ORIGINAL PAPER

https://dx.doi.org/10.4314/njpr.v16i2.7

Nig. J. Pharm. Res. 2020, 16 (2) pp 163-175
ISSN 0189-8434 **e-ISSN 2635-3555** **Available online at http://www.nigjpharmres.com**

Chrysophyllum Albidum Accelerates Delayed Gastric Ulcer Healing in Rats Through Oxidative Stress Reversal and Proton Pump Inhibition

A.T. SALAMI*[1 A-F], A. D. FAMUREWA[1 B-C, F], T. P. OMAYONE[2 B-C,E-F], T. F. IYIOLA[1,3 B-C,E-F], S. B. OLALEYE[1 A-C,E-F]

[1]Gastrointestinal secretion and Inflammation Research unit,
Department of Physiology, Faculty of Basic Medical sciences, University of Ibadan, Ibadan, Oyo State
[2] Department of Physiology, School of Health and Health Technology, Federal University of Technology, Akure, Nigeria
[3] Department of Physiology, Faculty of Basic Medical Sciences, Adeleke University, Ede, Osun State

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: *Chrysophyllum albidum* has been documented to exert its gastric ulcer (GU) healing activities by modulating blood inflammatory mediators, however, other probable in-vivo underlying mechanisms are still vague which this study sought to investigate.

Materials and Methods: Male Wistar rats (120-130g) divided into 9 groups (n=15 for groups I-VII; n=5 for groups VIII & IX) viz: Groups I- positive control (*DUnA*); II and III–250 and 500mg/kg methanolic extract of *C. albidum (MeCaB)* bark respectively; IV, V and VI-100mg/kg fractions A, B and C respectively; VII–30mg/kg omeprazole; VIII-ulcerated untreated (baseline), IX-negative control. Chronic GU was induced experimentally and delayed using indomethacin with 14 days simultaneous drug treatment. Gastric ulcer score, mucin content, antioxidant and proton pump activities were evaluated by days 3, 7 and 14 of treatment. Data were expressed as Mean±SEM and $P \leq 0.05$ was significant.

Results: *C. albidum* and fractions treated groups significantly decreased gastric ulcer scores and lipid peroxidation compared with *DUnA*. Negative control, *C. albidum* and fraction treated groups significantly increased superoxide dismutase, catalase, glutathione levels and mucin content compared with *DUnA* group by days 3 and 7. *C. albidum*, Negative and baseline control groups significantly decreased H^+K^+ATPase activities compared with *DUnA* by day14.

Conclusion: *C. albidum* and its fractions facilitated the healing of gastric ulcer, probably by enhanced antioxidant levels, mucin content and decreased gastric H^+K^+ATPase activity.

Keywords: C. *albidum* and chromatographic fractions, gastric ulcer healing, mucin , antioxidant, H^+/K^+ATPase pump.

INTRODUCTION

Delayed gastric ulcer healing activities of *Chrysophyllum albidum* has been documented (Salami and Famurewa, 2017) and was proposed to confer this activity through modulating inflammatory blood markers. *Chrysophyllum albidum* (G.Don) *Sapotaceae* family has been reported to have varied acclaimed medicinal properties: antinociceptive (Idowu *et al*., 2006), anti-colitis (Salami *et al*., 2018) and antihelmintic potentials (Salami *et al*., 2015). This probably is as a result of the inherent phytochemical constituents such as flavonoids, alkaloids, cardenolides, anthraquinones etc. which have also been implicated to ameliorate some gastrointestinal disorders (Salami *et al*., 2015). Stomach homeostasis is constantly been maintained by a balance in the protective and aggressive mucosa factors; a tilt in favor of aggressive factors results in gastric ulcer disease (GUD) partly due to release of free radicals (Amaral *et al*., 2013). Proton pump inhibitors have been used over the years in management of gastric ulcer mostly in preventing gastric acidity. However, these synthetic proton pump inhibitors have been documented to cause adverse activities on some other systems of the body (Klatte *et al*., 2017), hence the search for natural safer proton pump inhibitors. Recently, the need to curb generation of free radicals and gastric acidity in the treatment and management of GUD as another probable mechanism has been on the rise (He *et al*., 2019). Due to these new lines of GUD management, the probable antioxidative activities of *Chrysophyllum albidum* during (delayed) gastric ulcer were investigated in this study.

METHODOLOGY

Plant materials, collection and identification

Fresh barks of *C. albidum* were harvested from Igbo Owe farm at Moniya, Akinyele local government of Oyo state, South-Western Nigeria; its natural habitat during its' flowering and fruiting season (between December – February). A voucher number FH1 107514 was given to the identified plant parts at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Preparation of plant extracts and fractions was by the earlier reported methods of Salami *et al*. (2015) briefly,

Plant bark sequential extraction

Powdered-coarse bark of *C. albidum* weighing 2.0kg was soaked in 3.0L of absolute n-hexane for 72 hours in a glass extraction jar; the concentrated filtrate was collected as hexane fractions. The obtained marc was thoroughly air-dried for 24 hours. The dried marc was turned gently into a glass extraction jar after which 2.5L of absolute dichloromethane was added and thoroughly mixed for 72 hours, after which the filtrate was decanted to give dicholoromethane extract on concentration. The marc obtained was carefully air dried for 24 hours. Thereafter, the dried marc was well macerated in 2.5L of absolute methanol in a glass extraction jar for 72 hours after which the whole mixture was filtered, the corresponding filtrate was tagged methanol extract on concentration. All filtrates were concentrated *in-vacuo* at 37- 40^{o}C.

Column chromatography separation for active phytochemicals

The following solvents: Ethyl acetate, hexane and methanol were used in the extraction and isolation by column chromatography. 50 grammes of the methanol partitioned extract was dissolved in methanol and loaded on silica gel in the column as done by Salami *et al*. (2015). Elution began with absolute ethyl acetate gradually increasing the polarity to 100% methanol to give 3 pooled fractions (A, B and C) depending on the observed distance spots under different UV wavelength light and spectra and thereafter subjected to drying.

Experimental animals

The same procedure and conditions as done in Salami and Famurewa, (2017) was strictly adhered to, briefly - Male Wistar strain rats (120-150g) were used for this study after two weeks acclimatization. They were housed in solid bottom polypropylene cages under standard environmental conditions in a well-ventilated room under a condition of room temperature (23-25°C), relative humidity (55-65%), and natural environmental light (12 light/dark cycle). Animals were allowed free access to water *ad libitum* and fed with standard rodent pellets (Ladokun Feeds Nigeria Limited, Ibadan, Nigeria) throughout the experiment. All experimental animals received humane care according to the ethics and protocol outlined in the Guide for Care and Use of Laboratory Animals prepared by the National Academy of Science (NAS) (2011) which is approved by the Institutional Animal Ethical Research Committee.

