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Gastric ulcer - healing promoting activity of cobalt chloride in rats.

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Abstract

Background: Gastric ulcer develops when aggressive factors overcome protective factors in the gastrointestinal tract. Cobalt chloride is used in the manufacturing of vitamin B_{12}, essential for folate and fatty acid metabolism. Information regarding probable effects of Cobalt Chloride on Ulcer healing is void despite its vast use which this study addresses.

Method: 70 Male Wistar rats (150-180g, n=10) were used and induced with ulcer using acetic acid (excluding control) before grouping: Groups 1- Control, 2 - ulcer alone, 3 and 4 were ulcerated treated with 62 and 25mg/kg b.w of Cobalt chloride ($CoCl_2$); 5, 6 and 7 were ulcerated treated with 1, 40 and 30mg/kg b.w of Misoprostol, Cimetidine and Omeprazole respectively for 2 weeks. Gastric acid secretion, ulcer scores and histopathology of ulcerated areas were evaluated on days 7, 14 and 21. Data were analyzed using ANOVA and considered significant at $p<0.05$.

Result: This study revealed significant decrease in gastric acid secretion (and pH) of control (0.65±0.02) and treatment groups - high (0.86±0.01) and low (0.85±0.02) $CoCl_2$ compared to ulcer untreated (1.22±0.04) by day 7 post ulceration. A significant decrease in ulcer index of treatment group (high and low $CoCl_2$) by day 7(90.18% and 91.21%) respectively and complete healing day 14 was observed. Histological evaluations of $CoCl_2$ treated group revealed intact epithelium with normal glands by days 7 and post ulceration. There was no ulcer reformation in examined stomach, by day 21 (day 7 post 25mg/kg b.w $CoCl_2$ treatment).

Conclusion: Probably, Cobalt chloride exerts its anti-ulcerogenic property by stimulating gastric protective activities.

Keyword: *Cobalt chloride, gastric ulcer healing, gastric acidity.*

Résumé

Contexte: L'ulcère gastrique se développe lorsque des facteurs agressifs surmontent les facteurs protecteurs du tractus gastro-intestinal. Le chlorure de cobalt est utilisé dans la fabrication de la vitamine B_{12}, essentielle pour le métabolisme des acides foliques et gras. L'information concernant les effets probables du chlorure de cobalt sur la cicatrisation des ulcères est nulle en dépit de son vaste utilisation qui fait l'objet de cette étude.

Méthode : 70 rats Wistar (150-180g, n = 10) ont été utilisés et induits avec un ulcère en utilisant de l'acide acétique (excluant le contrôle) avant le regroupement: groupes 1- contrôle, 2 - ulcère seul, 3 et 4 ulcérés traités avec 62 et 25 mg / kg pc de chlorure de cobalt ($CoCl_2$); 5, 6 et 7 ont été traités avec 1, 40 et 30 mg / kg pc de misoprostole, de cimétidine et d'oméprazole respectivement pendant 2 semaines. La sécrétion d'acide gastrique, les scores d'ulcère et l'histopathologie des zones ulcérées ont été évalués aux jours 7, 14 et 21. Les données ont été analysées en utilisant ANOVA et considérées significatives à $p <0,05$.

Résultat : Cette étude a révélé la diminution significative de la sécrétion d'acide gastrique (et pH) des groupes de contrôle (0,65 ± 0,02) et de traitement - élevé (0,86 ± 0,01) et faible (0,85 ± 0,02) $CoCl_2$ par rapport au groupe d'ulcère non traité (1,22 ± 0,04) au jour 7 après l'ulcération. Une diminution significative de l'indice d'ulcère du groupe de traitement ($CoCl_2$ élevé et faible) au jour 7 (90,18% et 91,21%) respectivement et la cicatrisation complète jour 14 a été observée. Les évaluations histologiques du groupe traité par $CoCl_2$ ont révélé un épithélium intact avec des glandes normales au jour 7 et post-ulcération. Il n'y a pas eu de réformation d'ulcère dans les estomacs examinés, au jour 21 (au jour 7 après traitement avec 25 mg / kg pc de $CoCl_2$).

