that killed a rat, D_0= highest dose that did not killed any rat.

Study Design and Grouping

The research was an experimental study design. After two weeks for acclimatization, the animals were grouped into six experimental groups, containing five wistar rats each.

Group 1 (Control): The animals received distilled water (1ml/kg) orally and served as control.

Group 2 (Indomethacin): The animals were fasted for 48 h before intraperitoneal injection of a single dose of indomethacin (50mg/kg) (Akpamu *et al.*, 2013).

Group 3 (Cimetidine+ Indomethacin): The animals received 100 mg/kg of cimetidine daily for 14 consecutive days orally and fasted for 48 h before intraperitoneal indomethacin (50mg/kg) injection (Adefisayo *et al.*, 2017).

Group 4 (100mg/kg AD+ 50mg/kg indomethacin): The animals received aqueous extract of *Adansonia digitata* (AD) leaves (100mg/kg) daily for 14 consecutive days orally and fasted for 48 h before intraperitoneal indomethacin (50mg/kg) injection (Basipogu *et al.*, 2018).

Group 5 (150mg/kg AD+ 50mg/kg indomethacin): The animals received aqueous extract of *Adansonia digitata* (AD) leaves (150mg/kg) daily for 14 consecutive days orally and fasted for 48 h before intraperitoneal indomethacin (50mg/kg) injection (Basipogu *et al.*, 2018).

Group 6 (200mg/kg AD+ 50mg/kg indomethacin): The animals received aqueous extract of *Adansonia digitata* (AD) leaves (200mg/kg) daily for 14 consecutive days orally and fasted for 48 h before intraperitoneal indomethacin (50mg/kg) injection (Basipogu *et al*., 2018).

Collection of gastric secretion by pyloric ligation.

The animals were fasted for 48 hours in separate cages with raised wide wire mesh to avoid coprophagia (Basso *et al.*, 1983), but with water given *ad libitum* as in gastric ulcer group. Under anesthesia by ketamine hydrochloride (50mg/kg) intraperitoneally, the abdomen of the rats were shaved and a midline incision was made extending downwards from the xiphoid, abdominal wall was opened, the pylorus identified, ligated and abdomen was then closed (Abdelaziz *et al.*, 2006). After 5 hours, the animals euthanized by decapitation under anaesthesia, abdomen opened again, the oesophagus ligated and the stomach was removed and an opening was made along the greater curvature and the gastric juice was drained into a graduated test tube, it was then centrifuged at 3000 g for 15 min, after which the supernatant fluid volume was recorded in (ml) (Shay *et al.*, 1954). Gastric secretion parameters was determined including gastric pH determined in the supernatant using a pH meter, gastric juice volume (ml), total acidity (Meq/L) and proteolytic activity by pepsin concentration (mg/ml).

Analysis of gastric juice

Determination of the volume of each sample after centrifugation.

The supernatant fluid volume (mL) of each gastric content was measured using graduated test tube after centrifugation.

Determination of gastric pH

The pH of gastric juice was determined using a digital pH meter (Gehan *et al.*, 2009).

Determination of Total gastric acidity.

Gastric juice supernatant (0.2mL) was titrated with 0.01N NaOH using an end point of pH 7.0 as determined by colorimetrically with phenolphthalein as an indicator Davenport (1977). The NaOH was titrated against the acidic solution in the beaker, and observed until pink colouration was obtained in accordance to the method of Gehan *et al.* (2009) and Wang *et al.* (2007). It was calculated as milliequivalent per liter (Meq/L) which is equal to the number of milliliters (ml) of 0.01N NaOH required to neutralize 1 ml of gastric juice. The total acidity (mEq/L) was calculated using the following formular below:

Total gastric acidity = Vol of 0.01N NaOH (ml) which neutralizes 1 ml of gastric juice×N×100.

where N= normality= 0.01eq/L (Fornai *et al.*, 2011).

Determination of Proteolytic activity of gastric secretion

Pepsin content which is responsible for the major part of proteolytic activity of gastric juice was determined spectrophotometrically according to the method of (Hawk *et al.*, 1960).

About 0.2ml of centrifuged gastric juice was added to 3ml of casein 3% for each rat test and blank. Then 10ml of 6% trichloroacetic acid added to blank to stop enzyme activity.

Both blank and test tubes were incubated in water bath with temperature 37°C for 30 minutes. Then 10ml of trichloroacetic acid added to test tubes, shaken well and filtered by no. 1 Whattman filter paper. Proteolytic activity determined spectrophotometrically by optical density measured at 280 wave length. The pepsin concentration was deduced from standard proteolytic activity Curve.

Statistical Analysis

Quantitative variables were summarised as Mean ± SEM. Data were compared by one-way analysis of variance (ANOVA), followed by a Tukey's post-hoc to determine the statistical significant difference between the groups using IBM SPSS version 22. The $p<0.05$ indicated a significance difference.