Animal Groups

Experimental animals were divided into 9 groups of fifteen (15) rats each [except baseline and negative control groups with n=5]: Groups 1- Positive control (Delayed untreated ulcer; *DUna*), 2- Delayed Ulcer + 500mg/kg *C. albidum (HMeCaB*), 3- Delayed Ulcer + 250 mg/kg *C. albidum (LMeCaB*), 4- Delayed

Ulcer + 100 mg/kg *C.albidum* chromatography fraction A *(CcFrA*), 5- Delayed Ulcer + 100 mg/kg *C.albidum* chromatography fraction B (*CcFrB*), 6- Delayed Ulcer + 100 mg/kg *C.albidum* chromatography fraction C (*CcFrC*), 7- Delayed Ulcer + 30 mg/kg Omeprazole (*DUOme*), 8- (Ulcerated control, *UnA*) - Day 0: Baseline data before administration of indomethacin. **(**These animals were induced with gastric ulcer and were sacrificed on the fifth day). 9- Negative control (Normal). Delayed gastric ulceration and scoring were as described by Salami and Famurewa, (2017). Briefly, on induction of chronic gastric ulcer with 30% acetic acid, well defined gastric ulcer was formed by day 5 thereafter indomethacin was injected subcutaneously daily for 14 days to delay healing of formed chronic gastric ulcer (Szelenyi et al., 1982, Okabe et al., 2015, Salami et al., 2015).

Biochemical assay

The stomach after gastric ulcer scoring was weighed and placed in phosphate buffer (10% of its weight in mLs). It was then homogenized using a Tefflon Homogenizer and resulting homogenates were centrifuged at 10,000 revolutions per minute (rpm) at 4°C for 10 minutes. The supernatant fraction was collected, well labeled and kept frozen until use for biochemical estimations.

Protein concentration was estimated using Biuret method according to Gornal *et al*. (1949), but with slight modification in which potassium iodide was added to the reagent to prevent precipitation of Cu^{2+} ions as cuprous oxide. Lipid peroxidation was estimated by determining the level of Malondialdehyde (MDA) which was according to the method of Varsheny and Kale, (1990). Superoxide dismutase (SOD) activity of each supernatant fractions was by the method of Misra and Fridovich, (1972). Catalase (CAT) activity was evaluated by the method of Claiborne, (1985). Estimation of reduced glutathione (GSH) level was as described by Beutler *et al.* (1963). Gastric H^+/K^+ATPase activities of each supernatant fraction were by the modified method of Bewaji *et al*. (1985). Evaluation of the gastric mucin content was according to the method of Winzler, (1955)

Statistical analysis

Results were expressed as Mean ± SEM. One-way ANOVA with Bonferroni post hoc test was used to analyze the differences among them using Statistical Graphpad Prism package 5 and was significantly at $P \leq 0.05$.

RESULTS

Gastric ulcer score

The mean ulcer score was significantly reduced in the low dose methanolic extract of *C.albidum* bark (*LMeCaB*) and *C.albidum* chromatographic fractions (*CcFrA, CcFrB* and *CcFrC*) treated groups compared with the delayed ulcerated untreated group. There were no visible gastric ulcers in the *HMeCaB* group by day 3, 7 and 14 of treatment. The details are shown in Table 1.

Total gastric mucosa protein

There was a significant decreased in the total gastric mucosa protein level of the (delayed) ulcer untreated group when compared with the baseline control and treated (*MeCaB* and fractions) groups on days 3, 7 and 14. The total protein for *CcFrC* treated group was significantly low when compared with all the other groups (*MeCaB* and fractions) by day 14. This was documented in Table 2.

Table 1: Effect of methanolic bark extract of *Chrysophyllum albidum* **(*MeCaB*)** and its chromatographic fractions on ulcer index in delayed acetic acid ulcers after treatment by day 3, 7 and 14.

Groups	Ulcer Score (mm^2)			Relative Ulcer Score (mm^2)			Percentage healing rate		
	Day 3	**Day 7**	**Day 14**	**Day 3**	**Day 7**	**Day 14**	**Day 3**	**Day 7**	**Day 14**
DUnA (Positive control)	212.10±1.57^{bcdefg}	96.87±2.6^{bcdefg}	119.9±28.01^{bcdefg}	24.09±1.015^{bcdefg}	10.33±1.69^{bcdefg}	10.01±0.15^{bcdefg}	0	0	0
LMeCaB	1.047±1.05^{ag}	0.00±0.00^{ag}	0.00±0.00^{a}	0.109±0.109^{acdefg}	0	0	99.51	100	100
HMeCaB	0.00±0.0^{ag}	0.00±0.00^{a}	0.00±0.00^{a}	0	0	0	100	100	100
CcFrA	6.28±1.57^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.850±0.192^{eg}	0	0	97.04	100	100
CcFrB	16.76±1.05^{a}	0.00±0.0^{a}	0.00±0.00^{a}	2.01±0.33^{acdefg}	0	0	92.09	100	100
CcFrC	7.331±2.09^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.85± 0.26^{eg}	0	0	96.54	100	100
DUOme	15.19±0.52^{a}	4.451±1.31^{a}	13.61±2.09^{a}	2.36±0.057^{abcdf}	0.47±0.15^{abcdef}	0.92±0.22^{abcdef}	92.84	95.41	88.65
(Baseline) UnA	218.3±3.927			28.68±0.68				0	
Normal (Negative C**ontrol)**	0.00+0.00				-			-	

Values are represented as Mean ±SEM and are significant when $p \leq 0.05$.
***Keys of Significance**: [a] compared with delayed ulcer untreated alone (DUnA) group, [b] compared with 250mg/kg C. albidum treated group (LMeCaB), [c] compared with 500mg/kg C. albidum treated group (HMeCaB), [d] compared with fraction A treated group (CcFrA), [e] compared with fraction B treated group (CcFrB), [f] compared with fraction C treated group (CcFrC), [g] compared with omeprazole treated group (DUOme), [h] compared with ulcer alone group (UnA), [i]compared with normal (no ulcer or treatment).*

Table 2: Effect of methanolic bark extract of *Chrysophyllum albidum* **(*MeCaB*)** and its chromatographic fractions on gastric mucosa MDA levels during delayed ulcer healing.

