Official Publication of the Faculty of Basic Medical Sciences University of Ibadan, Nigeria

*Arch. Bas. App. Med. 5 (2017) 77 – 87*
*www.archivesbamui.com*

**ARCHIVES OF BASIC & APPLIED MEDICINE**

*Research Article*

# Effects of *Chrysophyllum albidum* Bark Extract and its Fractions on Delayed Gastric Ulcer Healing and the Role of Blood Inflammatory Markers

***Salami A.T and Famurewa A.D**

*Gastrointestinal Secretion and Inflammation Research Unit, Department of Physiology, University of Ibadan, Ibadan, Nigeria*

*Received: July, 2017; Accepted in final form: September, 2017*

**Abstract**

Previous studies have reported the anti-inflammatory potentials of *Chrysophyllum albidum*. In this study, the effect of *Chrysophyllum albidum* on gastric ulcer healing with particular interest in the role of inflammatory mediators, was carried out. 120 male wistar rats (120-150g) were divided into 8 groups (n=15) viz: Groups I -delayed ulcerated untreated; II and III – 500 and 250mg/kg b.w methanolic bark extract of *C. albidum* (MeCaB) respectively; II, III and IV-100mg/kg b.w chromatographic fractions A, B and C (CFrA, B and C) respectively; VII –30mg/mg b.w Omeprazole and VIII-ulcerated untreated not-delayed (baseline group). Chronic gastric ulcer was induced experimentally and delayed using indomethacin; drug treatments occurred simultaneously for 14 days. Daily body weights of experimental animals were monitored; hematological variables; ulcer score, and histological analysis were evaluated by days 3, 7 and 14 of treatment. Data were expressed as Mean SEM, analyzed using one-way ANOVA and p 0.05 was considered statistically significant. The MeCaB, CFr A, B and C significantly increased body weights and haematological variable except (White blood cells) compared with untreated groups by days 3 and 7. The MeCaB and fractions significantly reduced Neutrophil/lymphocyte and Platelet/lymphocyte ratios (blood inflammatory makers) in this study. Furthermore, MeCaB (250 and 500mg/kg b.w) showed complete ulcer healing by day 3 with CFrA, B and C by day 7 of treatment. These observations were buttressed by histological evaluations. *Chrysophyllum albidum* bark extracts and its chromatographic fractions enhanced ulcer healing by increasing nitric oxide levels and heamatological variables to gastric tissue. Blood inflammatory makers were also ameliorated to a low level.

**Key Words:** *Chrysophyllum.albidum*, Star apple, Delayed ulcer healing, haematological variables, nitric oxide, blood inflammatory markers

## INTRODUCTION

The gastric epithelium is maintained by a balance between protective and aggressive factors with cell renewal and repair occurring during damages (Tulassay and Herszenyi, 2010; Laine *et al*., 2008). However, several synthetic drugs (Ito *et al*., 1994) and medicinal herbs (Suzuki *et al*., 2009) have been employed in the management of gastric ulcers based on several proposed mechanisms. Presently, there is still a continued search for a more effective cure due to various setbacks or adverse reactions from use of some synthetic drugs.

Gastric ulcer healing involves many processes all aimed at restoring the epithelium to normal but they can be grouped mainly into two: Inflammatory and resolution of inflammatory (i.e regenerative) responses. During the inflammatory phase of GUH, the ulcerated site undergoes a cascade of events in which hemostasis occurs, infiltrated with certain inflammatory cells (polymorphonuclear cells, macrophages and lymphocytes) after which the platelets released to the site are degranulated and lastly proliferation of fibroblasts and epitheliazation (Tarnawski, 2005). Substances (chemoattractant cytokines: IL-8) released from the degranulated platelets at about 48 hours after ulceration marks the beginning of the inflammatory phase of ulcer healing. During, platelet degranulation, nitric oxide plays an important role of vasodilatation (particularly through eNOS, nNOS and iNOS) to supply adequate oxygenation and nutrients to healing site (Ma and Wallace, 2000) as well as modulation of the released cytokines (Vodovotz and Barcellos-Hoff 2001) which enhances healing (Shabani *et al*., 1996). Inflammatory cells (neutrophils, lymphocytes) if well modulated help in the clearance of dead gastric cells at ulcerated sites and prevention of adverse microbial actions or infections. They also release their cytokines (TNF-α and IL-1) both of which are modulated by nitric oxide (Eigler *et al*., 1995). In all, the role of Nitric oxide can not be undermined during gastric ulcer healing as it acts as a pivot in both the inflammatory and regenerative phases (Frank *et al*., 2000; Ziche and Morbidelli, 2000).

Recently, some gastro-protective synthetic drugs have been designed to help release NO which facilitates healing by increasing vasodilatation and blood flow to ulcerated site (Brzozowska *et al*., 2004) for oxygenation, hemostasis and stimulation of growth factors as well as prostaglandin production (Brzozowska *et al*., 2002). However, there's little research on the probable role of hematinic (synthetic or herbal)

**Author for Correspondence:* +2348038267882 E-mail: adeolathabitha@yahoo.com

on GUH either in accelerating or modulating the healing process/phases.

*Chrysophyllum albidum* a medicinal plant, belonging to the family Sapotaceae (Bada, 1997), has been documented for various ethnomedicinal uses: haematinic (Adewoye *et al*., 2012), antiplasmodial (Adewoye *et al*., 2010), antihelmintic (Salami *et al*., 2015), anti-colitis (Salami *et al*., 2014, 2017), anti-diarrhea and stomach ache reliving (Adisa, 2000) activities. However, there is paucity of information on the probable gastric ulcer healing potentials of C.albidum and its fraction or its modulatory activities on blood inflammatory markers during delayed gastric ulcer healing which this study was aimed at.

## MATERIALS AND METHODS

**Experimental Animals:** Male Wistar strain rats (120-150 g) were used for this study. They were acclimatized for 2 weeks and housed in solid bottom polypropylene cages under standard environmental conditions of room temperature (23-25□C), relative humidity (55-65%) and natural environmental light (12/12-hour light/dark cycle). Animals had 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) (Guide for the Care and use of Animals, 2011) which is approved by the Institutional Animal Ethical Research Committee.

**Animal Groups:** Animals were divided into 8 groups of fifteen (15) rats as follows: Group 1- Delayed untreated ulcer (DUna), 2– Delayed ulcer + 500 mg/Kg *C. albidum* (HMeCaB), 3- Delayed ulcer + 250 mg/Kg *C. albidum* (LMeCaB), 4- Delayed ulcer + 100mg/kg CcFrA (CcFrA), 5- Delayed ulcer + 100mg/kg CcFrB (CcFrB), 6- Delayed ulcer + 100 mg/kg CcFrC (CcFrC), 7 – Delayed Ulcer + 30mg/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).

**Reagents:** Sodium chloride, Glacial acetic acid, Methanol, Ethyl acetate - all of BDH chemicals, England, Distilled water, Omeprazole drug (Kwality Pharmaceuticals Ltd. Nag Kalan, Majitha Road, Amritsar India).

**Plant materials, collection and identification:** *C. albidum* fresh barks were harvested from its natural habitat at Moniya, Oyo state, South-Western Nigeria. Harvested plant parts were identified and given a voucher number FH1 107514 at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria.

**Preparation of Extracts and Fractions:** The procedure ealier described by Salami *et al*. (2015) was essentially followed.
Partioning of the Plant Extracts: 2.0kg of powdered bark of *C. albidum* was macerated in 100% methanol for 72 hours; the filtrate was collected as crude methanol fraction. A 1.5kg of the powdered bark of *C. albidum* was soaked in 2.5L of 100% n-hexane for 72 hours. The filtrate was collected as hexane fractions. The residue were spread out evenly and allowed to dry for a day. To this resolve, 2.5L of 100% dichloromethane was added and well mixed for 72 hrs after which the filtrate was decanted and the residue properly dried for a day.
2.5L of 100% methanol was added to the residue for 72 hrs after which the whole mixture was filtered with Whatman filtered paper No.1, the filtrate was labeled methanol fractions. All the three different filtrates were evaporated in dryness in-vacuo.
Column Chromatography: Ethyl acetate, hexane and methanol were used in the extraction and isolation by column chromatography. 50 gm of the extract was dissolved in methanol and loaded on silica gel in the column.