RESULTS

Phytochemical screening

The aqueous extraction of phytochemical constituents of *A. digitata l.* leaves revealed the presence of alkaloids, flavonoids, saponins, steroids, and tannins (Table 4.1). The presence or absence of the compound were expressed as positive (+ve) or negative (-ve) respectively.

Table 1: Phytochemical components of *A. digitata l.* leaves.

Phytochemicals screened	Aqueous extract (Inference)
Tannins	+
Terpenoids	-
Flavonoids	+
Saponins	+
Alkaloids	+
Anthraquinones	-
Steroids	+

Keys: (+): Present; (-): not present.

Acute oral toxicity (LD_{50}) test of the aqueous extract of *Adansonia digitata l.* leaves

The result of acute toxicity test showed that there was sign of toxicity at 5000mg/kg dose in the second phase of the test. Signs of toxicity such as salivation, stretching of the entire body, weakness, decrease locomotion, writhing, decreased in sensitivity to touch, weight loss were noticed in the first 4 hours and subsequently 24 hours after extract administration and there were no death recorded throughout the study. Therefore, LD_{50} was found to be greater than 5000mg/kg as there was no mortality up to 5000mg/kg dose of the extract presented in Table 2 below.

Table 2: Acute oral toxicity test (LD_{50}), of the aqueous extract of *Adansonia digitata l.* leaves

Groups	No. of animals	Doses (mg/kg)	No. of Death	LD_{50} (mg/kg)
1st phase				
1	3	10	0	>5000
2	3	100	0	
3	3	1000	0	
2nd phase				
1	1	1600	0	
2	1	2900	0	
3	1	5000	0	

Therefore, LD_{50} of aqueous extract of *Adansonia digitata l.* leaves = >5000mg/kg body weight in adult Wistar rats.

Effect of aqueous extract of *Adansonia digitata l.* on gastric pH, total acidity, gastric juice volume and pepsin concentration

Table 3 below presents the result of the effect of aqueous extract of *A. digitata l.* on gastric secretion parameters. The result shows that there was statistical significant difference in gastric pH, total acidity, gastric juice volume and pepsin concentration (P=0.000, 0.001, 0.000, 0.000) respectively, across the groups. There was a significant decrease in gastric pH in IND group (1.16±0.17) while significant increase in pH in all the groups (3.70±0.15, 2.95±0.06, 3.47±0.18, 4.04±0.11) compared to control group (1.78±0.09). There was a significant increase in total acidity in IND group (77.40±13.72) compared to control group (50.80±3.02) while significant decrease in total acidity in cimetidine and extract treatment groups (36.80±2.58, 42.00±1.47, 36.50±2.33, 36.80±1.07) respectively compared to IND group (77.40±13.72). Similarly, the difference in gastric juice volume for 100mg/kg AD + IND extract (3.95±0.19) and control, IND, CMD+IND groups (1.70±0.49, 1.06±0.29, 0.74±0.20) respectively was highly significant (P<0.05). Also, there was a significant increase in pepsin concentration in IND, CMD+IND, and 100mg/kg AD+IND groups (10.60±0.22, 7.90±0.07, 4.67±0.33) respectively compared to control group (3.06±0.05) while significant decrease in pepsin concentration in all the groups (7.90±0.07, 4.67±0.33, 3.60±0.18, 3.54±0.23) compared to IND group (10.60±0.22).

Table 3: Effect of aqueous extract of *A. digitata* L. on gastric pH, Total acidity, gastric juice volume and pepsin concentration. (mean±SEM, n=5)

Variables	Group 1 (control)	Group 2 (IND)	Group 3 (CMD+IND)	Group 4 (100mg/kg AD+IND)	Group 5 (150mg/kg AD+IND)	Group 6 (200mg/kg AD+IND)	F-Value	P-Value
pH	1.78±0.09	1.16±0.17^{a}	3.70±0.15^{ab}	2.95±0.06^{abc}	3.47±0.18^{ab}	4.04±0.11^{abd}	75.176	0.000*
Total acidity (meq/mL)	50.80±3.02	77.40±13.72^{a}	36.80±2.58^{b}	42.00±1.47^{b}	36.50±2.33^{b}	36.80±1.07^{b}	6.583	0.001*
Gastric juice volume (mL)	1.70±0.49	1.06±0.29	0.74±0.20	3.95±0.19^{abc}	1.57±0.42^{d}	0.76±0.22^{d}	12.423	0.000*
Pepsin concentration (mg/ml)	3.06±0.05	10.60±0.22^{a}	7.90±0.07^{ab}	4.67±0.33^{abc}	3.60±0.18^{bcd}	3.54±0.23^{bcd}	257.503	0.000*

*= P<0.05. IND=Indomethacin, CMD= Cimetidine, AD=Adansonia digitata. a=There is significance diff. with control group, b= there is significance diff. with the indomethacin group, c= there is significance diff. with cimetidine group, d= there is significant diff. with the 100mg/kg AD extract, e= there is significant diff. with the 150mg/kg AD extract, f= there is significant diff. with the 200mg/kg AD exract. (P<0.05 indicate significance difference).