Groups	Day 3 (unit/mg protein)	Day 7 (unit/mg protein)	Day 14 (unit/mg protein)
DUnA (Positive control)	1.896 ± 0.213 *abcdefg*	2.26 ± 0.276 *abcdefg*	0.748 ± 0.135 *abcdefg*
LMeCaB	1.394 ± 0.229	1.685 ± 0.136	0.651 ± 0.0989
HMeCaB	1.314 ± 0.0522	1.167 ± 0.0396	0.634 ± 0.0787
CcFrA	1.522 ± 0.14	1.355 ± 0.0284	0.469 ± 0.0557
CcFrB	1.211 ± 0.0249	1.238 ± 0.00033	0.558 ± 0.0146
CcFrC	0.858 ± 0.0572	1.48 ± 0.055	0.572 ± 0.00889
DUOme	1.311 ± 0.136	1.263 ± 0.0389	0.697 ± 0.1143
(Baseline) UnA	0.856 ± 0.054 *abcdeg*		
Normal (Negative control)	0.5057±0.0067 *abcdefgh*		

Values are represented as Mean ±SEM and are significant when p≤0.05.
***Keys of Significance**: [a] compared with delayed ulcer untreated alone (DUnA) group, [b] compared with 250mg/kg C. albidum treated group (LMeCaB), [c] compared with 500mg/kg C. albidum treated group (HMeCaB), [d] compared with fraction A treated group (CcFrA), [e] compared with fraction B treated group (CcFrB), [f] compared with fraction C treated group (CcFrC), [g] compared with omeprazole treated group (DUOme), [h] compared with ulcer alone group (UnA), [i]compared with normal (no ulcer or treatment).*

Gastric mucosa malondialdehyde (MDA) levels

There was a significant increase in gastric malondialdehyde (MDA) levels of the positive control (delayed ulcer untreated) group when compared with baseline control, negative control and the treated (*MeCaB* and fractions) groups by days 3, 7 and 14. The gastric MDA levels for *CcFrC* and *HMeCaB* treated groups were significantly low when compared with the treated (*MeCaB* and fractions) groups by days 3 and 14 respectively (Table 3).

Table 3: Effect of methanolic bark extract of *Chrysophyllum albidum* **(*MeCaB*)** and its chromatographic fractions on gastric mucosa protein levels during delayed ulcer healing.

Groups	Day 3 (mg/dl)	Day 7 (mg/dl)	Day 14 (mg/dl)
DUnA (Positive control)	0.924 ± 0.014	0.961 ± 0.002	0.751 ± 0.031 *d*
LmeCaB	0.973 ± 0.015	0.992 ± 0.029	0.892 ± 0.028 *d*
HmeCaB	0.966 ± 0.042	1.101 ± 0.0143*ah*	1.014 ± 0.062*a*
CcFrA	1.013 ± 0.0079	1.04 ± 0.0268*h*	1.07 ± 0.0349*a*
CcFrB	1.044 ± 0.0079*h*	1.008 ± 0.045	1.044 ± 0.0283*a*
CcFrC	1.006 ± 0.0017	1.057 ± 0.0115h	0.82 ± 0.003*de*
DUOme	1.001 ± 0.029	1.045 ± 0.0036h	0.83 ± 0.0606*de*
(Baseline) UnA	0.88 ± 0.045		
Normal (Negative Control)	0.943±0.0167		

Values are represented as Mean ±SEM and are significant when p≤0.05.
***Keys of Significance**: [a] compared with delayed ulcer untreated alone (DUnA) group, [b] compared with 250mg/kg C. albidum treated group (LMeCaB), [c] compared with 500mg/kg C. albidum treated group (HMeCaB), [d] compared with fraction A treated group (CcFrA), [e] compared with fraction B treated group (CcFrB), [f] compared with fraction C treated group (CcFrC), [g] compared with omeprazole treated group (DUOme), [h] compared with ulcer alone group (UnA), [i]compared with normal (no ulcer or treatment).*

Gastric mucosa Superoxide dismutase, Catalase and Glutathione levels

There was a significant decrease in the gastric SOD activity of the positive control (delayed ulcer untreated) group when compared with all the baseline control, negative (normal) control and treated (*MeCaB* and fractions) groups by days 3 and 7. However, by day 14 of treatment, the gastric SOD

levels for the *HMeCaB*, *LMeCaB* and *CcFrA* groups significantly decreased when compared with the positive control (delayed untreated ulcer) group (Fig. 1).

There was a significant decrease in the gastric CAT activity of the positive control (delayed ulcer untreated) group when compared with all the treated (*MeCaB* and fractions) and negative (normal) control groups by days 7 and 14.

The gastric CAT activity for *LMeCaB* treated group was significantly decreased by day 7 when compared with the positive (delayed untreated ulcer) and negative control groups. The gastric CAT activities of *CcFrC* and *HMeCaB* treated groups were significantly high relative to all the treated groups by days 7 and 14 respectively except the negative (normal) control group (Fig. 2).

