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Antidepressant and Gastro-Protective Effects of Zinc Gluconate on Chronic Restraint Stress-Induced Peptic Ulcer Model in Adult Male Wistar Rats

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Abstract

Peptic ulcers are open sores or lesions that spread throughout the muscularis mucosae of the gastrointestinal mucosa. There is a reciprocal association between depression and peptic ulcer disease, with depressed people having a higher chance of developing the condition. There are several negative side effects associated with current antidepressant and anti-ulcer treatments and because of their adverse effects, including tolerance development, relapse rates, and medication interactions, these medicines are restricted. Therefore, developing more potent options for treating peptic ulcers has become imperative. Hence, this study investigated the antidepressant and gastro-protective effect of Zinc gluconate on chronic restraint stress-induced peptic ulcer model in adult male Wistar rats. The study was carried out using thirty-five (35) adult male Wistar rats (150 - 200 g) and were assigned to seven groups (n=5) and treated daily for a period of twenty-one (21) days. The normal control group (group I) received 1mg/ml of distilled water for 21 days, while, peptic ulcer was induced in the rats in groups II-VII using chronic restraint stress (CRS). Group II were exposed to CRS only without any treatment. However, groups III, IV, V, VI, VII were co-administered orally with 20 mg/kg of fluoxetine, 100 mg/kg of Cimetidine, 50 mg/kg, 100 mg/kg, and 200 mg/kg of Zinc gluconate, respectively for 21 days. Twenty-four hours after treatment was completed, the animals were subjected to neurobehavioral assays [forced swim test (FST), and Y-maze test]. Immediately, the animals were sacrificed and stomach tissues were collected and prepared for peptic ulcer parameters examination. The results showed that 50 mg/kg, 100 mg/kg, and 200 mg/kg of Zinc gluconate significantly ($p < 0.05$) decreased immobility time and increase spontaneous alternation ratio in the FST and Y-maze test respectively when compared to the CRS untreated group. The result also showed that 50 mg/kg, 100 mg/kg, and 200mg/kg of Zinc gluconate significantly ($p < 0.05$) increased gastric pH and gastric wall mucus content, and significantly ($p < 0.05$) decreased gastric volume, total acidity, and pepsin concentration, reduced ulcer index, and inhibited ulceration by 59.46%, 75.67%, and 94.59% respectively. Therefore, it can be concluded that Zinc gluconate significantly showed antidepressant and gastro-protective effect via reduction in immobility time, improved percentage alternation and reduced ulcer index, gastric juice volume, total acidity, pepsin concentration and improved percentage inhibition, pH, gastric wall mucus in CRS-induced peptic ulcer in male Wistar rats.

Keywords: Peptic ulcer, stress, antidepressant, Gastro-protection, Zinc gluconate

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1. Introduction

Peptic ulcers are open sores or lesions that spread throughout the muscularis mucosae of the gastrointestinal mucosa (Njoku *et al.* 2019). They are often characterized by varying stages of necrosis, neutrophil infiltration, decreased blood flow, elevated oxidative stress, and inflammation (Sharifi-rad *et al.*, 2018). Clinically, Peptic Ulcer Disease (PUD) is defined as a disruption of the gastrointestinal mucosal lining that manifests as lesions or sores (Zibima *et al.*, 2020). It is the most common chronic gastrointestinal condition in nature and mostly affects the stomach, esophagus, and initial portion of the small intestine (Otanwa *et al.*, 2015). Peptic ulcers occur when any portion of the gastrointestinal system loses its mucous membrane due to exposure to pepsin or the acidic contents of gastric juice (Otanwa *et al.*, 2015). Peptic ulcer pathophysiology is primarily caused by an imbalance between protective and aggressive factors, such as the mucus-bicarbonate barrier, phospholipids, prostaglandins (PGs), mucosal blood flow, cell renewal and migration, nonenzymatic and enzymatic antioxidants, and a few growth

factors, and aggressive factors like increased secretion of hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species (ROS), prolonged use of non-steroidal anti-inflammatory drug (NSAIDs), alcohol, helicobacter pylori infection, and oxidative stress conditions (Brito *et al.*, 2018; Sreeja *et al.*, 2018).

