



# WESTERN NIGERIA JOURNAL OF MEDICAL SCIENCES (WNIJMS)

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WISTAR RATS: ROLE OF BLOOD INFLAMMATORY MARKERS

## COBALT CHLORIDE ATTENUATED UNHEALED GASTRIC ULCERATION IN MALE WISTAR RATS: ROLE OF BLOOD INFLAMMATORY MARKERS

Salami AT<sup>1</sup>, Bello O<sup>1</sup>, Adelakun MA<sup>1,2</sup>, Udechukwu JC<sup>1,3</sup>

<sup>1</sup> Gastrointestinal Physiology and Inflammation Research Unit, Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria

<sup>2</sup>Department of Physiology, Federal University, Oye-Ekiti, Ekiti State, Nigeria

<sup>3</sup>Department of Physiology, Faculty of Basic Medical Sciences, Osun State University Osogbo, Nigeria

**Correspondences to:** Salami AT, Gastrointestinal Physiology and Inflammation Research Unit, Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria. E-mail: [adeolathabitha@yahoo.com](mailto:adeolathabitha@yahoo.com)

### ABSTRACT

**Introduction:** Gastric ulcer (GU) relapses in healing with symptoms of anaemia, mostly in those with prolonged use of antacids. The gastroprotective and facilitated healing of experimental gastric ulcer by Cobalt chloride (CoCl<sub>2</sub>) have been documented. However, its activities during GU relapse using the Delayed Gastric Ulcer (DGU) healing model are unknown which this study evaluates.

**Methods:** Seventy-five (75) male Wistar rats (120–150g; n=15) were grouped, 1-Delayed Ulcer untreated (DUn), II, III, IV, and V treated with 62mg/kg, 25mg/kg CoCl<sub>2</sub>, 1mg/kg vitamin C and 1mg/kg misoprostol respectively after inducing DGU. On treatment days 3, 7 and 14 ocular vein was puncture for blood collection and analysis; thereafter, animals were sacrificed while GU scoring, biochemical analysis and histological evaluation on excised stomach tissues were done. Results were analyzed statistically using analysis of variance.

**Results:** Haemoglobin content, PCV, RBC, and Platelet counts significantly increased by day 3 but WBC count reduced by days 7 and 14 in DUn compared with other groups. Neutrophil/lymphocytes, Monocytes/lymphocytes, and Platelet/lymphocyte significantly increased in DUn compared with CoCl<sub>2</sub> groups. Platelet/lymphocyte significantly increased in

CoCl<sub>2</sub>-treatment compared with DUn on days 14. The ulcer score, superoxide dismutase activities, nitric-oxide level, Parietal and mucosa cells significantly decreased but MDA level increased in DUn compared with CoCl<sub>2</sub> on days 3, 7, and 14. Histological evaluation revealed healing with preserved epithelial cells and architecture from day7 in CoCl<sub>2</sub> groups.

**Conclusion:** Cobalt chloride attenuated unhealed gastric ulcer and associated anemia via reduced blood inflammatory markers, nitric oxide levels, and mucous cell count.

**Keywords:** Gastric Ulcer Relapse, Anaemia, Cobalt chloride, Blood Inflammatory Markers, Parietal and Mucous Cell Counts

### INTRODUCTION

Gastric ulcers, tend to relapse or re-occur during healing and the major cause of this is still being unraveled by researchers.<sup>1-2</sup> Recently, there has been a sudden rise on relapse of healed gastric ulcer or delayed healing especially in the elderly,<sup>1-2</sup> and those on prolonged use of antacids for gastric ulcer treatment. Various experimental models such as unhealed or delayed healing of chronic gastric ulcer,<sup>3-4</sup> are being developed to understand the basis of healing and its enhancement by gastro-protective

medications. In unhealed or delayed gastric ulcer healing,<sup>5,6</sup> it has been documented that healing is hampered basically as a disruption of or prolonged phase of healing without progressing to the next phase. Mast cells in the gastrointestinal tract help maintain homeostasis within the GI barrier.<sup>7</sup> There are few culprits of gastric ulcer re-occurrence which are increased gastric acid secretion, reduced acidity, histamine, serotonin and prostaglandin E secretions. However, the precise mechanism is not fully unraveled. Histamine and serotonin are few of the secretions found in the gastric mast cells which enhances H<sub>2</sub> receptors functioning but do not facilitate ulcer formation.<sup>8</sup> Gastro-protective activities of cobalt chloride were hinged on its reduced gastric acid secretion and increased gastric pH towards alkalinity during experimental chronic gastric ulcer.<sup>9</sup> In a recent report,<sup>10</sup> cobalt stabilised mast cell degranulation with modulated iNOS-COX2 cross talk at the gastric epithelial cell thus preventing gastric ulcer formation during reserpine and pyloric ligation induced gastric ulceration. These gastro-protective observations of low dose of cobalt<sup>9-10</sup> further buttress the observation of.<sup>8</sup>

Anemia is a prominent co-morbidity disease condition during gastric ulcers,<sup>11</sup> which several synthetic medications do not address while exerting gastro-protective activities.<sup>12</sup> Anemia cases have increased in recurrent or unhealed gastric ulcer.<sup>13</sup> Gastric ulcers caused by prolonged use of NSAID,<sup>14</sup> or associated with *H. pylori*,<sup>15</sup> has been found to cause anaemia due to impaired iron absorption or availability to gastric tissues,<sup>16</sup> as most is used up by the *H. pylori* bacteria. However, bilirubin and iron overload,<sup>17</sup> chronic inflammation like peptic ulcer,<sup>18</sup> loss of iron due to hemorrhagic gastritis,<sup>19</sup> and active bleeding ulcers,<sup>20</sup> have been documented to cause anaemia. Cobalt is an integral component in the manufacture of haematinic, thus, prevents anemia through absorption of vitamin B12 within the body.<sup>21</sup> It is also documented as a trace element needed for

normal body cell functioning such as repair of the nerve myelin sheath.<sup>22,23</sup> It mimics the beneficial function of iron within the body and thus used in treating anemia in pregnant women and people undergoing long-term hemodialysis.<sup>23</sup> It is however used by athletes as a doping agent,<sup>24</sup> for stimulation of red blood cell production and muscle mass through mimicking the hypoxia pathway within the body system during track events.<sup>25,26</sup> This is possible as it has a little half-life within the body and it cannot be easily detected. Cobalt is excreted mainly in feces as the gut can absorb only 30% of ingested cobalt,<sup>27</sup> however, it is detectable in small amounts in the blood,<sup>28</sup> urine,<sup>29</sup> and possibly body fluids.<sup>30</sup>

Few reports have documented that certain plants (*Chrysophyllum albidum* bark extract,<sup>31</sup> manganese chloride,<sup>32</sup> *Bucholzia coriacea*,<sup>33</sup> *Kolaviron*,<sup>34</sup>) or heavy metal enhance gastric ulcer healing through modulated inflammatory blood markers: reduced Neutrophil/Lymphocyte ratio (N/L), Platelet/Lymphocyte (PLR) and Monocyte/Lymphocyte (MLR).<sup>35-36</sup> These (N/L), and (PLR) are indicative of reduced systemic inflammation and increased platelet counts which initiates ulcer healing during ulceration through platelet derived growth factors.<sup>37</sup>

Cobalt chloride at lower doses in a previous study was documented to facilitate Acetic acid induced gastric ulcer healing without reoccurrence by day 21 post ulceration (and 7 days post treatment).<sup>38</sup> The precise gastro-protective mechanism of action of cobalt chloride (at lower doses) during unhealed gastric ulcer remains unclear which was unraveled using experimentally induced delayed gastric ulcer (unhealed gastric ulcer) model in this study.

## METHODS

### *Animals*

Healthy male experimental rats of *Wistar* strain (100-120g) used in this study were

obtained from the central-animal house, department of Physiology, Faculty of Basic Medical Science, University of Ibadan. The experimental rats were acclimatized for 3 weeks under standard experimental-laboratory conditions: room temperature ( $23.0\pm 2^{\circ}\text{C}$ ), environmental-humidity ( $55.0\pm 15\%$ ); natural-environmental 12 hours light and dark cycle. They were allowed free access to water and standard commercial rat pellets (Ladokun Feeds Nigeria Limited, Ibadan, Nigeria).

### ***Ethics considerations***

The care of experimental rats was as documented by animal handling ethics conformity with the institution's guideline and criteria for human care (National institute of Health Guidelines for the care and Use of Laboratory Animals).<sup>39</sup> The University of Ibadan ethical approval number was UI-ACUREC/19/0003.

### ***Delayed experimental induced gastric ulceration***

Seventy-five (75) Wistar rats were equally divided into 5 groups of 15 animals each. Group I-Ulcer Untreated control (DUn), II-delayed-ulcer+62mg/kg bw of  $\text{CoCl}_2$  (DUHCo), III- delayed-ulcer+25mg/kg bw  $\text{CoCl}_2$  (DULCo), IV- delayed-ulcer+1mg/kg bw Vitamin C (DUvit.C), V- delayed-ulcer + 1mg/kg bw Misoprostol (DUmistol). Delayed experimental induced gastric ulceration was according to the procedure of Buonacera.<sup>35</sup> Two days post acetic acid ulceration; 2mg/kg indomethacin was injected subcutaneously once every day for two weeks to all the experimental *Wistar* rats, before oral treatments (cobalt and misoprostol) and sacrifice by days 3, 7 and 14 of treatments, (five Wistar rats from each group).