Elution started with pure ethyl acetate after which the polarity was reduced to 1% of 100% methanol. Fractions were collected and distilled off to obtain a named residue.
Gastric ulcer induction and Delayed gastric ulceration: Chronic gastric ulcer was produced by acetic acid (Okabe *et al*., 2010) induction. Non-steroidal anti-inflammatory drugs [(NSAIDs) e.g. Indomethacin] are known to delay healing of experimental induced gastric ulcers in rats (Szelenyi *et al*., 1982). "Delayed gastric ulcers" were produced with slightly modified method by Salami *et al*. (2015). Briefly, chronic gastric ulcer were induced in gastric subserosa layer in the glandular part of the antral wall of experimental rats with eye forceps using 30% acetic acid and by day 5 of well defined chronic gastric ulceration, indomethacin was administered subcutaneously (to individual rat) continuously for 14 days to delay gastric ulcer healing.

**Gastric ulcer scoring:** Gastric Ulcerated area (mm2) and percentage (%) GUH was obtained according to Salami *et al*. (2015).
Assessment of weight of animals: Daily animal weights were measured using a standard rat weighing scale (Camry EK3052 model with a capacity of 5g – 5000g).

**Determination of hematological parameters**: Collected blood samples were analysed according to method of Dacie and Lewis (1994).

**Gastric acid secretion:** Continuous perfusion method described by Ghosh and Schild (1958) and modified by Amure and Ginsburg (1964) was used.

**Histological procedure:** Stomach sections from each animal per group on experimental days (3, 7 and 14) were collected, fixed in 10% phosphate buffered formalin before histological evaluation.

**Statistical analysis:** Mean, Standard Deviation and Standard Error of Mean were calculated and expressed as Mean ± SEM. One-way ANOVA and Student's t-test was used to analyze the statistical differences; statistical difference was significant at $p<0.05$.

## RESULTS

Effect of Methanolic Bark Extract of *Chrysophyllum albidum* (MeCaB) and its Fractions on Percentage Body Weight Change: Body weights of groups that received MeCaB (LMeCaB and HMeCaB) and its chromatographic fractions (CcFrA, CcFrB and CcFrC) treatment significantly increased compared with the ulcer alone group after 2 weeks of treatment (Figure 1).

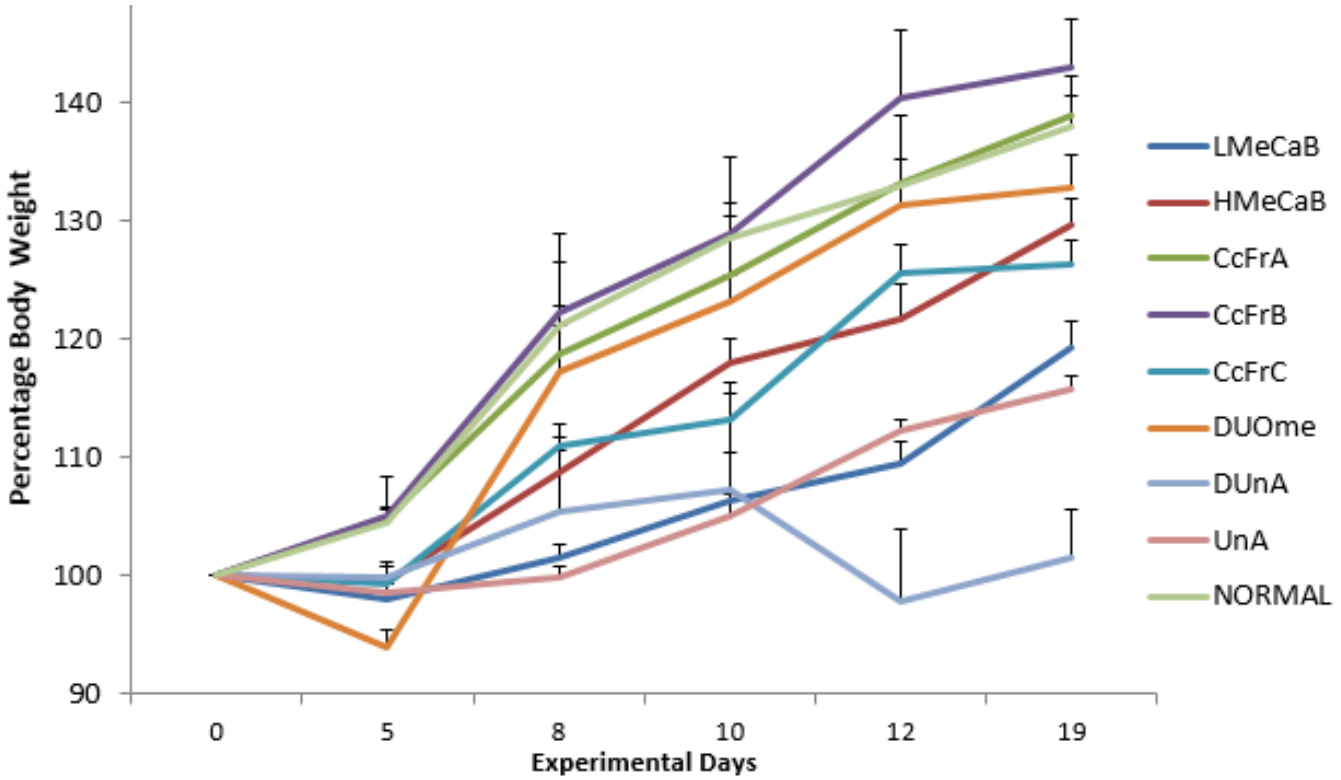



**Figure 1: Effect of Methanolic Bark Extract of *Chrysophyllum albidum* and its Chromatographic Fractions on Percentage Body Weight Change of Delayed Acetic Acid Induced Ulcer in Wistar Rats.**
Where: *LMeCaB*- Methanolic bark extract of *C. albidum* (250mg/kg b.w), *HMeCaB*- Methanolic bark extract of *C. albidum* (500mg/kg b.w), *CcFrA*- Methanolic bark extract of *C. albidum* Fraction A(100mg/kg b.w) *CcFrB*- Methanolic bark extract of *C. albidum* Fraction B(100mg/kg b.w), *CcFrC*- Methanolic bark extract of *C. albidum* Fraction C(100mg/kg b.w), *DUOme*- Omeprazole (30mg/kg b.w), *DUnA*- Delayed untreated ulcer group,*UnA*- Ulcer alone before indomethacin treatment.
***NOTE**: Day 5 is day of appearance of well-defined chronic ulceration and start of indomethacin injection. Day 8 is day 3 of delayed gastric ulcer healing, 12 is day 7 of delayed gastric ulcer healing and 19 is day 14 of delayed gastric ulcer healing*