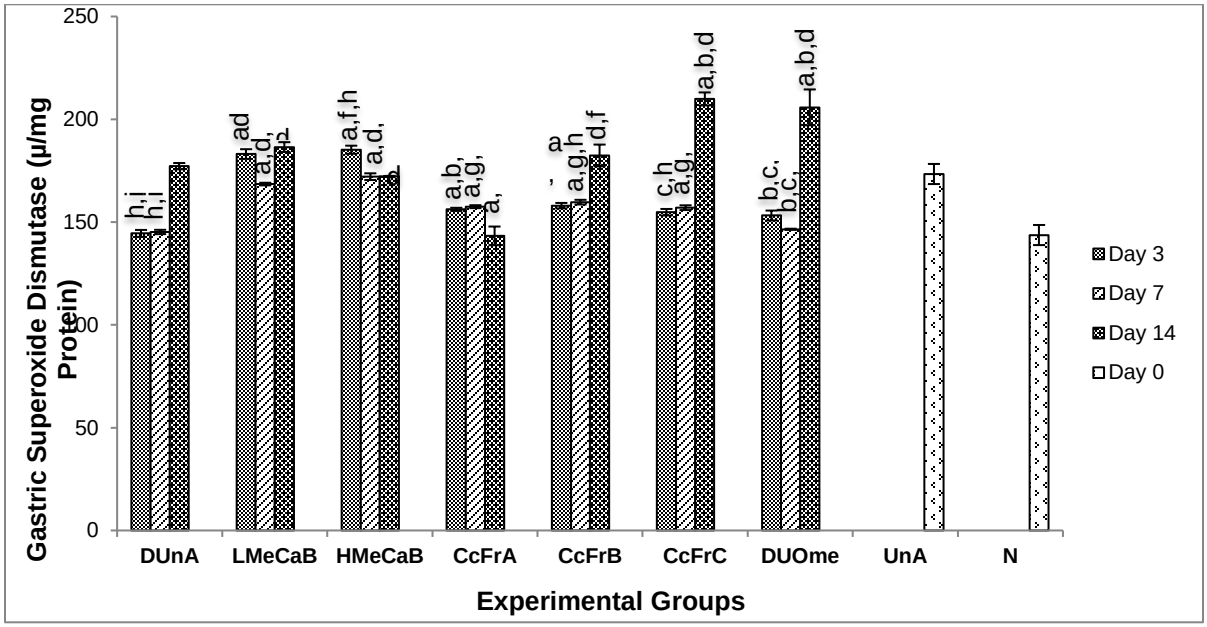

Figure 1. Effect of methanolic bark extract of *Chrysophyllum albidum* **(*MeCaB*)** and its chromatographic fractions on gastric mucosa superoxide dismutase (SOD) levels during delayed ulcer healing.

Values are represented as Mean ±SEM and are significant when p≤0.05.

***Keys of Significance**: [a] compared with delayed ulcer untreated alone (DUnA) group, [b] compared with 250mg/kg C. albidum treated group (LMeCaB), [c] compared with 500mg/kg C. albidum treated group (HMeCaB), [d] compared with fraction A treated group (CcFrA), [e] compared with fraction B treated group (CcFrB), [f] compared with fraction C treated group (CcFrC), [g] compared with omeprazole treated group (DUOme), [h] compared with ulcer alone group (UnA), [i]compared with normal (no ulcer or treatment).*

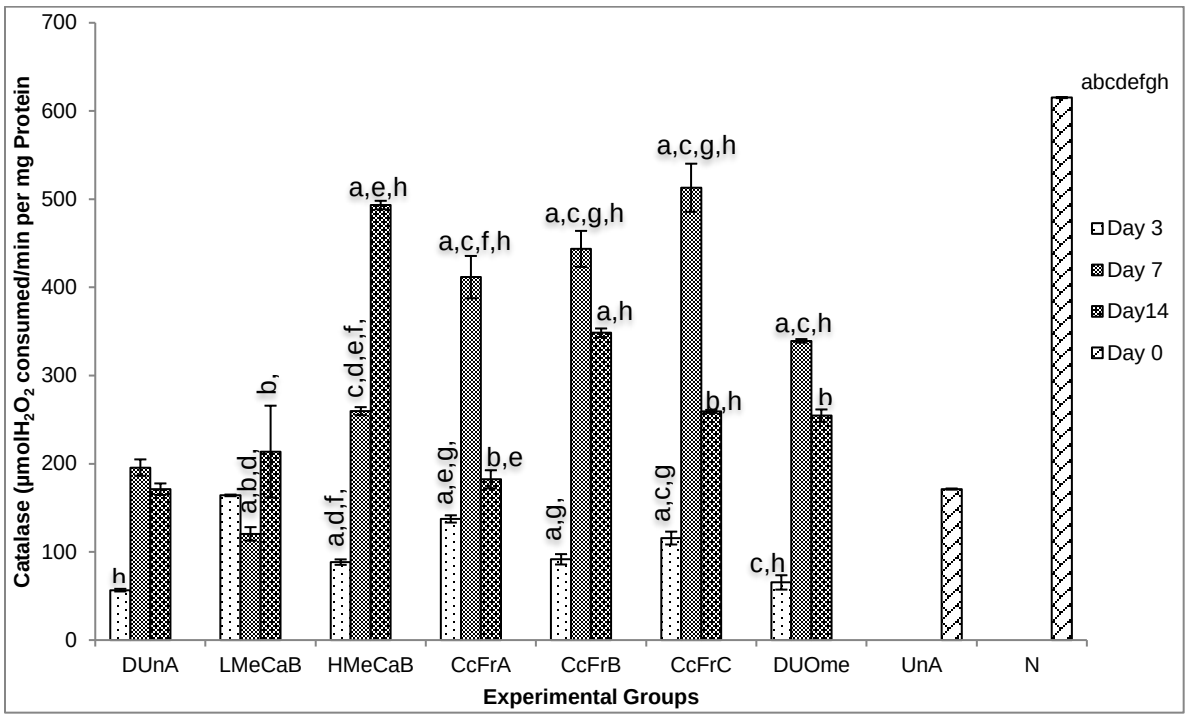

Figure 2. Effect of methanolic bark extract of *Chrysophyllum albidum* **(*MeCaB*)** and its chromatographic fractions on gastric mucosa catalase (CAT) levels during delayed ulcer healing

Values are represented as Mean ±SEM and are significant when p≤0.05.

***Keys of Significance**: [a] compared with delayed ulcer untreated alone (DUnA) group, [b] compared with 250mg/kg C. albidum treated group (LMeCaB), [c] compared with 500mg/kg C. albidum treated group (HMeCaB), [d] compared with fraction A treated group (CcFrA), [e] compared with fraction B treated group (CcFrB), [f] compared with fraction C treated group (CcFrC), [g] compared with omeprazole treated group (DUOme), [h] compared with ulcer alone group (UnA), [i]compared with normal (no ulcer or treatment).*

There was a significant decrease in the gastric GSH level of the positive (delayed ulcer untreated) control group when compared with the treated (*MeCaB* and fractions) and negative (normal) control groups on day 7. The gastric GSH level in *HMeCaB* and *LMeCaB* treated groups was significantly decreased compared with positive (delayed ulcer untreated) control group by day 3. There was a significant decrease in the gastric GSH activity of the treated (*MeCaB* and fractions) treated groups when compared with the positive (delayed ulcer untreated) control group on day 14. However, a significantly increased in GSH was observed in the *CcFrC* and *DUOme* groups when compared with the positive (delayed ulcer untreated) control group by day 14. A significant increase in gastric GSH activity of the normal group was observed when compared with all other groups by day 14 (as well as ulcerated untreated; baseline) (Fig. 3).