Conclusion: Probablement, le chlorure de cobalt exerce sa propriété d'anti-ulcération en stimulant des activités de protection gastrique.

Mot - clé : *Chlorure de cobalt, cicatrisation de l'ulcère gastrique, acidité gastrique .*

Introduction

In a normal scenario of a healthy stomach, there is usually a balance between protective factors (mucus and bicarbonate secretions) and aggressive factors (acid secretion and pepsin) [1]. Gastric ulcerations

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therefore develop when these aggressive factors overwhelm the protective mechanisms [2,3]. Gastric ulcers defined (peptic ulcers), as ulceration of the mucous membrane or stomach lining caused by hydrochloric acid (gastric digestive juice) action [4]. However, the major causative factor of peptic ulceration is a local decrease in the resistance of gastric mucosa to gastric juice digestive actions [5].

During gastric ulcer healing, a key factor is adequate blood flow and supply to ulcerated site in order to restore the damaged tissue components. This ensures oxygenation (red blood cells), removal of waste or necrotized tissues, prevention of infection (white blood cells) supply of nutrient and growth factors (platelets) to the ulcer site thus initiating angiogenesis [6].

Cobalt chloride has long been used for the treatment of anemia as it enhances erythropoiesis [7-10] especially those induced by hypoxia [11] or sickle cell [12]. It has been used by athletes to boost the endogenous erythropoietin levels [13]. It is inexpensive, readily available and very effective [14]. Various experiments have shown it to be effective in increasing physical performance (physical fitness) in rats [15], protect against ischemic injury and high altitude pulmonary edema [16] in rats.

Cobalt chloride has also been used as dietary supplements in ruminants [17] and observed to be protective against sodium thiosulfate during cyanide poisoning treatment [18]. Yildirim and Buyukbingol [19] observed that ascorbic acid enhanced the antioxidative effect of cobalt chloride by alleviating impaired oxidative stress in streptozotocin induced diabetes rats. Tephly and Hibbelin [20] observed that cobalt chloride (60mg/kg b.w) had an inhibitory effect on the synthesis of hepatic microsomal cytochrome P-450. It became a preferred choice [21] over uranium and tungsten alloys due to their toxicity [22] as well as its use (Cobalt-chromium alloys) in orthopedic hip replacement [23].

Cobalt has been found to cause several adverse effects when used in excess or prolonged exposure which might cause toxicity to various tissues and body system [24]. In man, ingestion of cobalt salts in excess for the treatment of certain refractory anemia produces nausea, vomiting, diarrhea, skin rashes and hot flushes in the short term [25] hence its none clinical prolong use [26, 24]. However its' over exposure leads to its toxicity [27] and uncontrolled adverse effects in the human tissue [28, 29]. Cobalt chloride has been reported to have varied effect on the respiratory tract, reproductive system, and bone tissue and heart- cardiomyopathy especially in beer drinkers [30] as it is used to stabilize foam in beer. There are however no reports on it being carcinogenic during oral exposure as it is poorly absorbed and excreted mostly through the faeces [31] (EFSA 2009). There has not been any beneficial report on its probable use in the gastrointestinal system despite its importance in the manufacturing of vitamin B12.

Vitamin B12 is important in the production of blood tonic or capsules generally referred to as hematinic which boosts hemoglobin production in anemia. Anemia has also been observed in some ulcer patients and has been treated with hematinic drug to boost their blood levels [32]. This study focuses on investigating the effect of cobalt chloride (in none lethal and sub lethal doses) [33] will exert on gastric ulcers healing.