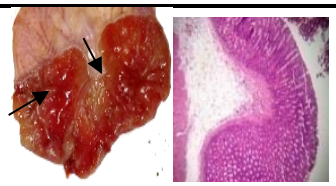
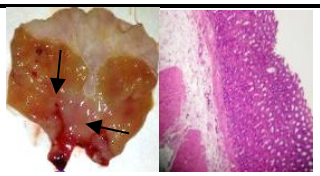
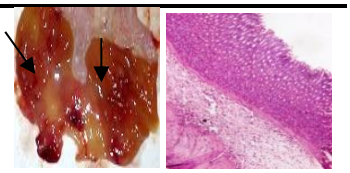
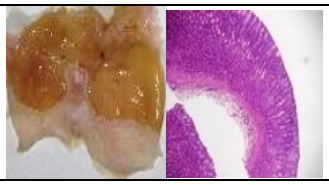
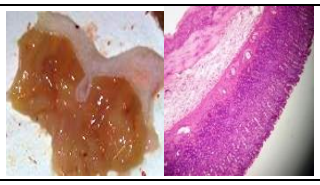
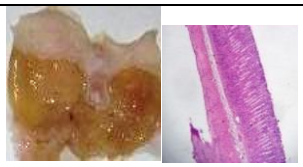
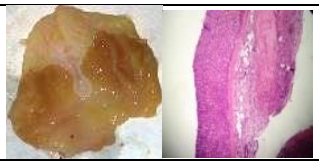
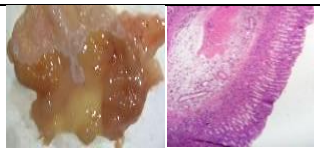
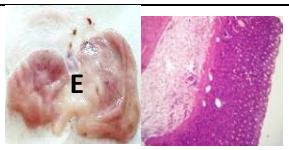
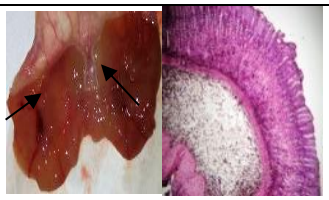
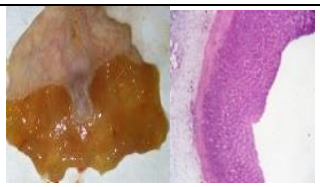
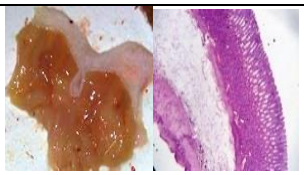
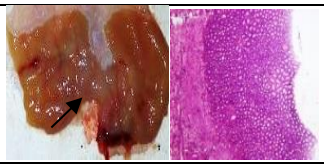
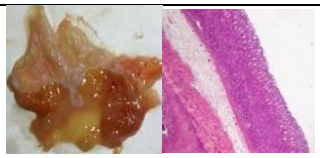
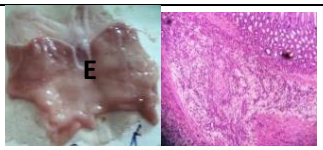
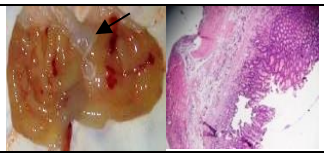
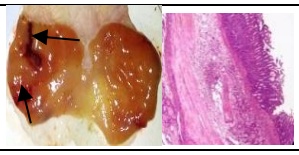
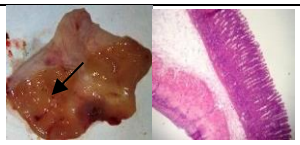
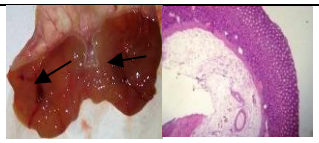
Effect of MeCaB and its Fractions on Mean Ulcer Score, Relative Ulcer Area, Percentage Healing Rate, Stomach and Relative Stomach Weights: Mean ulcer score and relative ulcer area were significantly reduced in MeCaB, CcFrA, CcFrB and CcFrC treated groups compared with DUna group. There were no visible gastric ulcers in LMeCaB and HMeCaB groups by day 3 of treatment, (Table 1). Healing rate was higher in MeCaB treated groups than DUna group (Table 2). Stomach weight of MeCaB treated groups was significantly higher compared with DUna group on days 7 and 14 (Table 2). It was evident that MeCaB, CFrA, CFrB and CFrC completely ameliorated delayed ulcer irrespective of indomethacin administration.

Effect of MeCaB and its Fractions on the Basal Acid Output (BGO), Acidity and Ph: A significant decrease was observed in the BGO and gastric acidity of CFrB treated group compared with all other experimental groups by day3. Gastric acidity of CFrA was significantly increased (tending towards neutral) compared with other experimental groups by day 3.

Gastric acidity was significantly increased in HMeCaB, CFrC and DuOme treated groups compared with other experimental groups by day 7. The CFrA and CFrB had increased gastric acidity (which was slightly above neutral) compared with other experimental groups by day 14 (Table 3).

**Effect of MeCaB and its Fractions on Hematological Profile and blood inflammatory markers.**
*a. Packed Cell Volume, Red Blood Cells, Hemoglobin and Platelet Count:* The MeCaB, CFrA, CFrB and CFrC treatment significantly increased RBC, Hb, PCV and Platelet count on days 3, 7 and 14 compared with DUna group, (Table 4).
*b.White Blood Cell (WBC), Neutrophil and Lymphocyte Counts:* The MeCaB, CFrA, CFrB and CFrC caused decrease in WBC on days 3 and 7 of study (Table 5). **Effect of MeCaB and its Fractions blood inflammatory markers:**
*a.Neutrophil/Lymphocyte Ratio (NLR):* The NLR significantly increased in DUna group compared with other groups by day 3. The LMeCaB, CcFrA and CcFrC groups showed significant increase in NLR by day 7 when compared with all other experimental groups. However by day 14, NLR in the DUna group had increased significantly compared with other experimental groups (Figure 2).
*b.Platelet/Lymphocyte Ratio (PLR):* The PLR significantly decreased in MeCaB, CFrA, CFrB and CFrC treated groups compared with DUna group (Figure 3).

**Table 1:**
Effect of Methanolic Bark Extract of *Chrysophyllum albidum* and its Chromatographic Fractions on ulcer index in delayed acetic acid ulcers after treatment by day 3, 7 and 14.

| Groups/ Treatment | Day 3 | Day 7 | Day 14 |
|---|---|---|---|
| **DUnA** |  |  |  |
| | 322.28±4.55 | 89.01±9.44 | 115.19±30.64 |
| **LMeCaB** |  |  |  |
| | 0.00±0.00[a,g] | 0.00±0.00[a] | 0.00±0.00[a] |
| **HMeCaB** |  |  |   |
| | 0.00±0.00[a,g] | 0.00±0.00[a] | 0.00±0.00[a] |
| **CcFrA** |  |  |  |
| | 7.86±1.57[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| **CcFrB** |  |  |   |
| | 9.16±3.34[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| **CcFrC** |  |  |  |
| | 8.90±3.19[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| **DUOme** |  |  |  |
| | 15.19±0.52[a] | 5.76±1.31[a] | 11.52±2.09[a] |

Keys: Ulcerated area are indicated with arrows; E- Erosion
*LMeCaB*- Methanolic bark extract of *C. albidum* (250mg/kg b.w),
*HMeCaB*- Methanolic bark extract of *C. albidum* (500mg/kg b.w),
*CcFrA*- Methanolic bark extract of *C. albidum* Fraction A(100mg/kg b.w)
*CcFrB*- Methanolic bark extract of *C. albidum* Fraction B(100mg/kg b.w),
*CcFrC*- Methanolic bark extract of *C. albidum* Fraction C(100mg/kg b.w),
*DUOme*- Omeprazole (30mg/kg b.w),
*DUnA*- Delayed untreated ulcer group,*UnA*- Ulcer alone before indomethacin treatment

**Table 2:**
Effect of Methanolic Bark Extract of *Chrysophyllum albidum* and its Chromatographic Fractions on Relative Ulcer Area, Percentage Healing Rate, Stomach Weight and Relative Stomach Weights by days 3, 7 and 14.

| Groups | Relative Ulcer Area | | | % Healing Rate | | | Stomach Weight | | | Relative Stomach weight | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 3 | Day 7 | Day 14 | Day 3 | Day 7 | Day 14 | Day 3 | Day 7 | Day 14 | Day 3 | Day 7 | Day 14 |
| **DUnA** | 33.92±2.86[h] | 10.33±1.70[h] | 9.71±1.44[h] | 0 | 0 | 0 | 0.71±0.02[h] | 0.61±0.02[h] | 0.73±0.05 | 0.71±0.02 | 0.61±0.02 | 0.73±0.05 |
| **LMeCaB** | 0.00±0.00[a,g,h] | 0.00±0.00[a,g,h] | 0.00±0.00[a,g,h] | 100.00 | 100.00 | 100.00 | 0.60±0.01[a,f,h] | 0.60±0.01[h] | 0.78±0.02 | 0.60±0.01[h] | 0.71±0.01 | 0.78±0.02 |
| **HMeCaB** | 0.00±0.00[a,g,h] | 0.00±0.00[a,g,h] | 0.00±0.00[a,g,h] | 100.00 | 100.00 | 100.00 | 0.67±0.01[h] | 0.70±0.00 | 0.77±0.04 | 0.67±0.01 | 0.70±0.00 | 0.77±0.04 |
| **CcFrA** | 0.66±0.19[a,g,h] | 0.00±0.00[a,g,h] | 0.00±0.00[a,g,h] | 98.05 | 100.00 | 100.00 | 0.65±0.01[h] | 0.63±0.03[h] | 0.80±0.01 | 0.65±0.01 | 0.63±0.03 | 0.80±0.01 |
| **CcFrB** | 1.08±0.32[a,g,h] | 0.00±0.00[a,g,h] | 0.00±0.00[a,g,h] | 96.82 | 100.00 | 100.00 | 0.63±0.00[h] | 0.65±0.03[h] | 0.80±0.05 | 0.63±0.00 | 0.65±0.03 | 0.80±0.05 |
| **CcFrC** | 0.78±0.44[a,g,h] | 0.00±0.00[a,g,h] | 0.00±0.00[a,g,h] | 97.70 | 100.00 | 100.00 | 0.71±0.01[h] | 0.72±0.02[h] | 0.78±0.07 | 0.71±0.01 | 0.72±0.02 | 0.78±0.07 |
| **DUOme** | 2.42±0.06[a,h] | 0.47±0.15[a,h] | 0.91±0.22[a,h] | 92.87 | 95.45 | 90.63 | 0.67±0.01[h] | 0.68±0.00[h] | 0.78±0.06 | 0.67±0.01 | 0.68±0.00 | 0.78±0.06 |
| **UnA** | Day 0 | | | | | | | | | | | |
| | 19.24 ± 1.49 | | | 0 | | | 0.83 ± 0.03 | | | 0.72 ± 0.06 | | |