### ***Ulcer Scoring***

The ulcerated area ( $\text{mm}^2$ ) was calculated as documented by Buonacera.<sup>35</sup>

Percentage Healing (relative to DUn group) Rate was calculated using the following formula:

$$\text{Healing \%} = [(\text{Control group UI} - \text{Treated group UI}) / (\text{Control UI})] \times 100.^{40}$$

### ***Blood Collection and Determination of Hematological variables***

1mL of a blood sample from each Wistar rat was collected on days 3, 7, and 14 of treatment via retro-orbital puncture using microhematocrit capillary into an Ethylene-Diamine-Tetra-Acetic acid (EDTA) bottle. The collected blood was analyzed for the determination of hematological variables using the method of Dacie.<sup>41</sup>

### ***Biochemical assay***

On cervical dislocation sacrifice of the rat after blood collection, a larger section of the excised, cleaned stomach samples were weighed and homogenized in 10 volume (weight) of iced-cold 0.1M pH 7.4 phosphate buffer. The resulting homogenates were centrifuged and the collected supernatant kept in  $-20^{\circ}\text{C}$  until use.<sup>42</sup>

### ***Biochemical Assay***

Determination of gastric Protein concentration was by means of Biuret method;<sup>43</sup> Lipid peroxidation, Superoxide dismutase (SOD) activity, and indirect total nitrite were measured using methods in earlier studies.<sup>44-46</sup>

### ***Histological stains and procedures***

Gastric Mucous and Parietal cells histopathological evaluation on the phosphate-buffered formalin fixed stomach samples from each experimental group was by the method of Oluwole et al,<sup>47</sup> using PAS, Hematoxylin and Eosin stains respectively. Identification, characterization and counting of both mucous and parietal cells was done using motic software by two blinded observers.

### ***Statistical analysis***

Data were analysed using descriptive statistics, one-way ANOVA with Bonferri post hoc test on Graph pad Prism version 8.0. Values are represented as mean $\pm$ SEM and statistical significance at  $p\leq 0.05$

## RESULTS

### **EFFECT OF COBALT CHLORIDE ON PACKED CELL VOLUME (PCV), RED BLOOD CELL (RBC) COUNT, HEMOGLOBIN CONTENT (Hb), AND THROMBOCYTE COUNTS (Platelets) DURING DELAYED GASTRIC ULCER HEALING**

Table 1 shows the effect of cobalt chloride on the PCV, RBC, Hb Content, and Platelet Counts. By day 3 of treatment an unusual significant increase in the PCV of the ulcerated delayed untreated (DUn) group was observed when compared with all other experimental groups. By days 7 and 14, there was a significant increase in the PCV of all experimental groups except , ulcerated delayed misoprostol (DUMisol) by day 7 and vitamin C-treated (DUvit.C) groups by day 14 compared with ulcerated delayed untreated group.

The RBC count significantly increased in the ulcerated delayed untreated (DUn) group compared with all experimental groups by day 3. Ulcerated delayed groups treated with varying doses of cobalt chloride (DUHCo and DULCo) showed a significant increase in RBC count by days 7 and 14 of treatment compared with ulcerated delayed untreated and vitamin c (DUvit.C) treated groups by day 14.

The platelet count in the ulcerated delayed low cobalt chloride (DULCo) and vitamin C (DUvit.C) treated groups were significantly decreased compared with other experimental groups by day 3. The platelet count for the ulcerated delayed untreated (DUn) group was significantly increased compared with other experimental groups by day 3. A significantly decreased platelet count was observed in the ulcerated delayed low cobalt chloride, vitamin C and Misoprostol treated (DULCo, DUvit.C and DUMisol respectively) groups compared with other experimental groups by day 7. The ulcerated delayed low cobalt chloride (DULCo) treated group had a significantly increased platelet count compared with other experimental groups by day 14. The ulcerated delayed untreated (DUn) and misoprostol treated (DUMisol) groups had a significantly

reduced platelet count compared with other experimental groups by day 14.

### **EFFECT OF COBALT CHLORIDE ON WHITE BLOOD CELL COUNTS, NEUTROPHIL COUNT, AND LYMPHOCYTE COUNT DURING DELAYED GASTRIC ULCER HEALING**

There was a significant decrease in the WBC ulcerated delayed high dose cobalt chloride, Vitamin C, and Misoprostol ulcerated treated (DUHCo, DUvit.C, DUMisol respectively) groups compared with ulcerated delayed untreated (DUn) low dose cobalt chloride treated (DULCo) groups by day 3, (Table 2). The WBC count significantly increased in the ulcerated delayed high dose cobalt chloride treated (DUHCo) group compared with all experimental groups by day 7, (Table 2). The WBC count was significantly decreased in the ulcerated delayed untreated Dun group by day 14 compared with all other experimental groups, (Table 2).

The neutrophil count was significantly decreased in the ulcerated delayed high cobalt chloride treated (DUHCo) group compared with the ulcerated delayed untreated (DUn) and ulcerated delayed low dose cobalt chloride treated (DULCo) groups by day 3, (Table 2). The neutrophil count was significantly increased in the ulcerated delayed untreated (DUn) group by day 7 compared with all experimental groups, (Table 2). The neutrophil count was significantly decreased in the ulcerated delayed vitamin C treated (DUvit.C) group compared with all experimental groups by day 14.

There was a significant decrease in the lymphocyte count of ulcerated delayed untreated (DUn) and ulcerated delayed low dose cobalt chloride treated (DULCo) groups compared with other experimental groups by day 7. The lymphocyte count of ulcerated delayed vitamin C-treated (DUvit.C) groups was significantly increased by day 14.

There was a significant increase in the monocyte count of the ulcerated delayed low cobalt chloride treated (DULCo) group compared with all other groups by day 3 while the ulcerated delayed untreated (DUn) group

**TABLE 1: EFFECT OF COBALT CHLORIDE ON PACKED CELL VOLUME (PCV), RED BLOOD CELL (RBC) COUNT, HAEMOGLOBIN (Hb) CONTENT, AND THROMBOCYTES COUNT DURING DELAYED GASTRIC ULCER.**

Groups / Days	PACKED CELL VOLUME (%)			RED BLOOD CELL COUNTS. (millions/cu mm)			HAEMOGLOBIN CONTENT. (g/dL)			THROMBOCYTES COUNT (PLATELETS). (millions/cu mm)		
	3	7	14	3	7	14	3	7	14	3	7	14
<b>DUn</b>	50.67± 0.67 <sup>d,e</sup>	41.00± 0.00	41.00± 0.00	8.67±0 .03 <sup>d,e</sup>	6.48±0 .17 <sup>b,c,d</sup> e	6.90±0. 02 <sup>b,c</sup>	17.03± 0.13 <sup>d,e</sup>	12.80± 0.10 <sup>b,c</sup> d,e	13.67±0 .07 <sup>b,c</sup>	11800 0.00±3 055	114000. 00±2.0 0	67000. 00±60 00 <sup>b,c,d</sup>
<b>DUHCo</b>	47.33± 0.33	44.00± 1.00 <sup>a,e</sup>	46.67± 2.40 a,d,e	7.73±0 .20	7.53±0 .11	8.15±0. 47	15.63± 0.12	14.97± 0.47	16.33±0 .98	11000 0.00±1 732	112000. 00±100 70	82670. 00±83 73 <sup>b</sup>
<b>DULCo</b>	45.33± 2.33	44.67± 0.88 <sup>a,e</sup>	44.00± 1.16 a,d,e	7.79±0 .46	7.61±0 .11	7.55±0. 05	15.00± 0.81	15.17± 0.23	14.73±0 .61	78670. 00±12 030 <sup>a,b,d</sup>	72000.0 0±3215 a,b	11300 0.00±7 234
<b>DUvit.C</b>	43.00± 0.58	44.00± 0.58 <sup>a,e</sup>	40.67± 2.19	7.28±0 .17	7.57±0 .64	6.91±0. 35 <sup>b,c</sup>	14.10± 0.15	15.00± 0.38	13.97±0 .77 <sup>b</sup>	92670. 00±10 770	51670.0 0±1968 0 <sup>a,b,c</sup>	89330. 00±78 60 <sup>b</sup>
<b>DUmisol</b>	43.67± 1.67	41.67± 0.88	41.33± 2.40	7.13±0 .22	7.54±0 .14	7.14±0. 31	14.23± 0.58	14.17± 0.32	14.03±0 .86	71670. 00±13 870 <sup>a,b,d</sup>	66330.0 0±8373 a,b	69670. 00±26 03 <sup>b,c,d</sup>

Values are expressed as Mean±SEM and are significant when  $p \leq 0.05$ . Keys of significance; <sup>a</sup>compared with ulcerated delayed untreated (DUn), <sup>b</sup>compared with ulcerated delayed treated high dose cobalt chloride (62mg/kg b.w) (DUHCo), <sup>c</sup>compared with ulcerated delayed treated low dose cobalt chloride (25mg/kg b.w) (DULCo), <sup>d</sup>compared with ulcerated delayed treated vitamin C (1mg/kg b.w) (DUvit.C), <sup>e</sup>compared with ulcerated delayed treated misoprostol (1mg/kg b.w) (DUmisol). (This applies to all tables and figures)

**TABLE 2: EFFECT OF COBALT CHLORIDE ON WHITE BLOOD CELLS COUNT, NEUTROPHIL COUNT, AND LYMPHOCYTE COUNT DURING DELAYED GASTRIC ULCER.**

GROUPS / DAYS	WHITE BLOOD CELL COUNT. (millions/cu mm)			NEUTROPHIL COUNT.			LYMPHOCYTE COUNT.			MONOCYTE COUNT.		
	3	7	14	3	7	14	3	7	14	3	7	14
<b>DUn</b>	6667.00± 233.30	4817.00± 166.70	4350.00± 550.00 <sup>b,c</sup> d,e	73.00±5. 57	74.00±5 .51 <sup>b,d,e</sup>	53.61±5. 33	23.67±6 .17	31.00±2 .00 <sup>b,d,e</sup>	47.33±6 .67	1.67+ 0.33 <sup>c</sup>	2.67+ 0.33 <sup>bd</sup>	2.75+ 0.43
<b>DUHCo</b>	4933.00± 172.90 <sup>a,c</sup>	6050.00± 583.80 <sup>a,c</sup> d,e	5450.00± 1405.00	53.33±0. 67 <sup>a,c</sup>	46.00±1 .00	53.00±2. 00	36.33±2 .33	52.33±1 .67 <sup>c</sup>	42.33±1 .45	2.00+ 0.00 <sup>c</sup>	0.33+ 0.33	2.00+ 0.00
<b>DULCo</b>	6550.00± 293.00	4817.00± 669.20	6717.00± 1270.00	72.00±3. 46	66.33±4 .91	58.33±5. 78	24.33±3 .18	31.00±4 .73 <sup>d,e</sup>	37.00±6 .03	3.33+ 0.33 <sup>de</sup>	1.67+ 0.33	1.67+ 0.33
<b>DUvit.C</b>	4617.00± 924.80 <sup>a,c</sup>	3433.00± 833.80	5100.00± 568.60	66.00±4. 04	48.67±0 .33	37.00±3. 79 <sup>a,b,c,e</sup>	33.33±3 .67	50.00±1 .00	57.00±3 .00 <sup>c</sup>	1.00+ 0.00	0.33+ 0.33	3.67+ 0.33 <sup>bc</sup>
<b>DUmisol</b>	4267.00± 594.70 <sup>a,c</sup>	4067.00± 381.20	5417.00± 1123.00	69.00±3. 06	51.67±7 .06	60.67±3. 93	27.67±3 .67	51.33±2 .33	33.33±3 .33	1.33+ 0.33	1.33+ 0.33	2.67+ 0.33

had a significantly increased monocyte count compared with the ulcerated delayed cobalt chloride vitamin C treated (DUHCo, DULCo, and DUvit.C, respectively) groups by day 7. By day 14 there was a significant increase in the monocyte count of the ulcerated delayed vitamin C-treated (DUvit.C) group compared with ulcerated delayed high and low Cobalt chloride treated (DUHCo and DULCo) groups, (Table 2).