DISCUSSION

In this study, the phytochemical screening of aqueous extract of *Adansonia digitata l.* leaves indicated the presence of tannins, flavonoids, saponins, alkaloids and steroids while terpenoids and anthraquinones were absent. The finding conforms with the study of Zagga *et al.* (2018), who reported the presence of saponins, flavonoids, alkaloids, tannins, cardiac glycosides, total phenols and absence of terpenoids, triterpenes and gelatin. This study investigated the acute oral toxicity test of aqueous extract of *Adansonia digitata l.* leaves and the median lethal dose (LD_{50}) was found to be greater than 5000mg/kg as presented in table 2 which conforms to the study of Christian *et al.* (2012), which showed that *Adansonia digitata l.* leaves aqueous extract has the LD_{50} of 5000 mg/kg. Non-toxicity of *Adansonia digitata l.* explains why most of the plant parts, seeds, fruit pulps, stem and leaves are consumed by many communities (Kamatou *et al.*, 2011, and Nguta *et al.*, 2011).

According to toxicity classes of Hodge and Sterner (2005), any compound with oral LD_{50} (rat) of 5000mg/kg or more should be

considered as practically harmless. Hence, oral administration of aqueous extract at a dose of less than or equal to 5000mg/kg could be safe (Abdulmalik and Magashi, 2016).

Gastric pH was significantly increase in CMD and extract treated groups ($P<0.05$), with statistically significant decrease in indomethacin group ($P<0.05$) when compared to the control group. This corroborates with the findings of Sabiu *et al.* (2015), Ibraheim (2019) and Katary and Salahuddin (2017), who reported a significant decrease in gastric pH in indomethacin group when compared with control group and is in line with the study of Raji *et al.* (2011), Usman *et al.* (2014) and Basipogu *et al.* (2018) who found a significant increase in gastric pH in cimetidine group and aqueous extract of *A. digitata l.* treated groups compared with control group.

The pH gives an idea of the level of acidity and volume of gastric secretions. Low pH value is a manifestation of increased hydrogen ion concentration in gastric juice and increase in volume of gastric juice (Sabiu *et al.,* 2015). Indomethacin induced aggressive factors via a decrease in gastric juice pH, indicating altered hydrophobicity (Salahuddin and Katary, 2017). This has been linked to pathogenesis of ulcer and gastric damage in experimental animals (Lullmann *et al.,* 2000). The cimetidine effects on gastric pH can be explained as H_2-receptor antagonists that act directly on the gastric mucosa to decrease acid secretion which causes subsequent increase in gastric pH and inhibit ulcer formation (Waldum *et al.,* 2000). Alkaloid compounds are reported to have potent activity against gastric ulcers (Malgave *et al.,* 2019), *A. digitata l.* extract provided significant protection against ulcer as indicated by an increase in pH of gastric content which indicates increase in alkalinity and reduces the acidic nature of the gastric juice (Kaur *et al*., 2014).

There was significant increase ($P<0.05$) in total acidity in IND group when compared with the control group which corroborate to the findings of Katary and Salahuddin (2017), and Oluwabunmi and Abiola (2015), that showed a significant increase in total acidity in indomethacin group compared with control group. Following administration of indomethacin in the ulcerated rats this may be attributed to either free radicals formation or inhibition of prostaglandin synthesis (Sabiu *et al.,* 2015). Decreased prostaglandin level has been attributed to causes elevation of gastric acid secretion, reduced mucosal blood flow, and bicarbonate secretion which impaired gastroprotection which are important events in the etiology of mucosal ulceration and indomethacin was reported to have caused alterations in gastric secretions of rats (Sabiu *et al.,* 2015).

This study, there was significant decrease ($P<0.05$) in total acidity in (CMD+IND) group and extract treated groups when compared with indomethacin group which is in line with the study of Raji *et al.* (2011) and Adefisayo *et al.* (2017) who reported a significant decrease in total acidity in cimetidine group when compared with indomethacin group and Basipogu *et al.* (2018) who reported a significant decrease in total acidity in extract treatment groups compared to indomethacin group. Possible mechanism of action of cimetidine (H_2 receptor blocker) on gastric acid secretion is by blocking H_2 receptor leading to inhibition of histamine release whose stimulatory action on gastric acid secretion had been established (Raji *et al.,* 2011). It is established that inhibition of histamine through H_2 receptors, inhibit intracellular adenylate cyclase, Na^+-K^+ ATPase and proton pump of parietal cells that eventually reduce the gastric acid secretion (Ayada, *et al.,* 2003). The reduction in gastric acid secretion seen in the groups treated with aqueous extract might be due to action of flavonoids which increases mucosal Prostaglandin content and inhibits histamine secretion (Malgave *et al.,* 2019). Histamine increases acid secretion through its binding to H_2 receptors (Schubert and Peura, 2008). Flavonoids's inhibitory action on histamine release may play a role in its effect on acid