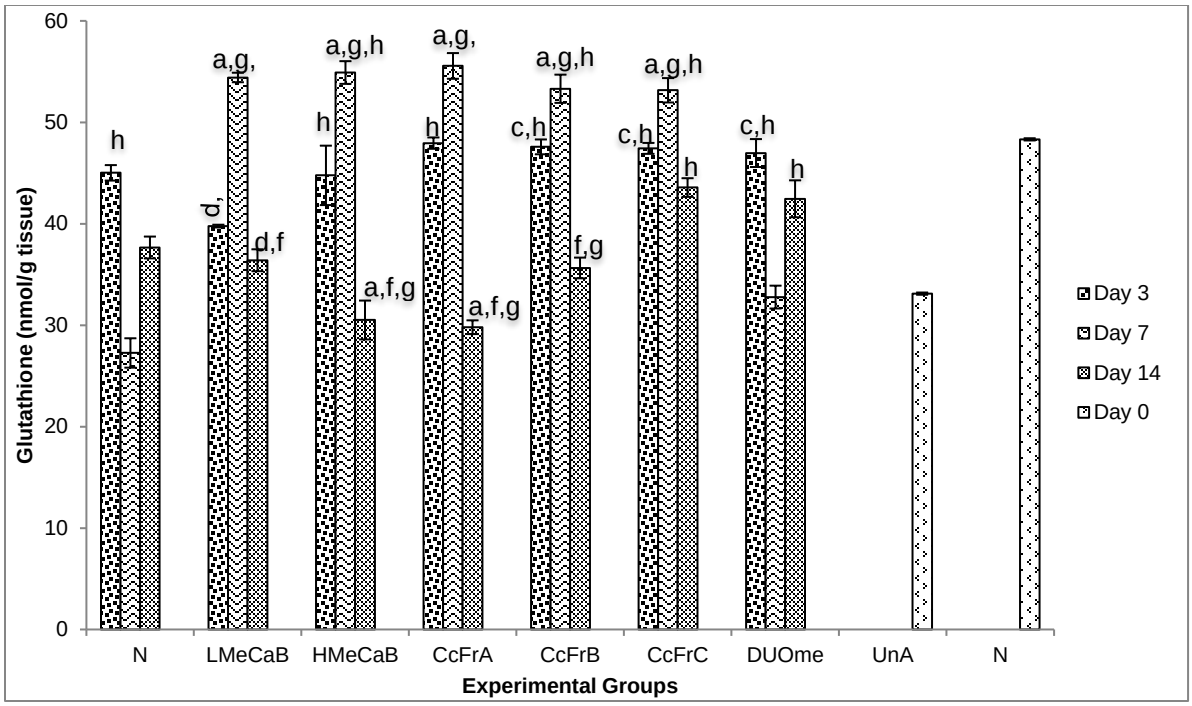

Figure 3. Effect of methanolic bark extract of *Chrysophyllum albidum* **(*MeCaB*)** and its chromatographic fractions on gastric mucosa glutathione (GSH) levels during delayed ulcer healing

Values are represented as Mean ±SEM and are significant when p≤0.05.

***Keys of Significance**: [a] compared with delayed ulcer untreated alone (DUnA) group, [b] compared with 250mg/kg C. albidum treated group (LMeCaB), [c] compared with 500mg/kg C. albidum treated group (HMeCaB), [d] compared with fraction A treated group (CcFrA), [e] compared with fraction B treated group (CcFrB), [f] compared with fraction C treated group (CcFrC), [g] compared with omeprazole treated group (DUOme), [h] compared with ulcer alone group (UnA), [i]compared with normal (no ulcer or treatment).*

Gastric mucosa mucin content

The gastric mucin content of the positive (delayed ulcer untreated) control group significantly decreased when compared with the negative (normal) control and treated (*MeCaB* and fractions) groups on days 3, 7 and 14. The gastric mucin content level for *CcFrB* and *CcFrC* groups was significantly decreased by day 14 when compared with the positive (delayed untreated ulcer) and negative control groups. This is shown in Fig. 4.

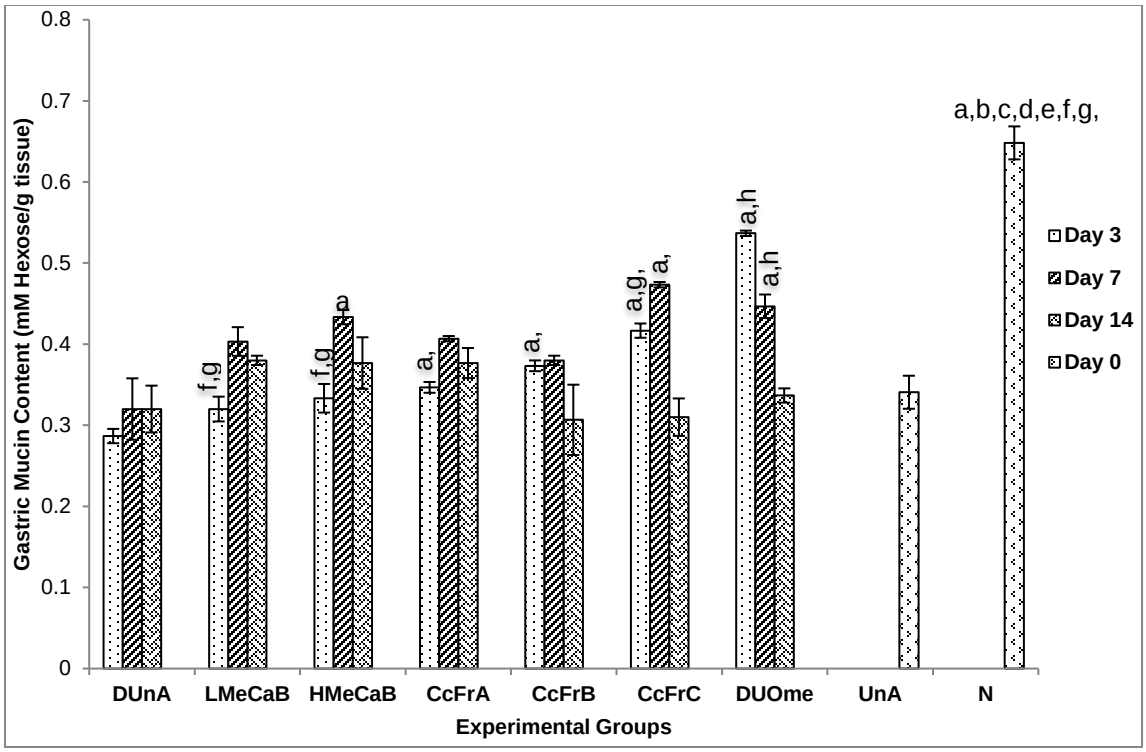

Figure 4. Effect of methanolic bark extract of *Chrysophyllum albidum* **(*MeCaB*)** and its chromatographic fractions on gastric mucosa mucin content during delayed ulcer healing

Values are represented as Mean ±SEM and are significant when p≤0.05.