**EFFECT OF COBALT CHLORIDE ON INFLAMMATORY BLOOD MARKERS: [(PLATELET/LYMPHOCYTE, (P/L), MONOCYTE/LYMPHOCYTE, (M/L), AND NEUTROPHIL/LYMPHOCYTE (N/L) RATIOS] DURING DELAYED GASTRIC ULCER HEALING**

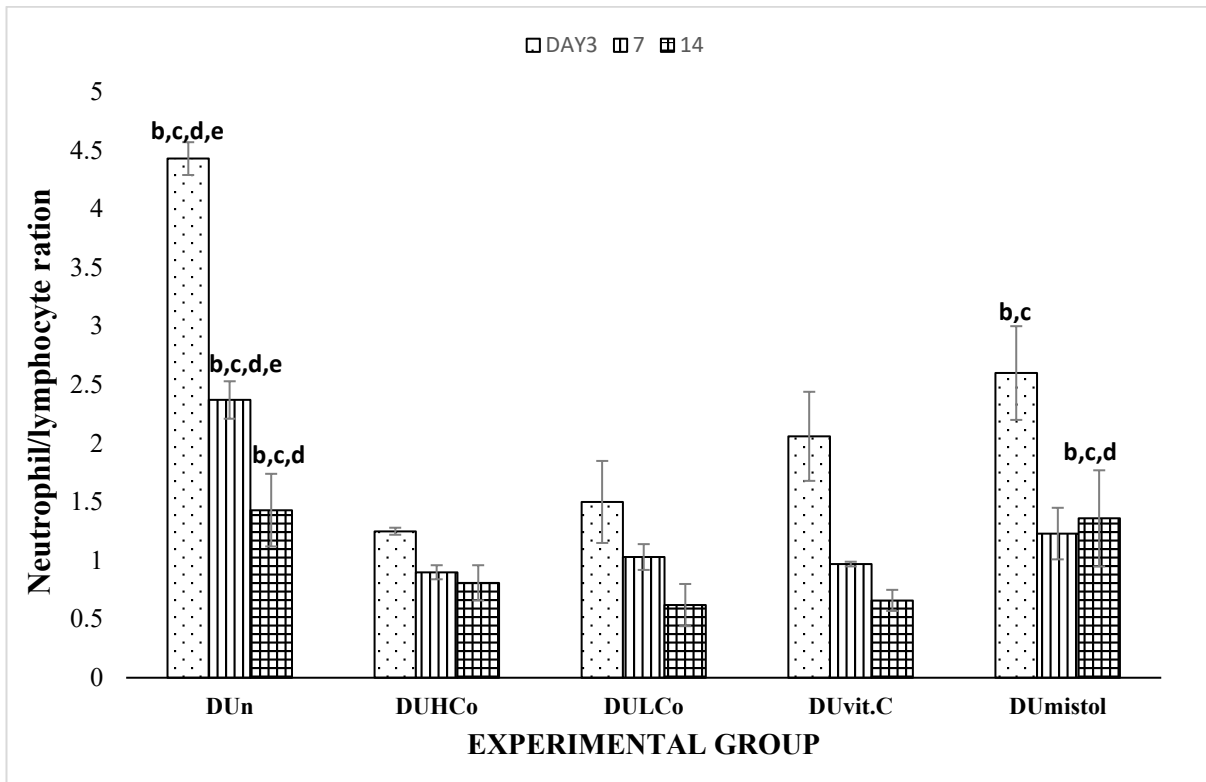
There was a significant increase in the P/L of the ulcerated delayed untreated (DUn) group compared with all other groups by day 3 of treatment. By day 7, the ulcerated delayed vitamin C treated (DUvit.C) group had a significantly increased P/L compared with all other groups. There was a significantly low P/L in the ulcerated delayed untreated (DUn) group compared with all other experimental groups by day 14, (Table 3)

The M/L in the ulcerated delayed high cobalt treated (DUHCo) group was significantly low compared with the ulcerated delayed untreated (DUn) and ulcerated delayed low cobalt chloride treated (DULCo) groups by day 3 while the ulcerated delayed untreated (DUn) group had a significantly high M/L compared with all experimental treated groups by day 7. By day 14 of treatment, the ulcerated delayed untreated (DUn) group had a significantly high M/L compared with all other experimental groups, (Table 3).

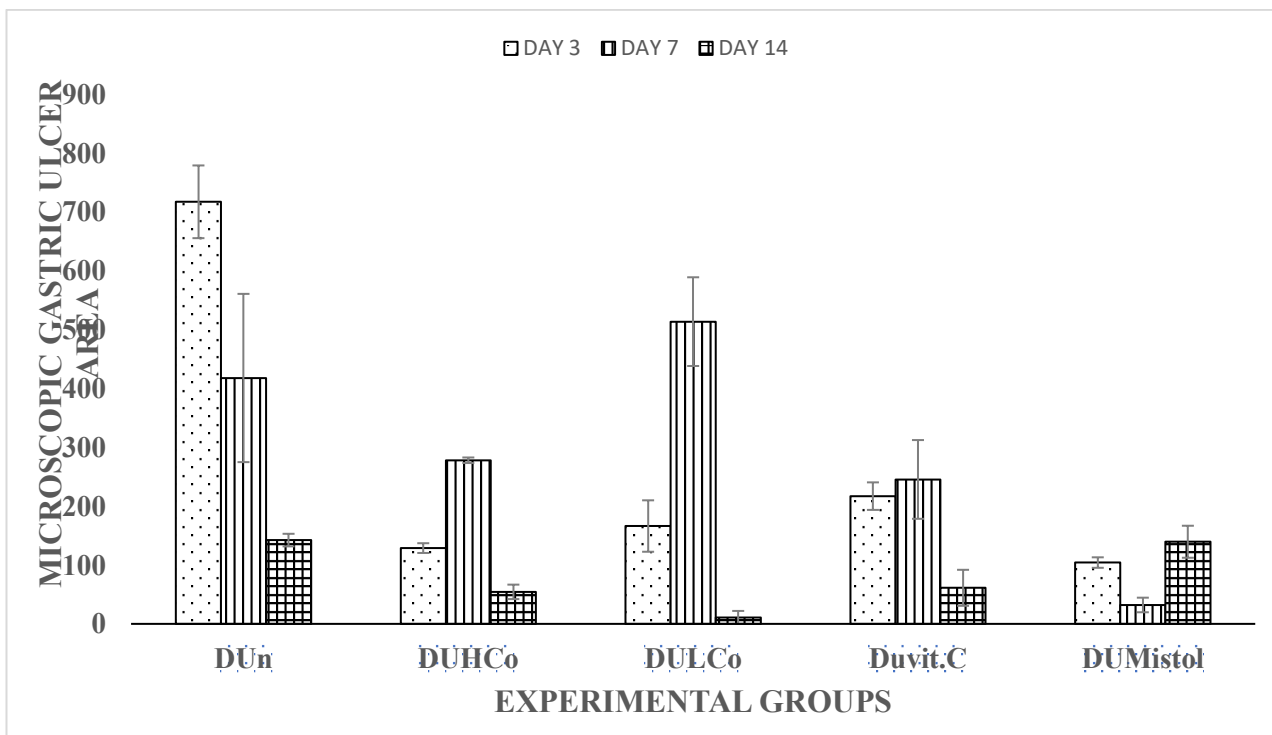
Figure 1 shows a significant decrease in the neutrophil-lymphocyte ratio in all treatment groups when compared with the ulcerated delayed untreated (DUn) group by days 3 and 7 of treatment. A significant increase was observed in the neutrophil-lymphocyte ratio of the ulcerated delayed untreated (DUn) and ulcerated delayed misoprostol treated (DUmisol) group when compared with the ulcerated delayed cobalt chloride and vitamin C treated (DUHCo, DULCo and DUvit.C respectively) groups by day 14, (Figure 1)

**TABLE 3: EFFECT OF COBALT CHLORIDE ON INFLAMMATORY BLOOD/SYSTEMIC MARKERS [(PLATELET/LYMPHOCYTE RATIO, (PLR) AND MONOCYTE/LYMPHOCYTE RATIO, (MLR)] DURING DELAYED GASTRIC ULCER.**

GROUPS / DAYS	PLATELET/LYMPHOCYTE (PLR)			MONOCYTE/LYMPHOCYTE (MLR)		
	3	7	14	3	7	14
DUn	6661.00±17.44 <sup>b</sup> cde	2425.00±72.05 b,e	1333.00±158.50	0.11±0.00 <sup>bde</sup>	0.10±0.01 <sup>b,d,e</sup>	0.18±0.03 <sup>bcd,e</sup>
DUHCo	2571.00±68.76	1969.00±80.15	1909.00±121.40	0.06±0.00 <sup>c</sup>	0.01±0.01 <sup>ce</sup>	0.04±0.00
DULCo	3070.00±264.00	2032.00±94.59 e	1620.00±18.73	0.14±0.01 <sup>de</sup>	0.07±0.01 <sup>d</sup>	0.03±0.00
DUvit.C	2112.00±226.80	2887.00±122.9 abce	1561.00±62.32	0.029±0.00	0.01±0.01 <sup>e</sup>	0.07±0.01
DUmisol	3364.00±476.30	1558.00±59.92	2305.00±72.77	0.03±0.01	0.05±0.01	0.06±0.01



**Figure 1: EFFECT OF COBALT CHLORIDE ON NEUTROPHIL/LYMPHOCYTE RATIO DURING DELAYED GASTRIC ULCER**



**Figure 2: EFFECT OF COBALT CHLORIDE ON GASTRIC ULCER AREA DURING DELAYED GASTRIC ULCER**

**EFFECT OF COBALT CHLORIDE ON ULCER AREA AND PERCENTAGE ULCER HEALING RATE DURING DELAYED GASTRIC ULCER HEALING**

Figure 2 and table 4 shows a significant decrease in macroscopic ulcer score in ulcerated delayed animals treated with cobalt (DUHCo, and DULCo) on days 3, 7 and 14, except ulcerated delayed 25mg/kg b.w cobalt treated (DULCo) group which shows no significant decrease by day 7.