Animal grouping

Seventy healthy adult male Wistar strain rats (150-170g) obtained from the Central Animal House, Department of Physiology, College of Medicine, University of Ibadan, Nigeria were used for this study. They were acclimatized for a period of 2 weeks and housed in cages at standard laboratory condition of room temperature (23±2°C), humidity (55±15%) with 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). The rats were handled according to the ethics of animal handling in compliance with the institution's guideline and criteria for human care (National institute of Health Guidelines for the care and Use of Laboratory Animals). The work was moderated by the Gastrointestinal Secretions and Inflammatory Research Unit, department of Physiology, University of Ibadan for thorough animal ethical humane compliance. These animals were then randomly divided into 7 groups of 10 animals each.

Group I (Normal control), Group II (Ulcer Untreated control), Group III ulcer+25mg/kg b.w $CoCl_2$, Group IV (ulcer + 62mg/kg b.w of $CoCl_2$, Group V (ulcer + 1mg/kg b.w Misoprostol), Group VI (ulcer + 40mg/kg b.w Cimetidine), Group VII (ulcer + 30mg/kg b.w Omeprazole)

Test and standard drugs

Cobalt chloride salts (manufactured and packed by Burgoyne, India Mumbai: batch number-24057) was purchased and used at two doses 25mg/kg b.w and 62mg/kg b.w.

Cimetidine capsule *(manufactured by Laborate Pharmaceutical (India) E-11,Ind. Area, Panipat – 132103; manufacturing date- 10-2012; expiry date- 09-2015)administered at* 40mg/kg b.w,
Mistoprotol tablets *(manufactured by JRP Co., Ltd. 34-40, Jeyakgongdan 2-gil, Hyangnam-eup, Hwaseong-si,Gyeonggi-do, Korea for Zolon healthcare limited, Isolo, lagos , Nigeria: manufacturing date-05-2013; expiry date-05-2016) administered at* 1mg/kg b.w and
Omeprazole tablets (*manufactured by Vee Excel Drugs and Pharmaceuticals Private Limited, Delhi, Ghaziabad No 703, Devika Tower, Ghaziabad – 201011, Uttar: manufacturing date- 09-2012; expiry date- 09-2015)* administered at 30mg/kg b.w respectively.

Gastric secretion

The gastric acid secretion was measured using the continuous perfusion method of Ghosh and Schild, [34], modified by Amure and Ginsburg, [35] at days 7 and 14 post ulceration after 24 hour fast but with access to clean drinking water alone.

Experimentally induced gastric ulceration

Gastric ulcer was produced by acetic acid via the release of histamine, which increases the capillary permeability and back diffusion of HCl as described by Okabe and Pfeifler [36]. Gastric ulcer were produced according to method of Jainu *et al.*, [37] and Okabe *et al* [38].

Measurement of ulcer index

The degree of ulceration was assessed by carrying out a microscopic examination with 2X magnification hand lens. Scoring of ulcerated area after gastric acid secretion was done by opening the stomach along the greater curvatures. The stomach was bathed in a normal saline and was then carefully spread out, pinned on a cock board for scoring. The ulcerated area was scored by plannimetry, represented in (mm^2), and then calculated according to the collection of the guiding principle of drug administration of ministry of health, Beijing using the equation $S=\pi (d_1/2) \times (d_2/2)$ [39,40].

Statistical analysis

Data obtained were analysed by Graph pad prism statistical package using descriptive statistics, ANOVA and t-test at p=.05 were significant.

Results

Effects of cobalt chloride on gastric acid secretion basal secretion and ph for days 7 and 14

Effect of cobalt chloride on gastric acid secretion:

Results from this study show that at day 7, there was a significant decrease in gastric acid secretion of groups treated with: High cobalt chloride (0.86±0.001), low cobalt chloride (0.85±0.01), control group (0.65±0.02), Misoprostol (0.83±0.01) and Cimetidine (0.96±0.01) compared with ulcer alone (1.22±0.04). A significant decrease in the gastric acid secretion in omeprazole group (0.46±0.003) compared with control group (0.650+01) by day 7 was also observed. The gastric acid secretion was further decreased in the Cobalt chloride and omeprazole treated groups compared with the ulcerated untreated group by day 14 post ulceration (Table 1).