***Keys of Significance**: [a] compared with delayed ulcer untreated alone (DUnA) group, [b] compared with 250mg/kg C. albidum treated group (LMeCaB), [c] compared with 500mg/kg C. albidum treated group (HMeCaB), [d] compared with fraction A treated group (CcFrA), [e] compared with fraction B treated group (CcFrB), [f] compared with fraction C treated group (CcFrC), [g] compared with omeprazole treated group (DUOme), [h] compared with ulcer alone group (UnA), [i]compared with normal (no ulcer or treatment).*

Gastric mucosa H^+/K^+-ATPase pump activity

The gastric H^+/K^+-ATPase pump activity for *CcFrC* and *DUOme* groups was significantly decreased by day 3 when compared with the other groups except the baseline and negative control groups. There was a significant decrease in gastric H^+/K^+-ATPase pump activity of positive control (delayed ulcer untreated) group when compared with the treated (L*MeCaB, HMeCaB*, *CcFrA* and *CcFrB*) groups by day 7. There was a significant decrease in the gastric H^+/K^+-ATPase pump activities of the baseline (UnA) and negative controls rats, as well as all the treated (*MeCaB* and fractions) groups when compared with the positive control (delayed ulcer untreated) group by day 14 (Fig. 5).

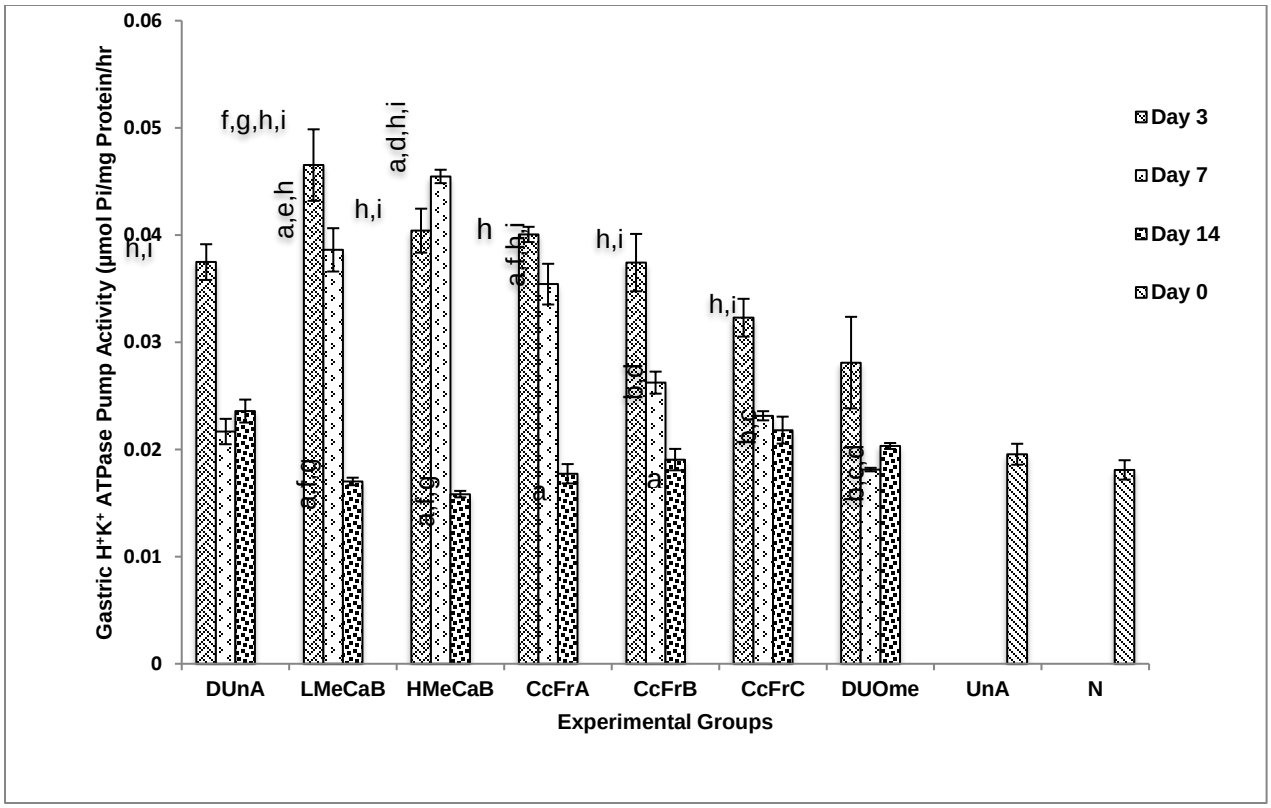

Figure 5. Effect of methanolic bark extract of *Chrysophyllum albidum* **(*MeCaB*)** and its chromatographic fractions on gastric mucosa H^+K^+ATPase activity during delayed ulcer healing

Values are represented as Mean ±SEM and are significant when p≤0.05.

***Keys of Significance**: [a] compared with delayed ulcer untreated alone (DUnA) group, [b] compared with 250mg/kg C. albidum treated group (LMeCaB), [c] compared with 500mg/kg C. albidum treated group (HMeCaB), [d] compared with fraction A treated group (CcFrA), [e] compared with fraction B treated group (CcFrB), [f] compared with fraction C treated group (CcFrC), [g] compared with omeprazole treated group (DUOme), [h] compared with ulcer alone group (UnA), [i]compared with normal (no ulcer or treatment).*

DISCUSSION

Similar gastric ulcer scores obtained in Salami and Famurewa, (2017) study were also recorded in this study which further confirmed the gastric ulcer healing properties of C.*albidum* and its fractions.

Salami and Famurewa, (2017) in their study documented that animals treated with C.*albidum* had increased body weights despite earlier reports that confirmed increased protein catabolism in gastric ulcer patients (Segawa *et al*., 1985). In this study also, there were notable increases in the protein concentrations of gastric tissue homogenate in the groups treated with *C. albidum* extracts. This observation further corroborates the protein anabolism activities of *C. albidum* and its chromatographic fractions as observed in earlier studies (Salami *et al*., 2018).