In table 4, there was a significant increase in the percentage healing of the ulcerated delayed cobalt treated (DUHCo and DULCo) groups by days 3 and 14 of treatment compared with other groups however, the ulcerated delayed low and high cobalt treated (DULCo and DUHCo) groups had a significantly reduced percentage ulcer healing by day 7 of treatment compared with other treatment groups. The ulcerated delayed untreated (DUn) groups had a significantly reduced percentage healing rate compared with other experimental treatment groups by days 3, 7 and 14.






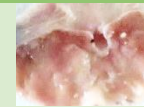



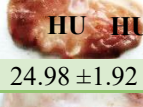





There was a significantly reduced relative gastric ulcer observed on days 3 and 14 in the

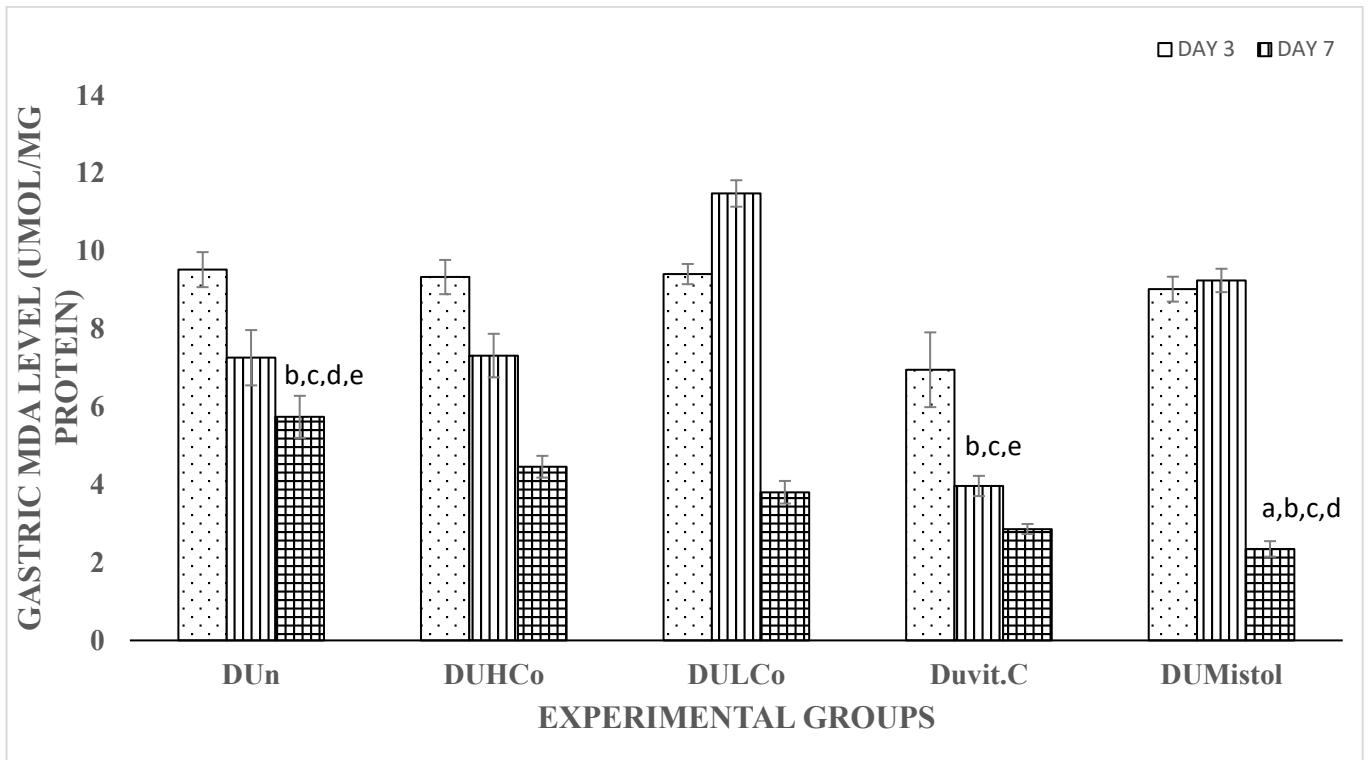
ulcerated delayed cobalt treated (DUHCo and DULCo) groups compared with ulcerated delayed untreated (DUn) groups. On day 7 of delayed gastric ulcer healing and treatment, the relative gastric ulcer was significantly reduced in the ulcerated delayed low cobalt (DULCo), misoprostol (DUmisol) and vitamin C treated (DUvit.C) groups compared with the ulcerated delayed untreated (DUn) group.

**EFFECT OF COBALT CHLORIDE ON GASTRIC PROTEIN LEVEL DURING DELAYED GASTRIC ULCER HEALING**

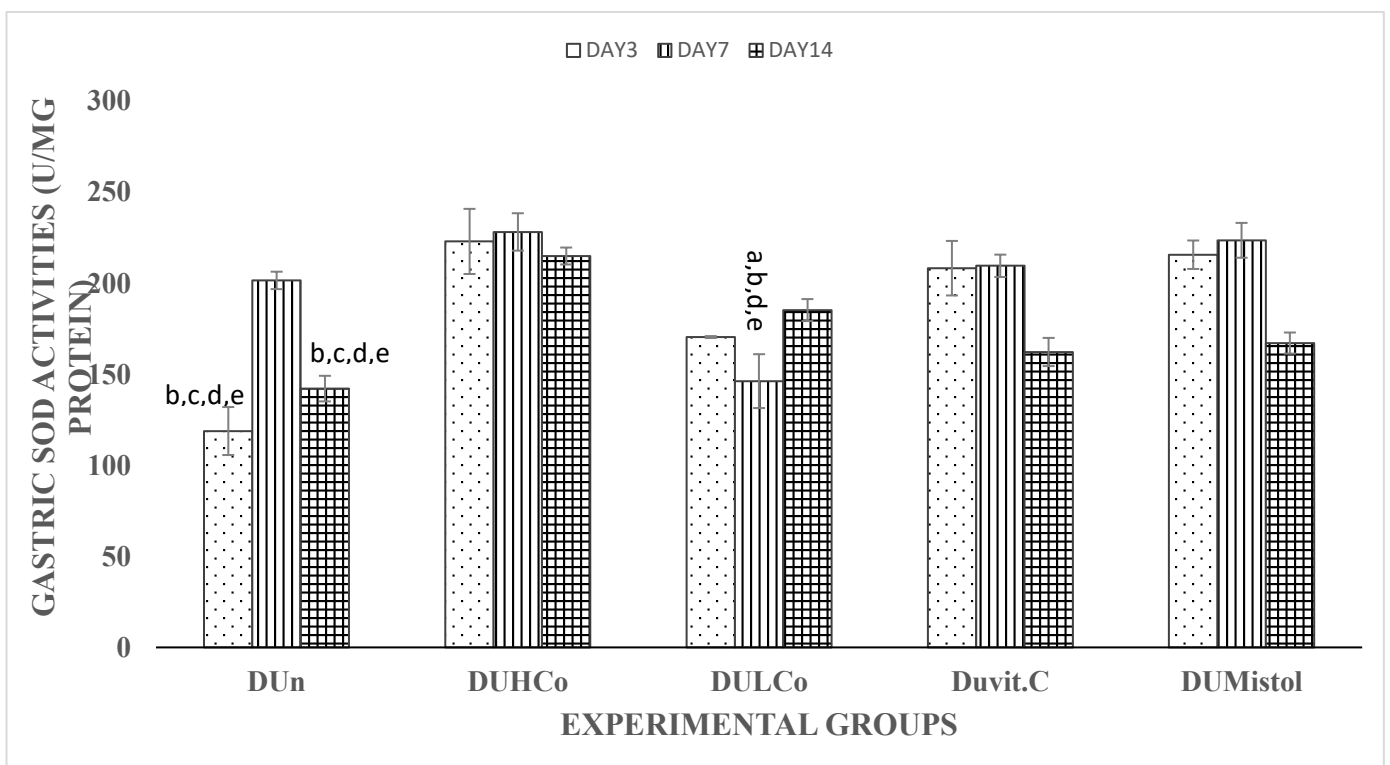
As shown in Table 6, there was a significant decrease at day 3 in the protein level of the ulcerated delayed high dose Cobalt (DUHCo) when compared with ulcerated delayed untreated (DUn) and ulcerated delayed low dose Cobalt (DULCo) groups. On day 7, the protein levels of the ulcerated delayed high dose Cobalt (DUHCo), ulcerated delayed vitamin C (DUvit.C) , and ulcerated delayed misoprostol (DUmisol) groups significantly reduced when compared with ulcerated delayed untreated (DUn) and ulcerated delayed low dose Cobalt (DULCo) groups.