Values are expressed as Mean ± SEM and are considered statistically significant when p value ≤ 0.05.
Keys of Significance: [a] compared with DUnA group, [b] compared with 500mg/kg *C. albidum* treated group (HMeCaB), [c] compared with 250mg/kg *C. albidum* treated group (LMeCaB), [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).

**Table 3:**
Effect of Methanolic Bark Extract of *Chrysophyllum albidum* and its Chromatographic Fractions on the Basal Acid Output, Acidity and pH in delayed acetic acid ulcers after treatment by day 3, 7 and 14.

| Groups | Basal Acid Output (ml/10mins) | | | Acidity ($\times 10^{-5}$mmol) | | | pH | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 3 | Day 7 | Day 14 | Day 3 | Day 7 | Day 14 | Day 3 | Day 7 | Day 14 |
| **DUnA** | 0.2±0.00 | 0.17±0.03 | 0.23±0.03 | 5.00±0.00 | 4.17±0.83 | 5.83±0.83 | 4.30±0.00 | 4.40±0.10 | 4.24±0.06 |
| **LMeCaB** | 0.2±0.00 | 0.2±0.00 | 0.2±0.00 | 5.00±0.00 | 5.00±0.00 | 5.00±0.00 | 4.30±0.00 | 4.30±0.00 | 4.30±0.00 |
| **HMeCaB** | 0.2±0.00 | 0.23±0.03 | 0.2±0.06 | 5.00±0.00 | 5.83±0.83[a] | 5.00±1.44 | 4.30±0.00 | 4.24±0.06 | 4.34±0.14 |
| **CcFrA** | 0.23±0.03 | 0.17±0.03 | 0.3±0.00 | 5.83±0.83[g] | 5.00±0.00 | 7.50±0.00[a,b,c,f] | 4.24±0.06 | 4.40±0.10 | 4.12±0.00 |
| **CcFrB** | 0.13±0.03[a,b,c,d,e] | 0.2±0.06 | 0.3±0.06 | 3.33±0.83[a,b,c,d,f] | 5.00±1.44 | 7.50±1.44[a,b,c,f] | 4.50±0.10 | 4.34±0.14 | 4.14±0.09 |
| **CcFrC** | 0.2±0.00 | 0.27±0.03 | 0.27±0.03 | 5.00±0.00 | 5.83±0.83[a] | 5.83±0.83 | 4.40±0.10 | 4.24±0.06 | 4.24±0.06 |
| **DUOme** | 0.17±0.03 [a,b,c,d,e] | 0.23±0.03 | 0.23±0.03 | 4.17±0.83 | 5.83±0.83[a] | 6.67±0.83 | 4.40±0.10 | 4.24±0.06 | 4.18±0.06 |

Values are expressed as Mean ± SEM and are considered statistically significant when p value ≤ 0.05.
*Keys of Significance: [a] compared with DUnA group, [b] compared with 500mg/kg C. albidum treated group (HMeCaB), [c] compared with 250mg/kg C. albidum treated group (LMeCaB), [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).*

**Table 4:**
Effect of Methanolic Bark Extract of *Chrysophyllum albidum* and its Chromatographic Fractions on Packed Cell Volume, Red Blood Cells, Hemoglobin and Platelet Counts In Acetic Acid Induced Ulcerated Rats by Days 3, 7 and 14.

| Groups | PCV(%) | | | RBC (millions/mm$^3$) | | | Hb (g/dL) | | | Platelet (millions/mm$^3$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Day 3** | **Day 7** | **Day 14** | **Day 3** | **Day 7** | **Day 14** | **Day 3** | **Day 7** | **Day 14** | **Day 3** | **Day 7** | **Day 14** |
| ***DUnA*** | 35.33±1.20 | 40.33±1.20 | 38.00±0.00 | 5.92±0.24 | 6.81±0.22 | 6.45±0.04 | 11.97±0.49 | 13.53±0.44 | 12.67±0.09 | 116,667±6,692 | 118,333±40,482 | 74,000±8,000 |
| ***LMeCaB*** | 36.00±1.16$^{e}$ | 43.3±0.88$^{h}$ | 40.67±1.76 | 6.47±0.32 | 7.20±0.04$^{h}$ | 6.89±0.33 | 12.07±0.24$^{e}$ | 14.80±0.35$^{h}$ | 13.77±0.58 | 122,000±8,000 | 131,000±4,359$^{e}$ | 267,333±53,333$^{a}$ |
| ***HMeCaB*** | 38.67±1.33$^{e}$ | 45.3±1.45$^{h}$ | 40.33±1.45 | 6.32±0.13 | 7.24±0.34$^{h}$ | 6.58±0.03 | 13.13±0.57$^{d,e,h}$ | 16.10±0.98$^{g,h}$ | 13.53±0.52 | 125,667±9,387 | 157,000±6,658$^{e}$ | 172,667±44,047 |
| ***CcFrA*** | 35.67±0.33$^{e}$ | 42.33±1.45 | 38.00±0.58 | 6.15±0.02 | 7.13±0.17$^{h}$ | 6.82±0.32 | 11.60±0.00$^{e,f}$ | 14.60±0.47$^{h}$ | 12.87±0.27 | 222,000±46,231 | 124,000±25,007$^{e}$ | 116,000±20,108 |
| ***CcFrB*** | 47.00±0.58$^{a,f,h}$ | 43.33±0.33 | 37.67±1.76 | 6.57±0.28 | 7.51±0.13$^{h}$ | 6.71±0.08 | 15.77±0.03$^{a,f,h}$ | 14.57±0.03$^{h}$ | 12.70±0.58 | 160,667±21,263 | 323,333±13,333$^{a,h}$ | 144,333±4,702 |
| ***CcFrC*** | 39.67±0.33$^{a,h}$ | 42.33±0.67 | 43.33±2.73 | 6.26±0.04 | 7.24±0.23$^{h}$ | 7.14±0.31$^{h}$ | 13.23±0.22$^{g,h}$ | 14.40±0.31 | 14.17±0.63$^{h}$ | 222,000±46,231 | 225,000±20,648 | 152,000±9,609 |
| ***DUOme*** | 36.00±0.58$^{e}$ | 39.00±0.58 | 42.00±2.00 | 6.23±0.09 | 7.11±0.30$^{h}$ | 7.02±0.30$^{h}$ | 12.07±0.37$^{e}$ | 13.10±0.36 | 12.20±2.47 | 137,000±21,378 | 197,000±22,189 | 120,333±21,667 |
| ***UnA*** | **Day 0** | | | | | | | | | | | |
| | 35.00 ± 0.58 | | | 5.62 ± 0.24 | | | 11.60 ± 5.33 | | | 169,333 ± 40,371 | | |

Values are expressed as Mean ± SEM and are considered statistically significant when p value ≤0.05.
*Keys of Significance: [a] compared with DUnA group, [b] compared with 500mg/kg C. albidum treated group (HMeCaB), [c] copared with 250mg/kg C. albidum treated group (LMeCaB), [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).*