C.*albidum* and its fractions ameliorated the exacerbated malondialdehyde (MDA) in gastric tissue to a minimal level though this reduction was more remarkable in *HMeCaB*. This might probably suggest that the ability of C.*albidum* to ameliorate lipid peroxidation (Devaki *et al*., 2004) is dose dependent. The observed reduction in gastric lipid peroxidation might have been as a result of decreased production of neutrophils (which mostly initiates inflammatory stress) and its counter-activities by C.*albidum* as earlier observed in Salami and Famurewa, (2017).

The body cells are well equipped to maintain a variety of defenses against oxygen toxicity especially reactive oxygen species (ROS) mainly by antioxidant enzymes and other endogenous antioxidant (Glutathione (GSH, superoxide dismutase, SOD and catalase, CAT) sources (Nilesh *et al*., 2010). Result from this present study showed that *MeCaB* and its fractions enhanced the production of gastric glutathione, superoxide dismutase (SOD) and catalase (CAT) enzymes, hence preventing injury to the gastric mucosa caused by ROS during gastric

ulceration and probably its healing. This increase was comparable with the negative control. Superoxide dismutase (SOD) is one of the most important enzymatic antioxidant defense system (Miller, 2004). It has been documented that flavonoids increase intracellular glutathione level by transactivation of the γ-gluthamylcysteine synthetase catalytical subunit promoter (Mari *et al*., 2002). This protective effect may be associated with the presence of flavonoids and phenolics in *C. albidum* extracts. Flavonoids have been documented to confer and or enhance anti-oxidative properties during inflammatory conditions (He *et al.,* 2019). It may be that the observed increases of these endogenous anti-oxidative enzymes in C.*albidum* treated animals might have also contributed to the observed ameliorated blood inflammatory markers in the previous study Salami and Famurewa, (2017).

Treatment with the *MeCaB* and its fractions enhanced production of mucin content found in the gastric tissues; this probably conferred more protection on the gastric mucosa from damage (due to facilitated mucus production). Mucus has been documented to act as an antioxidant, which helps in reducing mucosal damage mediated by oxygen free radicals (Allen and Flemstrom, 2005). The decreased mucin secretion in the positive control (delayed ulcerated untreated) group in this study indicated reduced ability of the mucosal membrane to protect the mucosa from physical damage and back diffusion of hydrogen ions. An increase in mucus production usually assists healing process by protecting the ulcer crater against irritant stomach secretions (acid and pepsin) thereby enhancing the rate of the local healing process.

Previous studies have documented that continuous administration of indomethacin after induction of chronic gastric ulcer impairs wound contraction (at the ulcer base), without which the process of gastric ulcer healing is impossible. For wound contraction to occur, there must be the presence of wound contraction aiding substances released to the ulcerated site. Few of these substances include serotonin, histamine and prostaglandin which are released from surrounding cells of ulcerated sites. Of interest in this model of (delayed) gastric ulcer is histamine which is also important for stimulation of the H^+K^+ ATPase (Shin *et al*., 2009) needed for gastric acid secretion. In this study, it was observed that the (delayed) gastric ulcer untreated group had reduced H^+K^+ ATPase activity which might been as a result of the non- availability of histamine to activate the H^+K^+ ATPase. The lack of histamine (Shin *et al*., 2009) at the ulcerated stomach of the delayed ulcerated untreated animals also exacerbated the ulcer hence preventing wound contraction necessary for healing. The lack of wound contraction was observed in the (delayed) gastric ulcer score as there were large unhealed ulcers in the (delayed) ulcerated untreated groups in both Salami and Famurewa, (2017) and this study. However, the groups treated with C.*albidum* and its fractions showed enhanced H^+K^+ ATPase activities by days 3 and 7 compared with (delayed) ulcerated untreated group; these are the days that constitute the inflammatory and proliferative phases of gastric ulcer healing respectively. This implies that C.*albidum* and its fractions probably uncoupled the inhibitory activities of indomethacin on histamine availability (in this model of gastric ulcer induction) thus making it available for wound contraction which resulted in healed gastric ulcers by days 3 and 7 in both Salami and Famurewa, (2017) and this study. However by day 14, there was greater inhibition of the H^+K^+ ATPase activities in all the C.*albidum* and fraction treated groups unlike the (delayed) ulcerated untreated group. In this study, it was shown that omeprazole inhibited H^+K^+ ATPase activity on days 3, 7 and 14, the gastric ulcer scores were pronounced but it further buttressed it as a known proton pump inhibitor.

H^+K^+ ATPase inhibitor (proton pump inhibitor) could be used as a pharmacological target for drug development against the disturbances of gastric acid production (Miyazakia *et al*., 2018) which could be beneficial to gastric ulcer healing. This proton pump inhibiting activity of C.*albidum* might have been responsible for the low basal gastric output (BGO) and gastric acidity production observed in the previous study Salami and Famurewa, (2017). Earlier reports Salami *et al.,* (2018) documented C.*albidum* to exert inhibitory H^+K^+ ATPase activities as well as acting as a probiotic during the inflammatory phase of colitis healing.

Previous studies reported that C.*albidum* contain flavonoids (earlier observed by Salami *et al*. (2015) and its contained active phytochemical have been identified and isolated (Idowu *et al.,* 2016) to be; procyanidin B5, epigallocatechin and epicatechin. In a study (Mota *et al*., 2009), flavonoids have been documented to exert inhibitory activities on H^+K^+ATPase found on the parietal cells of stomach. It can therefore be suggested that flavonoid contents of *MeCaB* might have been responsible for the observed proton pump inhibitory activities in this study and probably previous studies.

This study highlighted more probable mechanism of action by which treatment with methanolic bark extract and chromatographic fractions of *Chrysophyllum albidum* enhances delayed gastric ulcer healing. These mechanisms include enhancing gastric mucus production, acting as an oxidative stress modulator by facilitating production of

endogenous antioxidant and proton pump inhibitor. C.*albidum* and its chromatographic fractions also facilitated ulcer healing by reducing gastric tissue inflammation and lipid peroxidation of the cell membrane.

REFERENCES

Allen, A., Flemström, G. (2005). Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin, American Journal of Physiology – Cell Physiology. 288: C1- C19.