**Table 4: EFFECT OF COBALT CHLORIDE ON HEALING RATE, MACROSCOPIC ULCER SCORE AND STOMACH WEIGHT DURING DELAYED ULCER HEALING.**

GROUPS /DAYS	% Healing Rate			Relative Ulcer Area (mm <sup>2</sup> )			Stomach Weight (g)		
	3	7	14	3	7	14	3	7	14
DUn	-	-		 37.71 ± 5.42 <sup>b,c</sup>	 25.54±9.10 <sup>c,d,e</sup>	 27.63±11.19 <sup>b,c,d,e</sup>	1.90±0.0 2 <sup>b,c,d,e</sup>	1.00±0.03	1.45±0.08 <sup>c,e</sup>
DUHCo	64.47	1.84	86.72	 13.40± 0.64	 25.07±1.41	 3.67±0.72	1.03±0.0 8	0.81±0.05 <sup>c</sup>	0.85±0.06
DULCo	64.04	21.46	95.73	 13.56±3.93	 20.06±0.43	 1.18±1.18	1.23±0.0 3	1.39±0.13	1.52±0.13
DUvitC	33.76	59.00	56.64	 24.98 ±1.92	 10.47±5.24	 11.98±3.20	0.93±0.1 0	0.95±0.03	1.23±0.06
DUmisol	33.04	51.49	56.86	 25.25±7.18	 12.39±4.50	 11.92±2.70	0.84±0.0 5	0.99±0.03	1.24±0.04



**Figure 3:** EFFECT OF COBALT CHLORIDE ON GASTRIC MDA LEVEL DURING DELAYED GASTRIC ULCER



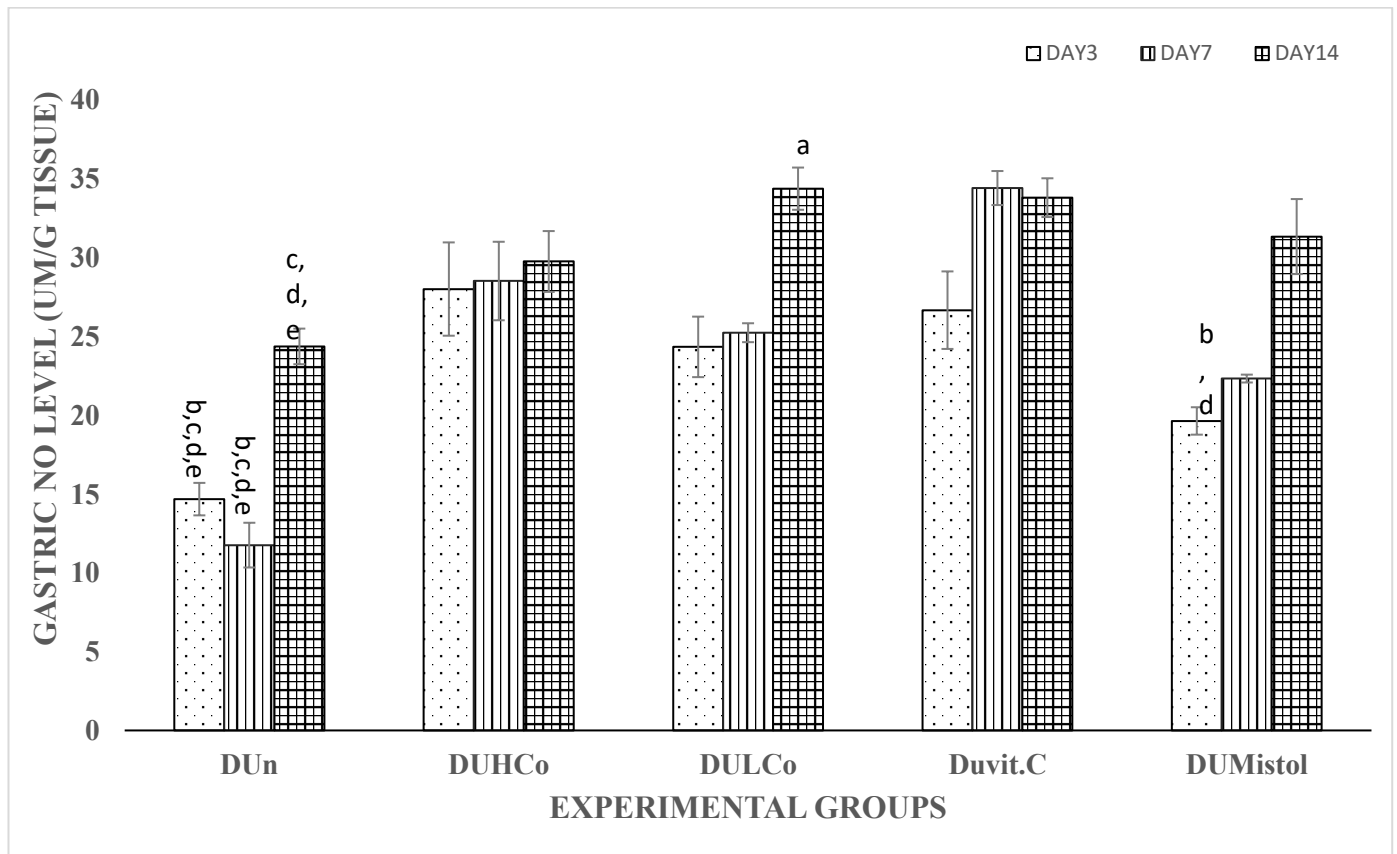
**Figure 4:** EFFECT OF COBALT CHLORIDE ON GASTRIC SUPEROXIDE DISMUTASE (SOD) DURING DELAYED GASTRIC ULCER

**EFFECT OF COBALT CHLORIDE ON GASTRIC MALONDIALDEHYDE (MDA) LEVEL DURING DELAYED GASTRIC ULCER HEALING**

A significant decrease in MDA level of the ulcerated delayed vitamin C treated (DUvit.C) group when compared with other experimental groups was observed by day 3. There was a significant increase in the MDA level of the ulcerated delayed Low dose Cobalt (DULCo) and Misoprostol treated (DUMisol) groups compared with other experimental group by day 7. The MDA level of the ulcerated delayed Vitamin C treated (DUvit.C) group was significantly reduced compared with other experimental groups by day 7. By day 14, all treated groups showed a significant decrease in MDA level compared with ulcerated delayed untreated (DUUn) group. The ulcerated delayed Misoprostol treated (DUMisol) group had the lowest MDA levels compared with all other experimental groups by day 14, Figure 3

**EFFECT OF COBALT CHLORIDE ON GASTRIC SUPEROXIDE DISMUTASE (SOD) LEVEL DURING DELAYED GASTRIC ULCER HEALING**

There was a significantly increased SOD level in the ulcerated delayed high dose cobalt treated (DUHCo) group compared with all experimental groups during the period of study. Of all the treated groups the low dose cobalt (DULCo) had a significantly lower SOD level by day 3. By day 7 of this study, the ulcerated delayed low dose cobalt treated (DULCo) group had a significantly lowered SOD value compared with all other experimental groups. However, the ulcerated delayed untreated (DUUn) groups had significantly reduced SOD levels compared with all other experimental groups on days 3 and 14, (Figure 4).



**Figure 5: EFFECT OF COBALT CHLORIDE ON GASTRIC NITRIC OXIDE LEVEL DURING DELAYED GASTRIC ULCER**

### **EFFECT OF COBALT CHLORIDE ON GASTRIC NITRIC OXIDE LEVEL DURING DELAYED GASTRIC ULCER HEALING**

There was a significant decrease in the nitric oxide level of ulcerated delayed untreated (DUn) group by days 3,7 and 14 compared with all experimental groups. The ulcerated delayed misoprostol treated (DUmisol) group had a significantly low NO level compared with other treatment groups by days 3 and 7. The ulcerated delayed Vitamin C treated (DUvit.C) group had a significant increase in the NO levels compared with other treatment groups by day7 while the ulcerated delayed low dose cobalt treated (DULCo) group had a significantly increased NO levels by day 14 compared with other treatment groups (Figure 5).

### **EFFECT OF COBALT CHLORIDE ON PARIETAL AND MUCOUS CELL COUNTS DURING DELAYED GASTRIC ULCER HEALING**

The ulcerated delayed untreated (DUn) group had a significantly low parietal cell count compared with all other experimental groups on days 3, 7 and 14. The ulcerated delayed high cobalt treated (DUHCo) group had a significantly low parietal cell count compared with the ulcerated delayed vitamin C treated (DUvit.C) group by days 3 and 7 (table 5)

The mucous cell count was significantly increased in the ulcerated delayed vitamin C

treated (DUvit.C) group compared with all other experimental groups by day 3. The mucous cell count in the ulcerated delayed untreated (DUn) group was significantly lower compared with the ulcerated delayed low cobalt chloride treated (DULCo) group by days 3 and 7, (Table 5). By day 7, both the ulcerated delayed untreated (DUn) and ulcerated delayed misoprostol treated (DUmisol) groups had significantly lower mucous cell counts compared with all other experimental groups while the ulcerated delayed low cobalt treated (DULCo) group had a significantly high mucous cell count by day 14 (Table 5).

### **EFFECT OF COBALT CHLORIDE ON GASTRIC CELL COUNTS DURING DELAYED GASTRIC ULCER HEALING**

**By DAY 3 (H&E STAIN MAGX100) SHOWING: ULCERATED DELAYED UNTREATED (DUn):** There are large focal areas of mucosa ulcer (white arrow), the mucosa epithelial cells layer are eroded, the gastric glands and laminal propria are severely infiltrated. The submucosal layer shows severe infiltration of inflammatory cells, the circular muscle layer shows mild infiltration.

**TABLE 5: EFFECT OF COBALT CHLORIDE ON PARIETAL AND MUCOUS CELL COUNTS DURING DELAYED GASTRIC ULCER AFTER TREATMENT.**

GROUPS / DAYS	PARIETAL CELL COUNTS			MUCOUS CELL COUNTS		
	3	7	14	3	7	14
DUn	258.30±31.81 <sup>cde</sup>	176.70±33.46 <sup>bcde</sup>	205.70±9.82 <sup>b,c,d,e</sup>	113.70±5.24 <sup>cd</sup>	110.00±2.08 <sup>b,c,d</sup>	112.50±1.44
DUHCo	377.30±13.62	309.30±4.807 <sup>d</sup>	431.50±22.27	205.50±24.19 <sup>d</sup>	252.70±37.17	102.30±6.93 <sup>ac</sup>
DULCo	432.70±19.22 <sup>a</sup>	373.30±29.95	420.00±25.24	310.00±53.20 <sup>d</sup>	273.00±29.70	147.50±4.09
DUvitC	478.00±9.539	441.50±31.56	361.30±28.59	512.50±52.83	327.30±26.67	131.50±2.59 <sup>c</sup>
DUmisol	418.00±53.45	376.20±18.57	347.70±17.13	165.50±10.64 <sup>d</sup>	124.80±7.34 <sup>bcd</sup>	107.50±8.61 <sup>d</sup>

**ULCERATED DELAYED HIGH DOSE CoCl<sub>2</sub> TREATED (DUHCo):** Poor architecture, of the mucosa epithelial cells layer are moderately sloughed (white arrow), the mucosa layer shows moderate infiltration of the gastric glands, the laminal propria are mildly infiltrated as well. The submucosal layer shows mild infiltration of inflammatory cells, the circular muscle layer appears normal.