**Table 5:**
Effect of Methanolic Bark Extract of *Chrysophyllum albidum* and its Chromatographic Fractions on White Blood Cell, Neutrophil and Lymphocyte Counts In Acetic Acid Induced Ulcerated Rats By Days 3, 7 and 14

| Groups | WBC (millions/cu mm) | | | Neutrophils | | | Lymphocytes | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Day 3** | **Day 7** | **Day 14** | **Day 3** | **Day 7** | **Day 14** | **Day 3** | **Day 7** | **Day 14** |
| **DUnA** | 9,133±1,798 | 10,450±2,976 | 5,733±881.9 | 29.00±2.08 | 32.20±1.20 | 38.00±2.52$^{b,c,e}$ | 66.33±2.03 | 57.00±1.73 | 55.00±2.65 |
| **LMeCaB** | 5,167± 898.80 | 8,917±245.50 | 12,800±3,299 | 26.00±2.00 | 42.67±1.45$^{b,e,h}$ | 28.33±0.88 | 68.00±2.00$^{f}$ | 53.33±1.20 | 67.67±0.88 |
| **HMeCaB** | 7,800±1,355 | 8,200±852 | 17,517±2,300$^{h}$ | 26.67±0.67$^{e}$ | 30.67±2.33$^{d,f}$ | 24.00±2.52 | 70.00±3.18$^{f}$ | 64.67±1.67 | 69.00±1.53 |
| **CcFrA** | 8,450±2,505 | 9,950±4,450 | 9,517±3,419 | 24.33±1.45 | 44.33±1.20$^{e,g,h}$ | 33.33±3.18 | 69.67±0.88$^{f}$ | 50.67±0.88$^{h}$ | 63.33±2.60 |
| **CcFrB** | 9,117±2,822 | 9,533±3,148 | 7,933±470.20 | 17.33±1.20$^{a,h}$ | 31.67±1.76$^{f}$ | 28.67±5.67 | 74.67±1.20$^{f}$ | 62.33±1.20$^{h}$ | 66.67±5.36 |
| **CcFrC** | 7,233±2,987 | 9,367±566.70 | 6,467±1,560 | 19.33±0.67$^{a}$ | 45.00±1.16$^{g,h}$ | 30.67±1.20 | 58.00±2.08$^{g,h}$ | 51.00±2.08 | 64.33±0.67 |
| **DUOme** | 5,883±294.90 | 10,400±1,100 | 10,267±4,669 | 25.33±1.67 | 32.67±1.45 | 33.67±8.82 | 70.67±0.88 | 51.33±6.69$^{h}$ | 62.00±8.15 |
| **UnA** | **Day 0** | | | | | | | | |
| | 3,950 ± 540.8 | | | 26.67 ± 1.76 | | | 69.67 ± 2.33 | | |

Values are expressed as Mean ± SEM and are considered statistically significant when p value ≤0.05.
*Keys of Significance: [a] compared with DUnA group, [b] compared with 500mg/kg C. albidum treated group (HMeCaB), [c] compared with 250mg/kg C. albidum treated group (LMeCaB), [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).*

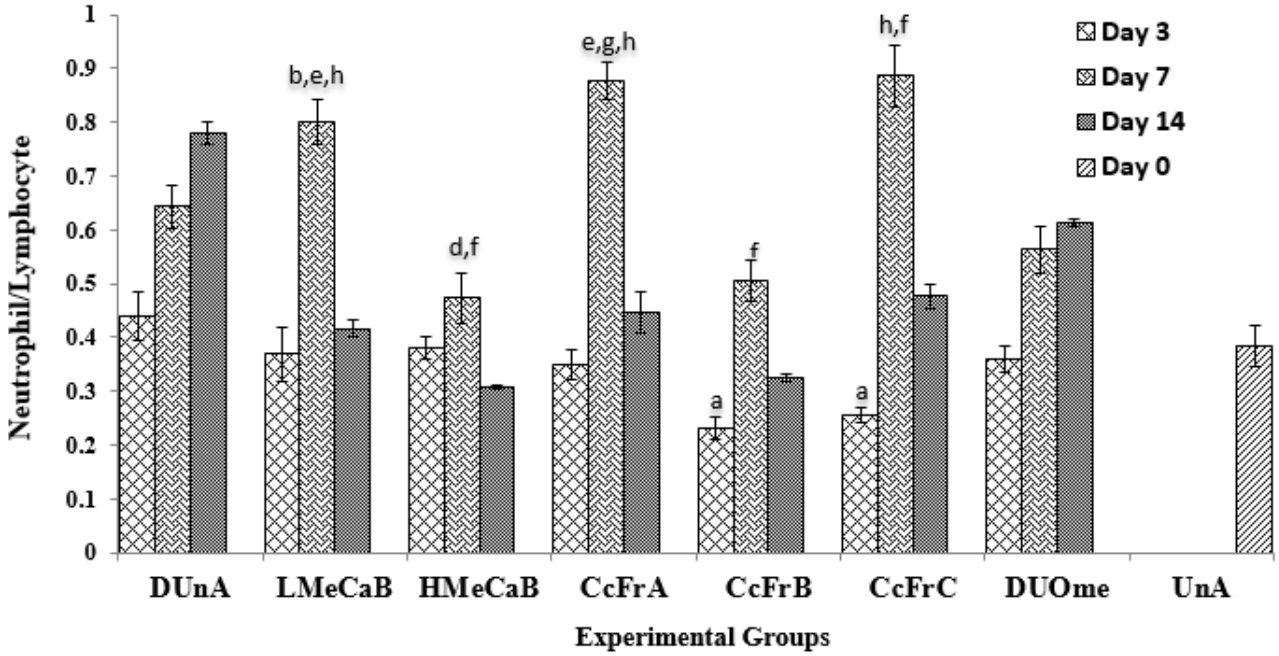



**Figure 2:**
**Neutrophil / Lymphocyte (N/L) Ratio in Methanolic Bark Extract of *Chrysophyllum albidum* and its Chromatographic Fractions Treated, Acetic Acid Induced Gastric Ulcerated Rats.**
Vertical bars represents Mean ±SEM. Values are significant when p≤0.05. *Keys of Significance: [a] compared with DUnA group,[b]compared with 500mg/kg C. albidum treated group (HMeCaB), [c]compared with 250mg/kg C. albidum treated group (LMeCaB), [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).*