Effects of cobalt chloride on gastric pH

There was a significant decrease in pH of the high cobalt chloride (3.67±0.01), low cobalt chloride

Table 1: Effects of cobalt chloride on gastric acid secretion basal secretion, pH and acidity for days 7 and 14

Group	Basal Secretion		pH	
	Day 7	Day 14	Day 7	Day 14
Control	0.65±0.02	0.53±0.01	3.79±0.01	3.90±0.01
Ulcer untreated	1.23±0.04[a]	1.18±0.01[a]	3.52±0.02	3.54±0.01[a]
High $CoCl_2$	0.86±0.01[ab]	0.85±0.01[a]	3.67±0.01[ab]	3.67±0.01[a]
Low $CoCl_2$	0.85±0.02[ab]	0.77±0.03[ac]	3.68±0.01[ab]	3.72±0.01[abc]
Misoprostol	0.83±0.01[ab]	0.91±0.01[abd]	3.69±0.01[ab]	3.65±0.01[abe]
Cimetidine	0.96±0.01[ab]	0.91±0.01[abd]	3.62±0.01[ab]	3.78±0.01[abce]
Omeprazole	0.46±0.03[abcdef]	0.51±0.01[bcdef]	3.98±0.01[ab]	3.99±0.01[bcdef]

Values are represented as Mean ± SEM and significant at $p<0.05$

Keys for significance: [a] compared with control, [b]- compared with ulcer untreated control, [c]- compared with high $CoCl_2$, [d]- compared with low $CoCl_2$, [e]- compared with Mistoprotol, [f] compared with Cimetidine and [g] - compared with Omeprazole .

Table 2: Effect of cobalt chloride on body and stomach weight, ulcer index and percentage healing.

Group	Day 7				Day 14			
	Animal Weight (G)	Stomach Weight (G)	Ulcer Index	% Healing	Animal Weight (G)	Stomach Weight (G)	Ulcer Index	Percentage Healing
Control	170.8± 1.42	0.94 ± 0.02	0	100	171.8±1.59	0.93±0.07	0	100
Ulcer untreated	162.6 ± 1.75[a]	1.00 ± 0.09	9.78± 0.89	0	167.0±0.95	1.09±0.06	2.39±0.49	0
High $CoCl_2$	162.4 ± 1.69[a]	0.97 ± 0.06	0.96± 0.59	90.18	162.8 ±1.24[a]	0.86±0.05	0	100
Low $CoCl_2$	162.4 ± 1.54[a]	1.00±0.02	0.86± 0.53[b]	91.21	164.2 ±1.16[a]	1.26±0.05	0	100
Misoprostol	166.6± 2.60	0.95±0.05	0[bcdfg]	100	169.2 ±1.02[c]	1.10±0.05	0	100
Cimetidine	159.6 ± 1.33[a]	0.90±0.09	0.47± 0.47[bcd]	95.19	160 ±1.27[abc]	1.08±0.03	0	100
Omeprazole	151.4 ± 0.75[abcdef]	0.97±0.04	0.45±0.45[bcd]	95.39	153.4 ±1.89[abcdef]	1.48±0.07	0	100

Values are represented as Mean ± SEM and significant at $p<0.05$ *Keys for significance:* [a]- *compared with control,* [b]- *compared with ulcer untreated control,* [c]- *compared with high* $CoCl_2$, [d]- *compared with low* $CoCl_2$, [e]- *compared with Mistoprotol,* [f]- *compared with Cimetidine and* [g] - *compared with Omeprazole .*