**ULCERATED DELAYED LOW DOSE CoCl<sub>2</sub> TREATED (DULCo):** Poor architecture, of the mucosa epithelial cells layer are moderately sloughed and shows focal area of ulcer. The mucosa layer and the crypts show mild infiltration, the gastric glands and laminal propria are mildly infiltrated. The submucosal layer shows mild infiltration of inflammatory cells, the circular muscle layer (red arrow) appears normal.

**ULCERATED DELAYED VITAMIN C TREATED (DUvit.C):** Moderately preserved mucosa epithelial cells layer (white arrow), the mucosa layer show mild infiltration of the gastric glands and laminal propria by inflammatory cells. The submucosal layer shows edema and moderate infiltration of inflammatory cells (blue arrow), the circular muscle layer appears normal.

**ULCERATED DELAYED MISOPROSTOL TREATED (DUmiso):** Poor architecture, of the mucosa epithelial cells layer are severely eroded with focal area of ulcer (white arrow), the gastric glands and laminal propria are mildly infiltrated. The submucosal layer shows mild to moderate infiltration of inflammatory cells, the circular muscle layer appears normal.

**By DAY 7 (H&E STAIN MAGX100) SHOWING: ULCERATED DELAYED UNTREATED (DUn):** There are moderately preserved mucosa epithelial cells layer (white arrow), the mucosa layer show mild infiltration of the gastric glands and laminal propria. The submucosal layer shows mild infiltration (blue arrow), the circular muscle layer appears normal. **ULCERATED DELAYED HIGH DOSE CoCl<sub>2</sub> TREATED (DUHCo):** Fairly normal architecture, of the mucosa epithelial cells layer are moderately preserved (white arrow), the mucosa layer and the crypts show mild infiltration. The submucosal layer shows mild infiltration of inflammatory cells (blue arrow) with mild fibrosis, the circular muscle layer (red arrow) appears normal.

**TABLE 6: EFFECT OF COBALT CHLORIDE ON GASTRIC PROTEIN LEVEL DURING DELAYED GASTRIC ULCER.**

GROUPS/DAY	3	7	14
DUn	0.54±0.02	0.55±0.02	0.61±0.04
DUHCo	0.46±0.01 <sup>ac</sup>	0.46±0.01 <sup>ac</sup>	0.71±0.05
DULCo	0.54±0.01	0.54±0.01	0.71±0.06
DUVitC	0.51±0.02	0.49±0.01 <sup>a</sup>	0.72±0.03
DUmisto	0.47±0.02	0.49±0.01 <sup>a</sup>	0.65±0.01

**ULCERATED DELAYED LOW DOSE CoCl<sub>2</sub> TREATED (DULCo):** Moderately normal architecture, of the mucosa epithelial cells layer are moderately preserved, (white arrow), the gastric glands and lamina propria are mildly infiltrated. The submucosal layer shows moderate infiltration of inflammatory cells (blue arrow), the circular muscle layer (red arrow) appears normal.

**ULCERATED DELAYED VITAMIN C TREATED (DUvit.C):** Normal architecture, there are well preserved mucosa epithelial cells layer (white arrow), the mucosa layer shows no infiltration of the gastric glands and lamina propria. The submucosal layer shows mild infiltration of lymphocytes, the circular muscle layer appears normal.

**ULCERATED DELAYED MISOPROSTOL TREATED (DUMisol):** Moderately normal architecture, of the mucosa epithelial cells layer are mildly eroded (white arrow), the mucosa layer and the crypts show no infiltration, the gastric glands and lamina propria are normal (slender arrow). The submucosal layer shows mild infiltration of inflammatory cells, the circular muscle layer appears normal.

**By DAY 14 (H&E STAIN MAGX100) SHOWING ULCERATED DELAYED UNTREATED (DUn):** Moderately normal architecture with epithelial erosion, there are moderately preserved mucosa epithelial cells layer (white arrow), the mucosa layer shows no infiltration of the gastric glands and lamina propria. The submucosal layer shows mild infiltration (blue arrow), the circular muscle layer (red arrow) appears normal.

**ULCERATED DELAYED HIGH DOSE CoCl<sub>2</sub> TREATED (DUHCo):** Moderately normal architecture, of the mucosa epithelial cells layer are moderately preserved (white arrow), the mucosa layer and the crypts show mild infiltration, the gastric glands are mildly infiltrated. The submucosal layer shows mild infiltration of inflammatory cells, the circular muscle layer appears normal.

**ULCERATED DELAYED LOW DOSE CoCl<sub>2</sub> TREATED (DULCo):** Moderately normal architecture, of the mucosa epithelial

cells layer are moderately preserved (white arrow), the mucosa layer and the gastric glands are not infiltrated. The submucosal layer shows no infiltration of inflammatory cells, there is mild vascular congestion. The circular muscle layer (red arrow) appears normal.

**ULCERATED DELAYED VITAMIN C TREATED (DUvit.C):** Moderately normal architecture, of there are well preserved mucosa epithelial cells layer (white arrow), the crypts are mildly infiltrated and the gastric glands and lamina propria are mildly infiltrated as well. The submucosal layer shows mild infiltration of lymphocytes, the circular muscle layer appears normal.

**ULCERATED DELAYED MISOPROSTOL TREATED (DUMisol):** Moderately normal architecture, of the mucosa epithelial cells layer are mildly eroded (white arrow), the mucosa layer and the crypts show no infiltration, the gastric glands and lamina propria are normal. The submucosal layer shows mild infiltration of inflammatory cells and mild vascular congestion (black arrow), the circular muscle layer appears normal.

## DISCUSSION

In this study (delayed gastric ulcers models), cobalt treated group had higher gastric ulcer healing percentages in similar manner as the previous study,<sup>9</sup> except on day 7 where the healing rate significantly reduced. During delayed gastric ulcer healing, the cobalt treated groups had abundance of mucous cell counts irrespective of the continuous subcutaneous administration of indomethacin (NSAID) to cause unhealed gastric ulcer. However, there was also an increase in the parietal cell count in the cobalt treated groups unlike in the delayed ulcerated untreated group which is a diseased condition called hypochlorhydria.<sup>48</sup> A reduction in gastric parietal cell leads to reduced gastric acid availability which reduces food digestion and nutrients.

During hypochlorhydria, there has been increased cases of iron deficiency anemia as

observed in the delayed ulcerated untreated (DUn) group of this study which had reduced hemoglobin levels on days 7 and 14 but with increased monocyte levels on days 3 through 14. This observation is similar to those reported in a study.<sup>49</sup> However, there was increased platelet and neutrophil counts as well as elevated platelet/lymphocyte level days 3 and 7 in the DUn group in this study indicative of thrombocytosis similar to observations by,<sup>50-51</sup> unlike in the ulcerated delayed cobalt treated group.

Polycythemia vera is a rare condition in which there is abnormally high level of PCV and red blood cell count as observed in the DUn group of this study.<sup>52-53</sup> It might be due to thrombocythemia in which the bone marrow of the rats in this group produce too many circulating platelets in an attempt to stimulate more platelet derived growth factors delivered at the ulcerated site for gastric ulcer healing. In the ulcerated delayed cobalt chloride treated group, there was no polycythemia vera, the platelet count were increased by day 3 and reduced or modulated (depending on the level of ulceration and healing) by days 7 and 14. The initial increase might have been due to a means of cobalt chloride enhancing haemostasis by days 3 to avoid anaemia during ulceration. Haemostasis, is a phase during gastric ulcer healing in which blood loss is managed or stopped by platelet aggregation.<sup>54,55</sup> All values were below the ulcerated delayed untreated (DUn) group in which thrombocytosis was observed.

During gastric ulceration there has been cases of increase systemic inflammation and probably low immunity response with indicators like high Platelet/Lymphocyte (PLR),<sup>56</sup> Neutrophil/Lymphocyte (N/L),<sup>57</sup> and Monocyte/Lymphocyte (MLR) ratios,<sup>58-59</sup>. In this study, the DUn group had elevated PLR, MLR and N/L levels which might be indicative of systemic inflammation or perforated gastric ulcers which is similar to an earlier report,<sup>60</sup> unlike in the ulcerated delayed cobalt chloride treatment groups.

Platelets enhance growth factor at ulcerated site for re-epithelisation which reduces ulceration which indicates healing and was

observed in the ulcerated delayed cobalt chloride treated groups by day 14 in this study. There were no elevated platelet/lymphocyte ratio in the ulcerated delayed cobalt chloride treated groups on days 3 and 7, however by day 14 there was an increase relative to the DUn group which might have resulted in the observed increased ulcer healing rate on this day. These observations validate the activity of cobalt in ameliorating induced anemia and systemic inflammation accompanying delayed or unhealed gastric ulceration in this study.

It has been documented that gastric ulceration is mostly accompanied with increased generation of free radical release. Increased lipid peroxidation was ameliorated in the cobalt treated group in a dose dependent manner. Inherent within most body cells are antioxidant defense systems (Superoxide dismutase which ameliorate adverse effects of released free radicals, enhancing gastro-protection and epithelial integrity as well as protecting the regenerating tissue.<sup>61</sup> Treatment with cobalt caused an increased production of superoxide dismutase, SOD activity which was dose and duration dependent.

Nitric oxide (NO) is thought to be mediator, not only of gastrointestinal mucosal defense causes vasodilatation resulting to increased supply of nutrients and oxygen to the ulcerated stomach thus prompting angiogenesis.<sup>62</sup> Cobalt facilitated the increased production of nitric oxide all through in delayed gastric ulcer healing in a dose dependent manner. This modulatory activity of Cobalt on nitric oxide might have resulted in the adequate supply of nutrients and oxygenation of ulcerated gastric tissue thus leading to observed gastric ulcer healing and prevention. It might well be that cobalt can modulate the release of nitric oxide in favour of protecting the gastric epithelium which is another probable mechanism for facilitating gastric ulcer healing.