acid secretions, which eventually inhibits intracellular adenylate cyclase, Na^+-K^+ ATPase responsible for gastric acid secretion (Malgave *et al*., 2019). Tannin tends to compete with adenosine triphosphate at the ATP hydrolysis site, thereby causing the inhibition of gastric H^+-K^+ ATPase that is necessary for gastric acid secretion (Adefisayo *et al.,* 2017). This suggests that *Adansonia digitata l.* leaves aqueous extract has inhibitory effect on gastric acid secretion and its inhibitory action might mimic cimetidine effect on gastric acid secretion.

In this study there was no significant difference (P>0.05) in gastric juice volume in indomethacin group compared to control group which is in contrary to the study of Sabiu *et al.* (2015), Oluwabunmi and Abiola, (2015) and Katary and Salahuddin (2017), who showed significant increase (P<0.05) in gastric juice volume in (indomethacin) group compared with control group. Indomethacin induced aggressive factors via increased gastric juice volume indicating altered hydrophobicity Katary and Salahuddin (2017). However, indomethacin caused decreased acid output with major mucosal damage and it is possible that the damage of cells and glands by indomethacin have also affected the acid producing parietal cells which eventually caused decrease in volume of gastric juice secreted from the parietal cells. It is also known that the capacity of the stomach to secrete HCl (Hydrochloric acid) is almost linearly related to parietal cell numbers which are damaged and decreases their number by indomethacin treatment and eventually decrease the volume of gastric juice (Yao and Forte 2003). Also this study showed significant increase (P<0.05) in gastric juice volume in (100mg/kg AD+IND) group when compared with control, indomethacin, cimetidine and 150mg/kg AD+IND and 200mg/kg AD+IND extract treated groups which is in contrary to the findings of Basipogu *et al.* (2018) who showed decrease in gastric juice volume in aqueous extract of *A. digitata l.* treated groups compared with control group.

Pepsin concentration, was significantly increase (P<0.05) in indomethacin treated group compared with control group. This agrees with the findings of Idowu *et al.* (2021), who found significant increase in pepsin concentration in indomethacin group compared to control group. Pepsin is responsible for proteolysis in the stomach and its activity depends on gastric acid output since gastric acid is required for its acidification from pepsinogen to pepsin (Abdallah *et al.,* 2011). The increase in pepsin concentration of ulcerated rats may have resulted from the activated proton pump which increased the gastric output and subsequently increasing the pepsin activity since gastric acid is required for acidification of this proteolytic enzyme (Idowu *et al.,* 2021). Additionally, pepsinogen secretion responds to enteric reflexes that could arise by aggravation of the gastric mucosa (Baharfar *et al.,* 2015), with aggressive agents such as indomethacin (Hernandez *et al.,* 2000). The present study found a significant decrease (P<0.05) in pepsin concentration in cimetidine group compared with indomethacin group which is in line with the study of Saleh *et al.* (2015) who found a significant decrease in pepsin concentration in cimetidine group compared with indomethacin group. Pre-treatment with the standard anti-ulcer drug however gave cyto-protective effects which is associated with decreased pepsin activity in the gastric mucosa (Nworgu *et al.,* 2019). Also, there was a significant decrease (P<0.05) in pepsin concentration among the rats treated with different concentrations of the extract compared with (IND) and CMD group, which corroborates with the findings of Malgave *et al.* (2019) who found a significant decrease in pepsin concentration in extract treated groups compared to the ulcerated group. The extract contains flavonoids which inhibits histamine secretion responsible for gastric acid secretion, thus inhibiting acid secretion which is responsible for the conversion of pepsinogen in to active pepsin and eventually decreases the pepsin concentration (Malgave *et al*., 2019).

CONCLUSION

The aqueous extract significantly decreases the aggressive factors which contribute to the formation of gastric ulcer such as total acidity, pepsin concentration and gastric juice volume and also increases the gastric pH which indicates the level of acidity, hence indication of gastro-protective effect of aqueous extract of *A. digitata l.* leaves.

Recommendation

Similar study should be carried out using methanolic, ethanolic extract of *Adansonia digitata l.* leaves to see whether different phytochemicals will be found and their respective effects. Further study should be conducted on the effect of aqueous extract of *Adansonia digitata l.* leaves on free radicals and lipid peroxidation.

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