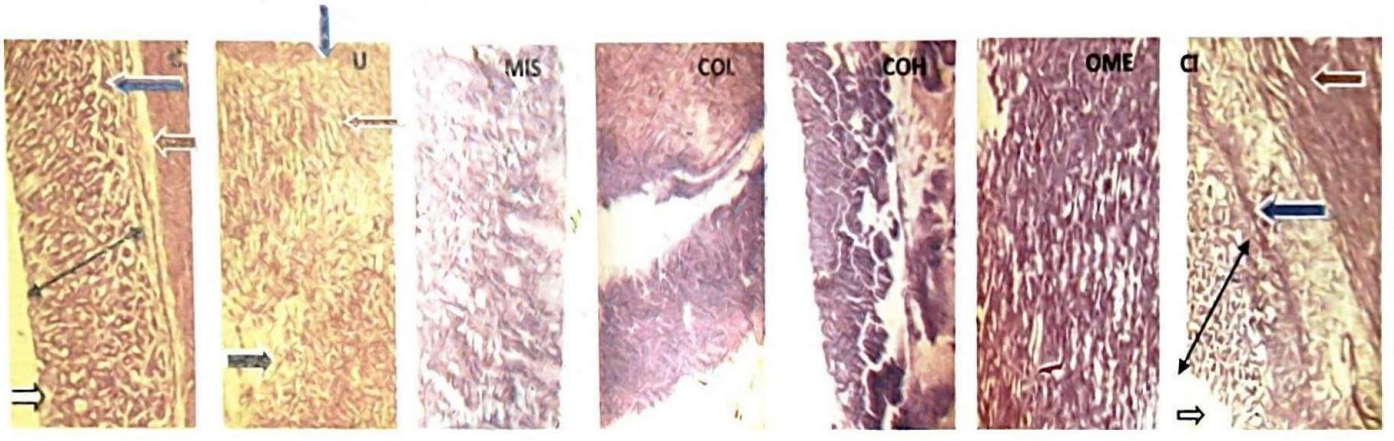

Plate 1: Photomicrograph of a stomach sections (MAG X 100) by day 7 showing **C (Control)**:- normal mucosa surface epithelial layer (white arrow). The mucosa layer (spanned) shows no infiltration of inflammatory cells.the gastric gland and lamina propria appear normal. The parietal cells appear normal (slender arrow). The circular muscle appears normal (red arrow): **U (ulcer untreated alone)**:- mucosa layer with mild ulcer (black arrow), the mucosa layer (spanned) shows severe infiltration of inflammatory cells.the gastric gland and lamina propria shows severe gastritis with severe infiltration (red arrow). The parietal cells appear normal but depleted. The submucosa layer shows moderate infiltration (blue arrow); **MIS (1 mg/kg b.w Mistoprotol)**:- intact surface epithelium, lamina muscularis mucosa, submucosa and muscularis externa. **COL (25 mg/kg b.w Cobalt chloride)**:- intact Lamina muscularis mucosa and submucosal. Fairly intact surface epithelium and increased mucous cells in the lamina propia. There are congested blood vessel in the submucosa. **COH (62 mg/kg b.w Cobalt chloride)**:- Intact surface epithelium, normal glands with mild amounts of resident neutrophils and macrophages at the base. Intact lamina muscularis mucosa, submucosa and muscularis externa. **CI (40 mg/kg b.w Cimetidine)**:- showing moderately preserved mucosa epithelial layer (white arrow), the mucosa layer (spanned) shows mild infiltration of inflammatory cells.the gastric gland and lamina propria shows moderate gastritis with mild infiltration (red arrow). There is no ulcer seen. The submucosa layer shows mild infiltration (blue arrow): **OME (30 mg/kg b.w Omeprazole)**:- widespread moderate erosion of the upper part of surface epithelium (slender black arrow), disrupted glands ( ), intact Lamina muscularis mucosa and widespread accumulation of neutrophils.

(3.68±0.01), misoprostol (3.69±0.01) and cimetidine (3.620±01) compared with the control group (3.79±0.01) day 7 as well as a significant increase in pH of the omeprazole treated group (3.98±0.01) compared with control group (3.79±0.01). A significant decrease in the gastric pH of ulcerated groups treated with high cobalt chloride, low cobalt chloride, misoprostol and cimetidine compared with control group was observed by day 14 (Table 1).