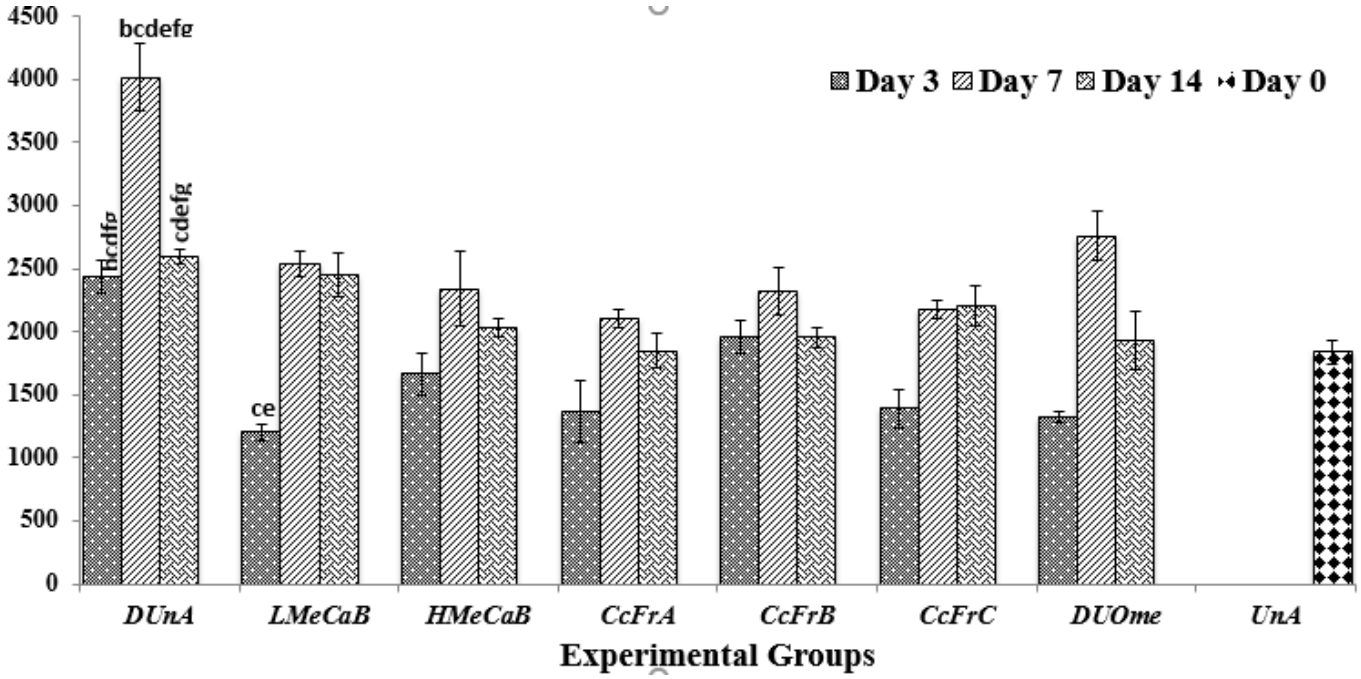



**Figure 3:**
**Effect of Methanolic Bark Extract of *Chrysophyllum albidum* and its Chromatographic Fractions on Platelet/Lymphocyte ratio during Delayed Ulcer Healing.**
Vertical bars represents Mean ±SEM. Values are significant when p≤0.05. *Keys of Significance: [a] compared with DUnA group, [b] compared with 500mg/kg C. albidum treated group (HMeCaB), [c] compared with 250mg/kg C. albidum treated group (LMeCaB), [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).*

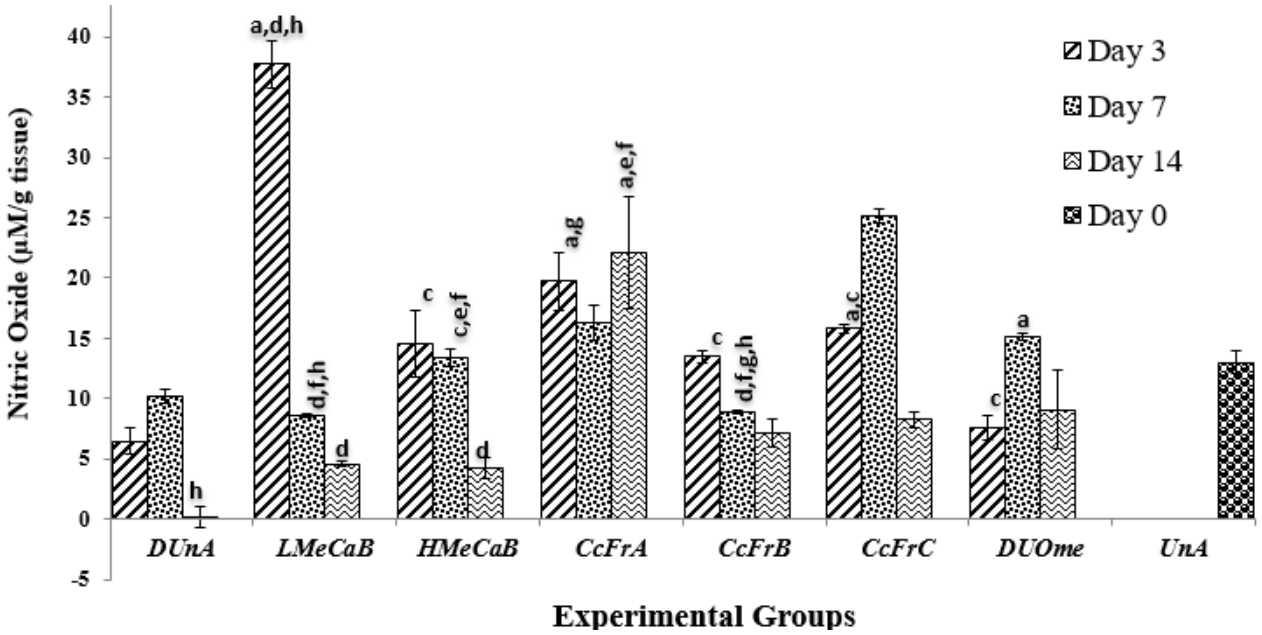



**Figure 4:**
**Effect of Methanolic Bark Extract of *Chrysophyllum albidum* and its Chromatographic Fractions on gastric mucosa NO levels during Delayed Ulcer Healing.**
Vertical bars represents Mean ±SEM. Values are significant when p≤0.05. *Keys of Significance: [a] compared with DUnA group, [b] compared with 500mg/kg C. albidum treated group (HMeCaB), [c] compared with 250mg/kg C. albidum treated group (LMeCaB), [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).*

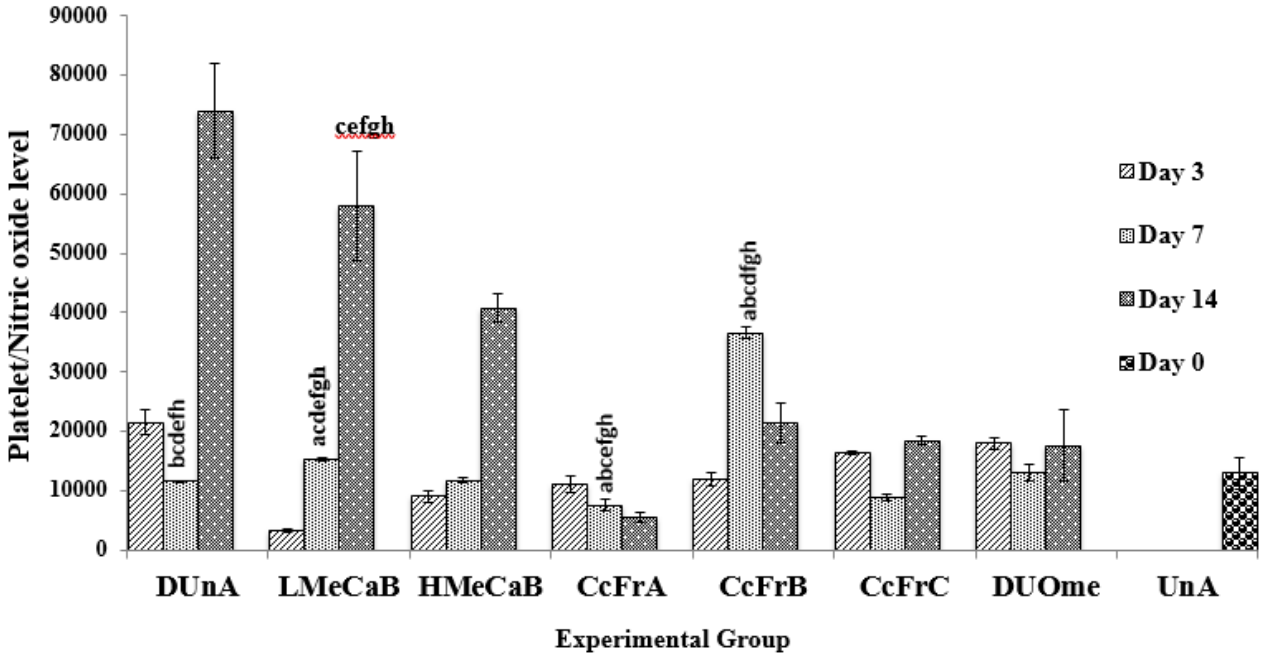



**Figure 5:**
**Platelet / Nitric oxide (P/NO) Ratio in Methanolic Bark Extract of *Chrysophyllum albidum* and its Chromatographic Fractions Treated, Acetic Acid Induced Gastric Ulcerated Rats.**
Vertical bars represents Mean ±SEM. Values are significant when p≤0.05. *Keys of Significance: [a] compared with DUnA group,[b]compared with 500mg/kg C. albidum treated group (HMeCaB), [c]compared with 250mg/kg C. albidum treated group (LMeCaB), [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).*

**Effect of MeCaB and its Chromatographic Fractions on gastric mucosa Nitric Oxide (NO) levels during Delayed Ulcer Healing:** There was a significant increase in the NO level of all the treated (MeCaB and fractions) groups when compared with DUna groups on day 3, 7 and 14. However, the NO level for LMeCaB and CcFrB groups was significantly decreased by day 7 when compared with DUna group. The NO level of LMeCaB, CcFrC and CcFrA groups were significantly high compared to all the treated groups on days 3, 7 and 14 respectively, (Figure 4).