The secretion of gastric acid which is involved in food digestion, bactericide and absorption of nutrients is carried out by the parietal cells in the gastrum. It has also been

documented that parietal cells are significant to gastroprotection, maintenance of gastric mucosa homeostasis, and the physiological repair of the stomach.<sup>63</sup> These functions are probably due to the activities of the growth factors secreted,<sup>64</sup> sonic Hedgehog,<sup>65</sup> and the regulation of stem cells progenitors.<sup>66</sup> Alterations in gastric mucosal architecture have been linked with loss of parietal cells.<sup>67</sup> The study showed that the production of parietal cells was promoted by Cobalt Chloride. The increase in parietal cells production observed in the cobalt groups probably facilitated ulcer healing through mucosal homeostasis and cell re-epithelization. Thus, it is suggestive that ulcer healing of Cobalt is predicated on the production and migration of parietal cells in the gastrum.

Mucus represents the first line of defense (physical and immunological barrier); due to the presence of specific immunoglobulins and antimicrobials.<sup>68</sup> Mucous cells that are adjacent to injury site form “healing zone” that contains some growth factors that triggers re-epithelization.<sup>69</sup> Gastric ulcer recurrence can occur due to the increased production of neutrophils which release proteolytic enzymes that digest tissue.<sup>70</sup> On day 3, the mucous cell count of all the treatment groups were elevated and by day 7 a reduced count was recorded in all the treatment groups except the Cobalt high dose group. The growth factors that probably stimulated re-epithelization process (which was facilitated by Cobalt) are not only derived from the mucous cells but also from the parietal cells, platelets and macrophages. The progressive reduction in the mucous cell count in the treatment groups might be indicative of the nearing to completion of the healing process. This projects Cobalt as a substance that has the potential of stimulating quality gastric ulcer healing.

## CONCLUSION

This study demonstrated that cobalt chloride attenuated unhealed gastric ulcer and associated anemia via reduced blood

inflammatory markers, nitric oxide levels, and mucous cell count.

## REFERENCES

1. Battaglia G, Di Mario F, Piccoli A, Vianello F, Farinati F, Naccarato R. Clinical markers of slow healing and relapsing gastric ulcer. *Gut*. 1987; 28(2):210-5. doi: 10.1136/gut.28.2.210. PMID: 3557191; PMCID: PMC1432970.;
2. Wang SY, Wang HY, Wang TE, Wang HH, Chang WH, Chu CH, et al. Delayed healing of gastric ulcer is associated with downregulation of connexin 32 in the gastric mucosa, *Advances in Digestive Medicine* 2015; 2(2):67-73, ISSN 2351-9797, <https://doi.org/10.1016/j.aidm.2015.01.004>.
3. Okabe S, Amagase K. An overview of acetic acid ulcer models-the history and state of the art of peptic ulcer research. *Biol Pharm Bull*. 2005; 28(8):1321-41. doi: 10.1248/bpb.28.1321. PMID: 16079471.
4. Salami AT, Adeola BO, Iyiola TO, Omayone TP, Oluwole FS, Olaleye SB. Antioxidative action of Manganese treatment in delayed healing of acetic acid induced ulceration in rat stomach. *Journal of African Association of Physiological Sciences*. 2015; 3(2): 67-78
5. Wang JY, Yamasaki S, Takeuchi K, Okabe S. Delayed healing of acetic acid-induced gastric ulcers in rats by indomethacin. *Gastroenterology*. 1989; 96(2 Pt 1):393-402. doi: 10.1016/0016-5085(89)91563-1. PMID: 2910759.
6. Amang AP, Mezui C, Siwe GT, Emakoua J, Mbah G, Nkwengoua EZ, et al. Healing and Antisecretory Effects of Aqueous Extract of *Eremomastax speciosa* (Acanthaceae) on Unhealed Gastric Ulcers. *BioMed Research International*. 2017; 1:1924320. <https://doi.org/10.1155/2017/1924320>
7. Albert-Bayo M, Paracuellos I, González-Castro AM, Rodríguez-Urrutia A, Rodríguez-Lagunas MJ, Alonso-Cotoner C, et al. Intestinal Mucosal Mast Cells: Key Modulators of Barrier Function and Homeostasis. *Cells*. 2019; 8(2):135. <https://doi.org/10.3390/cells8020135>

8. Ito M, Segami T, Inagguma K, Suzuki Y. Cimetidine and omeprazole accelerate gastric ulcer healing by an increase in gastrin secretion. *Eur J Pharmacol.* 1994; 263:251–259
9. Salami AT, James PA, Iyiola, TO, Olaleye SB. Gastric ulcer-healing promoting activity of cobalt chloride in rats. *African Journal of Medicine and Medical Sciences.* 2018; 47(2): 115-122
10. Salami AT, Akpamu U, Iyiola TO, Olaleye SB. Cobalt Modulates Healing of Gastric Ulceration in male Wistar Rats: iNOS/COX-2 crosstalk. *Nigerian Journal of Pharmaceutical Research.* 2024; 20(2):167-184.
11. Obeagu EI, Obeagu GU. Exploring the intersection: Peptic ulcers and hemolysis—Unraveling the complex relationship. *Medicine.* 2024; 103(11):e37565. <https://doi.org/10.1097/MD.00000000000037565>
12. Papatheodoridis GV, Sougioultzis S, Archimandritis AJ. Effects of *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs on peptic ulcer disease: a systematic review. *Clin Gastroenterol Hepatol.* 2006; 4:130–142
13. DeLoughery TG. Microcytic anemia. *N Engl J Med.* 2014; 371:1324–1331. doi: 10.1056/NEJMra1215361
14. Cryer B, Mahaffey KW. Gastrointestinal ulcers, role of aspirin, and clinical outcomes: pathobiology, diagnosis, and treatment. *J Multidiscip Healthc.* 2014; 7:137–146
15. Haile K, Yemane T, Tesfaye G, Wolde D, Timerga A, Haile A. Anemia and its association with *Helicobacter pylori* infection among adult dyspeptic patients attending Wachemo University Nigist Eleni Mohammad Memorial Referral Hospital, Southwest Ethiopia: A cross-sectional study. *PLoS ONE,* 2021; 16(1):e0245168. <https://doi.org/10.1371/journal.pone.0245168>
16. Betesh AL, Santa Ana CA, Cole JA. Is achlorhydria a cause of iron deficiency anemia? *Am J Clin Nutr.* 2015; 102:9–19. 10.3945/ajcn.114.097394
17. Patel PK, Patel SK, Dixit SK, Rathore RS. Gastritis and peptic ulcer diseases in dogs: a review. *Int J Curr Microbiol App Sci.* 2018; 7:2475–2501.
18. Papatheodoridis GV, Sougioultzis S, Archimandritis AJ. Effects of *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs on peptic ulcer disease: a systematic review. *Clin Gastroenterol Hepatol.* 2006; 4:130–142.
19. Camaschella C. Iron-deficiency anemia. *N Engl J Med.* 2015; 372:1832–1843. doi: 10.1056/NEJMra1401038.
20. Tan H, Goh K. Extragastrintestinal manifestations of *Helicobacter pylori* infection: Facts or myth? *J Dig Dis.* 2012; 13:342–349
21. Jelkmann W. Erythropoietin. *Sports Endocrinology.* 2016; 47:115-127.
21. Jelkmann W. Regulation of erythropoietin production. *J Physiol.* 2011; 15; 589(Pt 6):1251-1258. doi: 10.1113/jphysiol.2010.195057.
22. Tvermoes BE, Unice KM, Paustenbach DJ, Finley BL, Otani JM, Galbraith DA. Effects and blood concentrations of cobalt after ingestion of 1 mg/d by human volunteers for 90 d. *Am J Clin Nutr.* 2014; 99(3):632-646. doi: 10.3945/ajcn.113.071449. Epub 2014 Feb 5. PMID: 24500148.
23. Czarnek K, Terpiłowska S, Siwicki AK. Selected aspects of the action of cobalt ions in the human body. *Central-European Journal of Immunology.* 2015; 40(2):236. <https://doi.org/10.5114/ceji.2015.52837>
24. Houlihan B, Vidar Hanstad D. The effectiveness of the World Anti-Doping Agency: developing a framework for analysis, *International Journal of Sport Policy and Politics.* 2019. 11(2):203-217. DOI: 10.1080/19406940.2018.1534257
25. Ebert B, Jelkmann W. Intolerability of cobalt salt as erythropoietic agent. *Drug Test Anal.* 2014; 6(3):185-189. doi: 10.1002/dta.1528.
26. Thevis M, Krug O, Piper T, Geyer H, Schänzer W. Solutions advertised as erythropoiesis-stimulating products were found to contain undeclared cobalt and nickel species. *Int J Sports Med.* 2016; 37:82-84. [doi:10.1055/s-0035-1569350](https://doi.org/10.1055/s-0035-1569350)
27. Nordberg M, Nordberg GF. Trace element research-historical and future aspects. *Journal of Trace Elements in Medicine and Biology.* 2016; 38:46-52. <https://doi.org/10.1016/j.jtemb.2016.04.006>