Effects of cobalt chloride on the body and stomach weight, ulcer index and percentage inhibition.Effect of cobalt chloride on body and stomach weight.

There was a significant decrease in the body weight of all the ulcerated treated and untreated groups compared with the control group by days 7 and 14 post ulceration (Table 2).

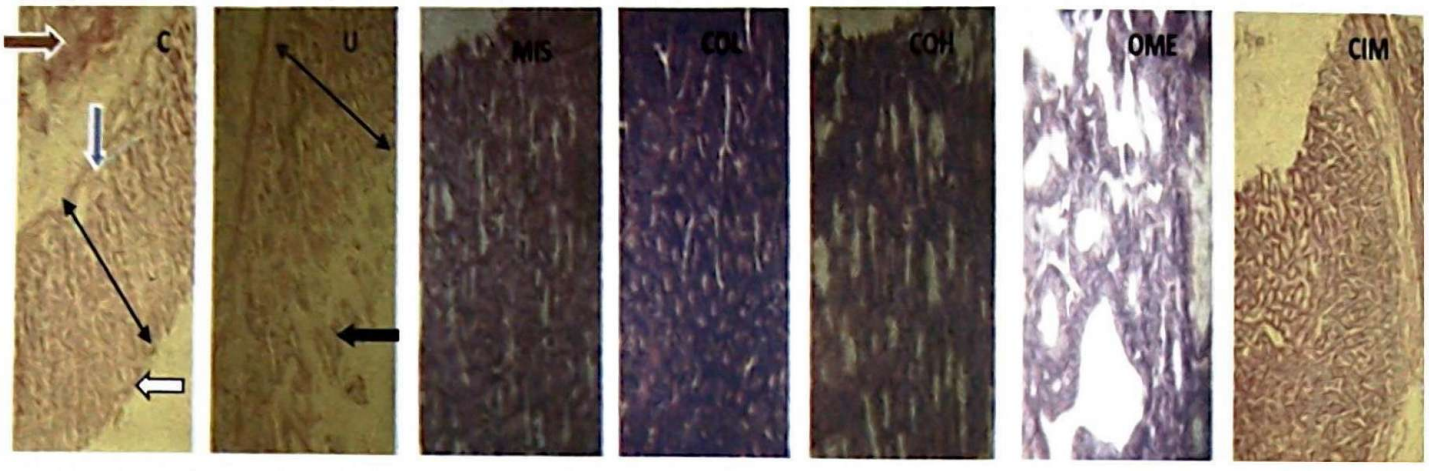

Plate 2: Photomicrograph of a stomach sections (MAG X 100) by day 14 Showing C (Control):- normal mucosa surface epithelial layer (white arrow). The mucosa layer (spanned) shows no infiltration of inflammatory cells.the gastric gland and lamina propria appear normal. The parietal cells appear normal. The circular muscle appears normal (red arrow). **U (Ulcer untreated alone):-** mucosa layer with moderate ulcer (black arrow), the mucosa layer (spanned) shows severe infiltration of inflammatory cells.the gastric gland and lamina propria shows severe gastritis with moderate infiltration (slender arrow).. The parietal cells appear normal but depleted. The submucosa layer shows moderate infiltration (blue arrow). **MIS (1 mg/kg b.w Mistoprotol):-** fairly normal surface epithelium, moderatly congested blood vessel in the lamina propia with moderate amount of neutrophils. The submucosa is expanded with very loose connective tissue. **COL (25 mg/kg b.w Cobalt chloride):-** There were no visible lessions in all the tunics. **COH (62 mg/kg b.w Cobalt chloride):-** there are few foci of eroded lips in the surface epithelium and other tunics are normal. **OME (30 mg/kg b.w Omeprazole):-** Mild atrophy of the muscle wall thickness, minimal inflammation **CI (40 mg/kg b.w Cimetidine):-** moderately preserved mucosa surface epithelium (white arrow) and normal mucosal layer (spanned) showing the gastric gland and lamina propria without infiltration. The eosinophilic parietal cells appear normal. There is no ulcer, no haemorrhage seen. The submucosa layer shows moderate inflammatory cells (blue arrow).