## DISCUSSION

Reports exist in literature to show that gastric ulceration leads to loss of body weight (Palmer, 1973) which may be due to increased catabolism of stored body fats or proteins during ulceration. Marcus *et al*. (2003) revealed that medicinal plants with minute quantities of tannins when administered induced an increase in body mass. This (observed increase in body weight of treated animals) might be due to the presence of minute quantities of tannins found in both the extract and chromatographic fractions.

It was evident that MeCaB, CFrA, CFrB and CFrC completely ameliorated delayed ulcer irrespective of indomethacin administration. *C. albidum* has been reported by Adewoye *et al*. (2010) and Salami *et al*. (2015) to contain certain phytochemicals among which is flavonoid. Idowu *et al*. (2016) isolated and characterized the identified peaks of flavonoids observed by Salami *et al*. (2015) to be epicatechin, procyanidin, epigallocatechin. Flavonoids have been documented to exert gastroprotective activities (Mota *et al*., 2009). It may well be that the observed gastroprotective activity by MeCaB, CFrA, CFrB and CFrC might be due to the presence and synergetic activities of these flavonoids amongst other phytochemicals (tannins and phenolics).

Decreased BGO and increased gastric acidity are important factors that have been documented to enhance gastric ulcer healing (Olsen *et al*., 1988). These observed activities by MeCaB, CFrA, CFrB and CFrC might have facilitated the observed increased healing rate in this study irrespective of indomethacin administration.

It has been documented that most cases of gastric ulceration are accompanied with anemia. Flavonoid contents of medicinal have been observed to cause increased haematological variables during anaemic conditions (Mota *et al*., 2009) The MeCaB, CFrA, CFrB and CFrC helped in ameliorating observed anemic condition in this study which was similar to reports from previous studies (Adewoye *et al*., 2012). It has been documented that *C. albidum* has high amount of flavonioids, which might have possibly conferred hematinic or erythropoietic potential activities. Platelets contain a wide range of factors capable of initiating angiogenesis these include vascular endothelial growth factor (VEGF) (Maloney *et al*., 1998), transforming growth factor-□ and platelet factor-4 (Schmassmann *et al*., 1995; Ma *et al*., 2005). It was observed in this study that platelet counts were notably increased in MeCaB, CFrA, CFrB and CFrC treated groups this (platelet) might have facilitated the rate of gastric ulcer healing.

Recent studies have shown an increased WBC count in ulcerated untreated animals (Khalid and Ali, 2014)) while this observed increase might have been as a result of physiological response by the host during diseased conditions in protecting mucosal tissue. This observed decrease in WBC has been attributed to migration of some of these cells from blood (leucocyte extravasation) into areas around the damaged mucosal tissue mainly as part of innate immune response in the body's defense mechanism. It might probably be that MeCaB, CFrA, CFrB and CFrC facilitated removal of dead gastric tissue from ulcerated site probably via observed increased lymphocyte cell counts.

Neutrophils are the major source of inflammatory mediators as they can release potent reactive species such as superoxide, hydrogen peroxidase and myeloperoxidase derived antioxidant. These reactive oxygen species are highly cytotoxic and can induce tissue damage. Shimuzi *et al*. (2000), has shown that reduction in neutrophil infiltration into ulcerated gastric tissue promotes GUH in rats. Asako *et al*. (1992b) also observed that NSAID like indomethacin, stimulate white blood cells especially neutrophil production during gastric ulceration. This increase in neutrophil infiltration causes a decreased mucosal blood flow due to obstruction of the capillaries (Gana *et al*., 1988). It was observed that MeCaB, CFrA, CFrB and CFrC reduced the rate of neutrophil infiltration to a minimal level during GUH. This counter-activity exerted by *C. albidum* (on neutrophil during NSAID treatment) might be another probable mechanism of action by which it accelerated GUH.

Inflammatory makers alert the physician of an acute inflammation during a diseased state and also help in diagnosing responsiveness of the subject to treatment regime. Few of these inflammatory makers are C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR), plasma viscosity, fibrinogen and some others which are less predictive of inflammation in certain diseased conditions such as gastric ulceration (Watson *et al*., 2012). However, about two new blood inflammatory makers have been identify which corroborates the results of CRP and ESR (Ahbap *et al*., 2016) these include NLR (Jing *et al*., 2015) as well as PLR (Turken, 2013) which are suited for gastrointestinal disorders (Alexander, 2016). In this study, it was observed that *C. albidum* treated animals had reduced levels of both NLR and PLR which might be an indication of its' ability to reduce blood inflammatory responses during GUH. It could probably be indicative of a good prognosis correlation by MeCaB, CFrA, CFrB and CFrC treated animals during gastric ulcer healing thus buttressing the medicinal efficacy of the plant.

In a study done by Salami *et al*. (2016), it was reported that thyroxine treated animals had reduced Platelet-Nitric oxide ratio (P/NO) levels during ischemia induced gastric ulcer and might be a mechanism by which thyroxine enhanced gastric ulcer healing compared with other groups. This probably was through increased vasodilatation of blood vessels to healing gastric ulcerated site as a result of regulated or moderately increased NO production. It was observed that the CcFrA, CcFrC and Omeprazole treated animals had reduced P/NO levels all through the study which might be a rapid cellular response to ameliorate delayed GUH. It also revealed the ability of the treatment to enhance vasodilatation to ulcerated site hence increasing the rate at which oxygenation and restorative blood cells (platelets) reach the healing site. This might also be a mechanism by which it (*C. albidum*) facilitates gastric ulcer healing via reduced or suppressed blood inflammatory markers.

In conclusion, this study suggests that treatment with methanolic bark extract and chromatographic fractions of *Chrysophyllum albidum* enhances gastric ulcer healing. The mechanism by which methanolic bark extract and fractions of *Chrysophyllum albidum* enhance ulcer healing could be by stimulating increased platelet formation (released into the body circulation and to ulcerated site). It could well be that the increased nitric oxide production in the C.albidum and fractions treated groups might have enhanced vasodilation to gastric tissues hence supplying more nutrient to aid regrowth of damaged mucosa. It may also be by modulating inflammatory reactions during the healing processes or and at the gastric ulcerated site.

**Acknowledgement:**
*The authors of this work acknowledge the efforts of Mr. Peter Otegbade of Histopathology department of the University College Hospital, Ibadan, in preparing and interpreting the microscopic slide*

**REFERENCES**

Adewoye, E.O., Salami, A.T., Emikpe, B.O (2012). Effect of the Methanolic Extract of *Chrysophyllum albidum* bark on hematological indices in mice with experimental hemorrhagic anaemia. Afr J Biomed Res 15: 85-91

Adewoye, E.O., Salami, A.T., Taiwo, V.O (2010). Antiplasmodial and Toxicological effects of methanolic bark extract of *Chrysophyllum albidum* in albino mice. Journal of Physiology and Pathophysiology, 1: 1-9

Adisa, S.A (2000). Vitamin C, Protein and mineral contents of African Apple (*Chrysophyllum albidum*) IN: Proceedings of the 18th annual conference of NIST. (Eds.) 141 -146

Ahbap, E., Sakaci, T., Kara, E., Sahutoglu, T., Koc, Y., Basturk, T., Sevinc, M., Akgol, C., Kayalar, A.O., Ucar Z.A., Bayraktar, F., Unsal, A. (2016). Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in evaluation of inflammation in end- stage renal disease. Clin Nephrol. 85 (4): 199-208.