28. Finley BL, Monnot AD, Gaffney SH, Paustenbach DJ. Dose-response relationships for blood cobalt concentrations and health effects: a review of the literature and application of a biokinetic model. *J Toxicol Environ Health B Crit Rev.* 2012;15(8):493-523. doi: 10.1080/10937404.2012.744287. Erratum in: *J Toxicol Environ Health B Crit Rev.* 2013;16(1):67. PMID: 23199219.
29. Hoffmeister T, Schwenke D, Krug O, Wachsmuth N, Geyer H, Thevis M, Byrnes WC, Schmidt WFJ. Effects of 3 Weeks of Oral Low-Dose Cobalt on Hemoglobin Mass and Aerobic Performance. *Front Physiol.* 2018; 19(9):1289. doi: 10.3389/fphys.2018.01289. PMID: 30283349; PMCID: PMC6157393.
30. Hoffmeister T, Schwenke D, Wachsmuth N, Krug O, Thevis M, Byrnes WC, Schmidt WF. Erythropoietic effects of low-dose cobalt application. *Drug testing and analysis.* 2019; 11(2):200-207.
31. Salami AT, Famurewa AD. Effect of *Chrysophyllum albidum* bark extract and fractions on delayed gastric ulcer healing and the role of blood inflammatory makers. *Archives of Basic and Applied Medicine.* 2017; 5(2):77-87.
32. Salami AT, Adeola BO, Iyiola TO, Omayone TP, Oluwole FS, Olaleye SB. Antioxidative action of Manganese treatment in delayed healing of acetic acid induced ulceration in rat stomach. *Journal of African Association of Physiological Sciences.* 2015;3(2):67-78.
33. Salami AT, Odukanmi OA, Faniyan OF, Omayone TP, Olaleye SB. Seeds of *Buchholzia coriacea* in Diet Mitigate Ischemic Reperfusion-Induced Gastric Ulceration in Experimental Rats. *Journal of dietary supplements.* 2018; 15(6):842–859. <https://doi.org/10.1080/19390211.2017.1404544>
34. Alabi QK, Akomolafe RO, Olukiran OS, Nafiu AO, Omole JG, Adefisayo AM. Et al. “Assessment of Haematological and Biochemical Effects of Kolaviron in Male Wistar Rats”, *Journal of Pharmaceutical Research International,* 2017; 16(3):1–14. doi: 10.9734/BJPR/2017/33517.
35. Buonacera A, Stancanelli B, Colaci M, Malatino L. Neutrophil to Lymphocyte Ratio: An Emerging Marker of the Relationships between the Immune System and Diseases. *International Journal of Molecular Sciences.* 2022; 23(7):3636. <https://doi.org/10.3390/ijms23073636>
36. Kim JK, Sun KH. Role of platelet-to-lymphocyte ratio at the time of arrival to the emergency room as a predictor of short-term mortality in trauma patients with severe trauma team activation. *Acute Crit Care.* 2024; 39(1):146-154. doi: 10.4266/acc.2023.01319
37. Tarnawski AS, Ahluwalia A. The Critical Role of Growth Factors in Gastric Ulcer Healing: The Cellular and Molecular Mechanisms and Potential Clinical Implications. *Cells.* 2021; 10(8):1964. doi: 10.3390/cells10081964. PMID: 34440733; PMCID: PMC8392882.
38. Salami AT, James PA, Iyiola TO, Olaleye SB. Gastric ulcer-healing promoting activity of cobalt chloride in rats. *African Journal of Medicine and Medical Sciences.* 2018; 47(2):115-122.
39. Clark B, Stewart GB, Panzone LA, Kyriazakis I, Frewer LJ. Citizens, consumers and farm animal welfare: A meta-analysis of willingness-to-pay studies. *Food Policy.* 2017; 68:112-127. <https://doi.org/10.1016/j.foodpol.2017.01.006>
40. Adinortey MB, Ansah C, Galyuon I, Nyarko A. In Vivo Models Used for Evaluation of Potential Antigastroduodenal Ulcer Agents. *Ulcers,* 2012; 2013(1):796405. <https://doi.org/10.1155/2013/796405>
41. Dacie JV, Lewis SM. *Practical Haematology* 7<sup>th</sup> edition. ELBS with Churchill Livingstone. Longman group UK. 1994; 5-82:160-175.
42. Lawal HO, Iyiola TO, Salami AT, Oluwole FS. Gastroprotective Activities of *Canarium schweinfurthii* Fruit and Oil Diets: Role of H<sup>+</sup>/K<sup>+</sup> ATPase Activity. *Archives of Basic and Applied Medicine.* 2024; 12:54-66
43. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. *J Biol Chem.* 1949; 177(2):751-766.
44. Vashney R, Kale RK. Effects of calmodulin Antagoniss. *Int. J Rad. Biol.* 1990; 58:733-743

45. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.* 1972; 247:3170-3175.
46. Ignarro L, Buga G, Wood K, Byrns R, Chaudhuri G. Endothelium derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences USA.* 1987; 84:9265-9269.
47. Oluwole SO, Saka MT. Effect of thyroid hormone on gastric mucous secretion around indomethacin-induced gastric ulcers in rats. *J Med Sci.* 2007; 7(4):678-681.
48. Filardo S, Scalese G, Virili C, Pontone S, Pietro MD, Covelli A, et al. The Potential Role of Hypochlorhydria in the Development of Duodenal Dysbiosis: A Preliminary Report. *Frontiers in Cellular and Infection Microbiology.* 2022; 12:854904. <https://doi.org/10.3389/fcimb.2022.854904>
49. Beckett AC, Piazuolo MB, Noto JM, Washington MK, Scott Algood HM, et al. Dietary Composition Influences Incidence of Helicobacter pylori-Induced Iron Deficiency Anemia and Gastric Ulceration. *Infection and Immunity.* 2016; 84(12):3338. <https://doi.org/10.1128/IAI.00479-16>
50. Qi X, De Stefano V, Shao X, Guo X. Thrombocytosis in a patient with upper gastrointestinal bleeding. *Intractable Rare Dis Res.* 2017; 6(1):69-71. doi: 10.5582/irdr.2016.01080. PMID: 28357187; PMCID: PMC5359359.
51. Kohutek F, Bystricky B, Kohutekova M. Thrombocytosis and leukocytosis: Are they negative prognostic factors in solid tumours? *Annals of Oncology.* 2018; 29:viii12 - viii13
52. Ohyashiki K, Akahane D, Gotoh A, Ito Y, Tauchi T, Miyazawa K, et al. Uncontrolled thrombocytosis in polycythemia vera is a risk for thrombosis, regardless of JAK2V617F mutational status. *Leukemia.* 2007; 21(12):2544-2545. <https://doi.org/10.1038/sj.leu.2404829>
53. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. *Am J Hematol.* 2020; 95(12):1599-1613. doi: 10.1002/ajh.26008. Epub 2020 Oct 23. PMID: 32974939.
54. Lu H, Chiang C. Turn over the new leaf of the treatment in peptic ulcer bleeding: A review of the literature. *Therapeutic Advances in Gastroenterology.* 2024; 17 <https://doi.org/10.1177/17562848241275318>
55. Seleem WM, Hanafy AS. The Additive Effect of Platelet-Rich Plasma in the Treatment of Actively Bleeding Peptic Ulcer. *Clinical Endoscopy* 2021; 54(6):864-871. DOI: <https://doi.org/10.5946/ce.2021.04>
56. Shimoyama Y, Umegaki O, Agui T, Kadono N, Minami T. Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio are superior to other inflammation-based prognostic scores in predicting the mortality of patients with gastrointestinal perforation. *JA Clin Rep.* 2017; 3(1):49.
57. Huabing R, Dong W. The differential diagnosis value of neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in upper digestive tract ulcer perforation. *J Clin Emerg.* 2020; 21(2):147-152. doi: [10.13201/j.issn.1009-5918.2020.02.010](https://doi.org/10.13201/j.issn.1009-5918.2020.02.010)
58. Farah R, Hamza H. A link between platelet to lymphocyte ratio and Helicobacter pylori infection. *Journal of Clinical Laboratory Analysis.* 2017; 32(1):e22222. <https://doi.org/10.1002/jcla.22222>,
59. Al-Yahri O, Saafan T, Abdelrahman H, Aleter A, Toffaha A, Hajjar M, et al. Platelet to Lymphocyte Ratio Associated with Prolonged Hospital Length of Stay Postpeptic Ulcer Perforation Repair: An Observational Descriptive Analysis. *BioMed Research International.* 2021:6680414. <https://doi.org/10.1155/2021/6680414>
60. Aydin O, Pehlivanli F. Is the platelet to lymphocyte ratio a potential biomarker for predicting mortality in peptic ulcer perforation? *Surg Infect(Larchmt).* 2019; 20(4):326-331,
61. Zheng M, Liu Y, Zhang G, Yang Z, Xu W, Chen Q. The Applications and Mechanisms of Superoxide Dismutase in Medicine, Food, and Cosmetics. *Antioxidants.* 2023; 12(9):1675. <https://doi.org/10.3390/antiox12091675>

62. Liang Y, Deng M, Li X, Xu X, Chen G. The role of nitric oxide in peptic ulcer: A narrative review. *Medical Gas Research*. 2021; 11(1):42. <https://doi.org/10.4103/2045-9912.310059>
63. Engevik AC, Kaji I, Goldenring JR. The Physiology of the Gastric Parietal Cell. *Physiological Reviews*, 2019; 100(2):573. <https://doi.org/10.1152/physrev.00016.2019>
64. Tarnawski AS, Ahluwalia A. The Critical Role of Growth Factors in Gastric Ulcer Healing: The Cellular and Molecular Mechanisms and Potential Clinical Implications. *Cells*. 2021; 10(8):1964. <https://doi.org/10.3390/cells10081964>
65. Engevik AC, Feng R, Yang L, Zavros Y. The Acid-Secreting Parietal Cell as an Endocrine Source of Sonic Hedgehog During Gastric Repair. *Endocrinology*. 2013; 154(12):4627. <https://doi.org/10.1210/en.2013-1483>
66. Karam SM. A focus on parietal cells as a renewing cell population. *World journal of gastroenterology*. 2010; 16(5):538–546. <https://doi.org/10.3748/wjg.v16.i5.538>
67. Bockerstett KA, Osaki LH, Petersen CP, Cai CW, Wong CF, Nguyen TM, et al. Interleukin-17A Promotes Parietal Cell Atrophy by Inducing Apoptosis. *Cell Mol Gastroenterol Hepatol*. 2018; 5:678–690. doi: 10.1016/j.jcmgh.2017.12.012.
68. Vllahu M, Voli A, Licursi V, Zagami C, D'Amore A, Traulsen J, et al. Inflammation promotes stomach epithelial defense by stimulating the secretion of antimicrobial peptides in the mucus. *Gut Microbes*. 2024; 16(1):2390680 <https://doi.org/10.1080/19490976.2024.2390680>
69. Tarnawski AS, Jones MK, Ahluwalia A. Dedifferentiation and reprogramming of epithelial cells during gastric ulcer healing is triggered by hypoxia and a well-coordinated, sequential activation of egfr signaling: Cross talk with IGF1 and COX2. *Gastroenterology*. 2021; 160:S77–S78. doi: 10.1016/S0016-5085(21)00932-X.
70. Arakawa T, Watanabe T, Tanigawa T, Tominaga K, Fujiwara Y. Quality of ulcer healing in gastrointestinal tract: Its pathophysiology and clinical relevance. *World Journal of Gastroenterology*. 2012; 18(35):4811. <https://doi.org/10.3748/wjg.v18.i35.4811>