Amaral, G.P., de Carvalho, N.R., Barcelos, R.P., Dobrachinski, F., Portella Rde L., da Silva, M.H., et al. (2013). Protective action of ethanolic extract of *Rosmarinus officinalis* L. in gastric ulcer prevention induced by ethanol in rats, Food Chem. Toxicol. 55: 48-55 DOI 10.1016/j.fct.2012.12.038.

Beutler, E., Duron, O and Kelly, B.M. (1963). Improved method for the determination of blood glutathione, J. Lab. Clin. Med. 61: 882–890.

Bewaji, C.O., Olorunsogo, O.O and Bababunmi, E.A. (1985). Comparison of the membrane-bound ($Ca^{2++}Mg^{2+}$)-ATPase in erythrocyte ghosts from some mammalian species," Comparative Biochemistry and Physiology. 82: 117–122.

Claiborne, A. (1985). Catalase activity. In: Handbook of methods for oxygen radical research. Greenwald RA (ed), CRC Press, Boca Raton, FL, pp 283-284.

Devaki, T., Raghavendran, H.R.B., Sathivel, A. (2004). Hepatoprotective nature of seaweed alcoholic extract on acetaminophen-induced hepatic oxidative stress, J. Hlth. Sci. 50: 42- 46.

Guide for the Care and Use of Laboratory Animals (2011) 8th ed. https://grants.nih.gov/grants/./Guide for-the-Care-and-use-of-laboratory-animals. pdf.

Gornal, A.G., Bardowill, C.J., David, M.M. (1949). Determination of serum protein by means of Biuret reaction, J. Biol. Chem. 177: 571-576.

He, H., Li, X., Yu, H., Zhu, S., He, Y., Komatsu, K., et al. (2019). Gastroprotective effect of araloside A on ethanol- and aspirin-induced gastric ulcer in mice: involvement of H+/K+-ATPase and mitochondrial-mediated signalling pathway, Journal of Natural Medicines. 73:339-352 DOI 10.1007/s11418-018-1256-0. Epub 2018 Dec 6.5.

Idowu, T.O., Iwalewa, E.O., Aderogba, M.A., Akinpelu, B.A., Ogundaini, A.O. (2006). Antinociceptive, Anti – inflammatory and antioxidant activities of Eleagnine: and alkaloid isolated from seed cotyledon of C.*albidum*, Jour. of Biological Sciences. 6: 1029–1034.

Idowu, T.O., Ogundaini, A.O., Adesanya, S.A., Onawunmi, G.O., Osungunna, M.O., Obuotor, E.M. et al. (2016). Isolation and characterization of chemical constituents from *Chrysophyllum albidum* G. Don holl. stem-bark extracts and their antioxidant and antibacterial properties, Afr. J. Tradit. Complement. Altern. Med. 13: 182–189.

Klatte, D.C.F., Gasparini, A., Xu, H., de Deco, P., Trevisan, M., Johansson, A.L.V., et al. (2017). Association between proton pump inhibitor use and risk of progression of chronic kidney disease, Gastroenterology. 153: 702–710.

Mari, C., Myhrstad, W., Carlsen, H., Nordstrom, O., Blomhoff, R., ∅ivind Moskaug, J. (2002). Flavonoids increase the intracellular glutathione level by transactivation of the γ- Glutamylcysteine synthetase catalytical subunit promoter, Free Radical Biology and Medicine. 32: 386-393.

Miller, A.F. (2004). Superoxide dismutases: active sites that save, but a protein that kills, Curr. Opin. Chem. Biol. 8: 162-8.

Misra, H.P and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 247: 3170-3175.

Miyazakia, Y., Ichimuraa, A., Satoa, S., Fujiic, T., Oishid, S., Sakaic, H., et al. (2018). The natural flavonoid *myricetin* inhibits gastric H^+K^+-ATPase. European Journal of Pharmacology. 820: 217–221.

Mota, K.S.L., Dias, G.E.N., Pinto, M.E.F., Luiz-Ferreira, A., Souza-Brito, A.R., Hiruma-Lima CA. (2009) Flavonoids with gastroprotective activity, Molecules. 14: 979–1012.

Nilesh, P., Babre, P., Debnath, S., Manjunath, Y.S., Parameshwar, P., Wankhede, S.V., et al. (2010), Antioxidant potential of hydroalcoholic Extract of *Barringtonia acutangula* Linn roots on Streptozotocin induced diabetic rats, Int. J. Pharm. Pharm. Sci. 2: 201-203.

Salami, A.T., Famurewa, A.D. (2017). Effects of *Chrysophyllum albidum* bark extract and its fractions on delayed gastric ulcer healing and the role of blood inflammatory markers, Arch. Bas. App. Med. 5: 77 – 87.

Salami, A.T., Odukanmi, A.O., Oshode, O.O., Olaleye, S.B. (2018). Modulatory activities of *Chrysophyllum albidum* and its fractions on microflora and colonic pump activities during inflammatory phase of colitis healing in experimental mice, Food Bioscience. 22: 38–49 DOI 10.1016/j.fbio.2017.12.015.

Salami, A.T., Jonah, D.C., Chukwudi, I.M., Attah, F.A., Adeyemi, A.A., Moody, J.O., Silva, O.D. (2015). *In vitro* Antihelmintic and kill kinetics activities of stem bark extracts and chromatographic fractions of *Chrysophyllum albidum* (G. don), Arch. Bas. App. Med. 3: 29-36.

Segawa, K., Nakazawa, S., Tsukamoto, Y., Kobayashi, E., Yamaguchi, H., Goto, H. (1985). Amino acid in gastric juice of peptic ulcer patients. Jpn. J. Med. 24: 34-38.

Shin, J.M., Munson, K., Vagin, O., Sachs, G. (2009). The gastric H^+K^+-ATPase: structure, function, and Inhibition, Pflugers. Arch. 457: 609–622. DOI 10.1007/s00424-008- 0495-4.

Varshney, R and Kale, R.K. (1990). Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes, Int. J. Radiat. Biol. 58: 733–743.

Winzler, R.J. (1955). Determination of serum glycoproteins. In: Glick DP, editor. Methods of biochemical analysis. New York: Interscience Publishers Inc.;279 – 311.

*Address for correspondence: Adeola T. Salami
Gastrointestinal secretion and inflammation research unit,
Department of Physiology,
University of Ibadan,
Ibadan,
Nigeria
Telephone: +234-8038-267882
E-mails: **adeolathabitha@yahoo.com**

Conflict of Interest: None declared

Received: August 1, 2020

Accepted: September 15, 2020