Amure, B.O., Ginsburg, M (1964). Inhibitors of histamine catabolism and the action of gastrin in the rat. Br J Pharmacol Chemother. 23: 476-485

Asako, H., Kubes, P., Wallace, J.L., Gaginella, T., Wolf, R.E., Granger, D.N (1992b). Indomethacin induced leukocyte adhesion in mesenteric venules: Role of lipoxygenase products. Am J Physiol 262: G903-8

Alexander, N.I (2016). Reference values of neutrophil/lymphocyte ratio, platelet/lymphocyte ratio and mean platelet volume in healthy adults in North Central Nigeria. Journal of Blood and Lymph 6: 1

Brzozowska, I., Targosz, A., Sliwowski, Z., *et al*. (2004). Healing of chronic gastric ulcer in diabetic rats treated with native aspirin, nitric oxide derivative of aspirin and cyclooxygenase (COX)-2 inhibitors. J Physiol Pharmacol., 55: 773-90

Brzozowska, I., Knoturek, P.C., Brzozowski, .T, *et al*. (2002). Role of prostaglandin, nitric oxide, sensory nerves and gastrin in acceleration of ulcer healing by melatonin and its' precursors, L-tryptophan. J Pineal Res 32: 149-62

Bada, S.O (1997). Preliminary information on the ecology of *Chrysophyllum albidum* done in the West and Central Africa; In proceedings of a National Workshop on the potentials of Star Apple in Nigeria, 16 – 25

Dacie, J.V., Lewis, S.M (1994). In: Practical Haematology 7th edition ELBS with Churchill Livingstone, Long man group Uk 5-82: 160-175

Eigler, A, Moeller, J., Endres, S (1995). Endogenous and exogenous nitric oxide attenuates tumor necrosis factor synthesis in the murine macrophage cell line RAW 264.7. J. Immunol. 154: 4048-4054.

Frank, S., Kamfer, H., Wetzler, C., Stallmeyer, B., Pfeilschifter, J (2000). Large induction of the chemotactic cytokine RANTES during cutaneous wound repair: a regulatory role for nitric oxide in keratinocyte-derived RANTES expression. Biochem. J. 347 (1): 265-273

Gana, T.J., MacPherson, B.R., Koo, J (1988). Gastric mucosal blood flow in misoprostol pretreated aspirin-induced ulceration. Ann Surg. 207: 327-334

Ghosh, M.N., Schild, H.O. (1958) Continuous recording of acid gastric secretion in the rat. Br. J. Pharm. 13:54-61

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

Idowu, T.O., Ogundaini, A.O., Adesanya, S.A., Onawunmi, G.O., Osungunna, M.O., Obuotor, E.M., and Abegaz B.M (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(5): 182–189

Ito, M., Sagami, T., Tsukahara, T., *et al*. (1994). Effect of cimetidine and omeprazole on gastric ulcer healing of rats with lSalami, 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

Jing, C., Dongsheng, H., You, Z., Peng, S (2015). Meta-analysis of association between neutrophil to lymphocyte ratio and prognosis of gastric cancer. World Journal of Surgical Oncology, 13: 122

Khalid, G.A., Ali, J.A. (2014). The protective role of Camel's milk on some haematological parameters of male rats infected with gastric ulcer. World Journal of Pharmaceutical Sciences. 29: 1465-1468.

Laine, L., Takeuchi, K., Tarnawski, A. (2008). Gastric mucosal defense and cytoprotection: bench to bedside. Gastroenterology, 135: 41-60

Ma, L., Wallace, J.L (2000). Endothelial nitric oxide synthase modulates gastric ulcer healing in rats. Am. J. Physiol. Gastrointest. Liver Physiol. 279: G341-G346.

Ma, L, McKnight, W., Dicay, M., Klein, A., Hollenberg, M.D., Wallace, J.L (2005). Proteinase-activated receptors 1 amd 4 counter-regulate endostatin and VEGF release from human platelets. Proc. Natl. Acad. Sci. U.S.A. 102: 216-220

Maloney, J.P., Silliman, C.C., Ambruso, D.R., Wang, J., Tuder, R.M., Voelkel, N.F (1998) In vitro release of vascular endothelial growth factor during platelet aggregation. Am J Physiol. 275: H1054-H1061

Marcus, C., Karin, L., Jain, G., Matthias, L., Jorns, F., Tilman, G., Jurgen, W.S (2003). Captive roe deer (Capreolus capreolus) select for low amount of tannic acid but not quebracho: fluctuation of preference and potential benefit. Com. Biochem and Physio. Part B: Biochem and Molec. Biolo. 136: 369-382

Mota, K.S., Dias, G.E., Pinto, M.E. *et al*. (2009). Flavonoids with gastroprotective activity. Molecules 14: 979-1012

Okabe, S., Kikuko, A., Koji, T. (2010). Acetic acid ulcer model-state of the art in 2010. Gastroentelogia Polska 17: 165-168

Olsen, S.P., Therkelsen, K., Poulsen, S.S (1988). Effect of omeprazole and cimetidine on healing of chronic gastric ulcers and gastric acid secretions in rats. Tohoku J Exp Med. 155: 305-310

Palmer, E.D (1973). Benign chronic gastric ulcer and weight loss. Am Fam Physician 8: 109

Salami, A.T., Odukanmi, A.O., Olagoke, C.O., Iyiola, T.O., Olaleye, S.B (2016). Role of nitric oxide and endogenous antioxidants in thyroxine facilitated healing of ischemia-reperfusion induced gastric ulcers. Nig J Pharm Res. 12: 189-206

Salami, A.T., Oyelami, AI., Omayone, T.P., Adewoye E.O., Olaleye, S.B. (2014). Effect of methanolic bark extracts of *Chrysophyllum albidum* on acetic acid induced colitis in rats. Proceedings of the 9th congress of European Crohn's and Colitis organization.

Salami, A.T., Odukanmi, A.O., Oshode, O.O. Olaleye, S.B (2017). 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, https://doi.org/10.1016/j.fbio.2017.12.015

Salami, A.T, Adeola B.O, Iyiola T.O, Omayone, T.P, Oluwole F.S., Olaleye S.B (2015). Antioxidative action of Manganese treatment in delayed healing of acetic acid induced ulceration in rat stomach. Journal of African Association of Physiological Sciences Vol. 3. (2): 67-78

Schmassmann A, Tarnawski A, Peskar BM, Varga L, Flogerzi B., Halter F (1995). Influence of acid and angiogenesis on kinetics of gastric ulcer healing in rats: interaction with indomethacin. Am. J. Physiol 268: G276-G285

Shabani, M., Pulfer, S.K., Bulgrin, J.P., Smith, D.J. (1996). Enhancement of wound repair with a tropically applied nitric oxide-releasing polymer. Wound Repair Regener. 4: 353-362

Shimuzi N, Wantabe T, Arakawa T, Fujiwara Y, Higuchi K, Kuroki T (2000). Pentoxifylline accelerates gastric ulcer healing in rats: roles of tumour necrosis factor and neutrophils during the early phase of ulcer healing. Digestion 6: 157-164

Suzuki, H., Inadomi, J.M., Hibi, T. (2009). Japanese herbal medicine in functional gastrointestinal disorders. Neurogastroenterology and motility, 21: 688-696

Szelenyi, I., Engler, H., Hwrzog, P., Postius, S., Vergin, H., Holtermuller, K (1982). Influence of non-steroidal anti-inflammatory compounds on healing of chronic gastric ulcers in rats. Agents and Actions 12: 180-182

Tarnawski, A (2005): Cellular and molecular mechanisms of gastrointestinal ulcer healing. Dig Dis Sci, 50, (1): S24-S33

Tulassay, Z., Herszenyi, L. (2010). Gastric mucosal defense and cytoprotection. Best Pract Res Clin Gastroenterol 24: 99-108

Turken, K (2013). Platelet to lymphocyte ratio: one of the novel and valuable platelet indices in hemodialysis patients. Hemodial Int. 17: 670

Vodovotz, Y., Barcellos-Hoff, M.H (200lammation1). Direct and indirect modulation of the inducible nitric oxide synthase by nitric oxide: feedback mechanisms in inflammation, in: Salvemini, D., Billiar, T.R., Vodovotz, Y (Eds), Nitric oxide and Inflammation, Birkhauser-Verlag, Basel, 41-58

Wallace, J.L., Dicay, M., McKnight, W. and Dudar, G.K. (2006). Platelets accelerates gastric ulcer healing through presentation of vascular endothelial growth factor. British Journal of Pharmacology 148: 274-278

Watson, J., Round, A. Hamilton, W (2012). Raised inflammatory markers. BMJ. 344: e454

Ziche, M., Morbidelli, L (2000). Nitric oxide and angiogenesis. J. Neurooncol. 50: 139-148.