Effect of cobalt chloride on ulcer index

The ulcerated high cobalt chloride (0.96±0.59) and low dose (0.86±0.53) treated groups had a significantly low ulcer index compared with ulcerated untreated group (9.78±0.89) by day 7 post ulceration (Table 2).

All the treatment groups (high dose Cobalt chloride, low dose Cobalt chloride; Misoprostol, Cimetidine and Omeprazole) had no visible ulcer by day 14 post ulcer induction (i.e the ulcers had healed) compared with the ulcerated untreated groups (Table 2).

Discussion and conclusion

Ulcer etiology is linked to a decrease in the level of mucosal cell count or erosion of the gastric mucus layer as a result of imbalance between the aggressive factor (Gastric acid secretion and acidity) and defensive factors (mucous cell count, prostaglandin) [2,41]. The model of acetic acid induction of gastric ulcer [42] is well noted for studying ulcer healing as it has been proven to produce ulcers in close resemblance as those found in humans [43] hence its' use for screening potential anti-ulcer or healing activities of drugs or treatment.

The capacity of the stomach to secrete acid is almost linearly related to parietal cell number/count and its attacking effect on the mucous is inversely related to the mucous cell population [44]. Observations from this study revealed that cobalt chloride decreased gastric acid secretion. The pH of cobalt chloride treated group increased reflecting decreased acidity of gastric secretion in these groups. It might well be that cobalt chloride exerts anti-secretory activities among others in promoting gastric ulcer healing. Certain factors (gastric defense mechanism) have been proven to be beneficial in accelerating and ensuring proper healing of gastric ulceration [45]. These factors: increased blood flow, decreased gastric acid secretion, growth factors, and antioxidant system all have a role in the ulcer prophylaxis or healing. Cobalt chloride helped in ameliorating the adverse effect of acetic acid induced ulceration by accelerating healing in a manner comparable to the control drugs – omeprazole and cimentidine (i.e decreased gastric acid secretion).

Histological evaluations of the varied doses of cobalt chloride administered revealed intact epithelium and increased mucous cells at the *lamina propia* on both days unlike the ulcerated untreated groups. This was similar to findings in omeprazole treated groups. It is however worthy to note that there

was no reformation of ulcer at the lower dose (25 mg/kg b.w) day 21 post ulcer induction) after withdrawal of treatment (cobalt chloride) from day 14 post ulceration. The mucous gel (first layer of defense) is secreted by surface epithelium (second layer of defence) [46] which is formed by water and mucin glycoprotein [47]. This gel helps in acid neutralization, accelerated epithelial repair and maintenance of mucosal blood flow. It may well be that cobalt chloride accelerated the rate of ulcer healing by increasing the level of mucous cells present thereby increasing the production of mucous gel layer which increased gastric acidity (pH) neutralization, enhance epithelial repair and preventing re-occurrence of ulcer after treatment withdrawal. Domschke *et al.*, [47] observed that decreased epithelial cell leads to a decreased mucous production and eventually ulceration. The intact epithelium of the cobalt chloride treated groups may also suggest another probable mechanism by which it (cobalt chloride) helped in mitigating the adverse effect of acid.

It may well be that cobalt chloride enhanced ulcer healing by conferring accelerated repair at the mucous gel and epithelial layer besides acting as an anti-secretory agent. Observations from the gastric tissue histology is also suggestive that the high dose though sub-lethal also conferred some level of gastric protection as observed during ulcer healing in this study. More work needs to be done to ascertain the probably mechanism by which cobalt chloride might be conferring this ulcer healing activities. It is also of interest as a result of the indiscriminate use of blood tonics (of which Cobalt chloride is an integral component) among many populaces – both old and young.

It can be concluded that cobalt has ulcer healing promoting potentials and work is ongoing to unravel its probable mechanism of action.

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