The majority of patients with peptic ulcers, especially those with gastric ulcers, experience somatic and psychotic symptoms in addition to depression. There seems to be a significant degree of overlap between the neural pathogenic pathways implicated in depression and ulcer development (Abdel-Hamed *et al.*, 2022). Ulcer development is linked to increased susceptibility to anxiety and depression in experimental animals, and this relationship also holds true for humans (Abdel-Hamed *et al.*, 2022). Lee *et al.* (2017) have proposed potential pathogenic processes for the development of PUD as a result of mental health issues. Firstly, through the brain-gut axis, the GI system and brain are closely connected via the autonomic nervous system. Stressful or depressive conditions can cause neurologic dysfunction, which can lead to increased stomach acid and pepsin release as well as mucosal damage. Secondly, through affecting the hypothalamic-pituitary-adrenal axis, psychological problems may alter cortisol secretion (Sandusky-Beltran *et al.*, 2018). Stressful conditions typically result in higher cortisol levels, which can raise the amount of stomach acid secreted (Lee *et al.*, 2017). The GI tract's natural inflammatory response may be hampered by the elevated levels of cortisol and stomach acid (Adefisayo *et al.*, 2018).

Peptic ulcers remain a significant medical challenge, currently affecting around 10% of the global population (Xie *et al.*, 2022). A high prevalence of peptic ulcer disease has been reported in sub-Saharan Africa (Archampong *et al.*, 2016). It continues to be one of the main causes of morbidity and mortality in Nigeria and many other developing nations, and it is mostly prevalent in the elderly and those with low incomes (Zibima *et al.*, 2020). Chronic stress is increasingly being identified as a contributing factor in the development of peptic ulcers (Elsaed *et al.*, 2018). Recent research has shown that chronic stress disrupts the balance between protective and harmful factors in the GI tract (Read *et al.*, 2017). Several studies suggested a relationship between psychological stress and gastrointestinal diseases (Zhang *et al.*, 2012). In line with this idea, depression was reported to increase the risk of peptic ulcer disease (Hsu *et al.*, 2015).
Despite numerous medications available to treat peptic ulcers and depression, these medications can fail to work because the aetiology of the conditions are influenced by a variety of factors (Abdel-Hamed *et al.*, 2022). Unfortunately, there are several negative side effects associated with current anti-ulcer and antidepressant treatments, including toxicities, and prolonged use could alter the body's metabolic processes (Sharath *et al.*, 2015). However, because of their adverse effects, including tolerance development, relapse rates, and medication interactions, these medicines are restricted (Njoku *et al.* 2019). Therefore, developing more potent options for treating patient with both peptic ulcers and depression has become imperative. Certain critical micronutrients and nutrients, like zinc compounds, vitamins, and herbal remedies, are suggested to support the integrity of the gastrointestinal mucosa and may also have an antidepressant impact (Otanwa *et al.*, 2015).

Zinc, an essential trace element, plays a crucial role in maintaining gastrointestinal health, and its deficiency has been linked to increased vulnerability to peptic ulcers (Yazdanpanah *et al.*, 2016). Studies have shown that zinc supplementation can enhance mucosal integrity, reduce oxidative stress, and modulate immune responses, thereby providing a protective effect against gastric ulcers (Prasad, 2013). However, the therapeutic potential of zinc in the context of stress-induced ulcers has not been fully elucidated (Shah *et al.*, 2019). Previous studies have provided conflicting results regarding the effectiveness of zinc supplementation in ameliorating stress-related gastric damage, with some reporting significant benefits while others have shown minimal or no effects (Yazdanpanah *et al.*, 2016). This inconsistency highlights the need for further investigation into the optimal dosage, duration, and possible mechanisms by which zinc exerts its protective effects on the gastric mucosa under chronic stress conditions.

Furthermore, the interaction between stress, zinc deficiency, and ulcerogenesis remains poorly understood (Yazdanpanah *et al.*, 2009). Stress-induced alterations in zinc metabolism, including changes in absorption, transport, and distribution within the gastrointestinal tract, may significantly influence the susceptibility to ulcers (Yazdanpanah *et al.*, 2016). Additionally, the role of zinc in modulating neuroendocrine pathways and inflammatory responses associated with stress-induced gastric injury warrants further exploration (Konturek, *et al*., 2011)**.** Therefore, this study was aimed to investigate the gastro-protective effects of zinc gluconate on CRS-induced peptic ulcers in rats, with a focus on elucidating the potential therapeutic benefits of zinc supplementation.

2. Materials and Methods

Ethical Considerations

The study was approved by Ahmadu Bello University Ethics Committee on Animal Use and Care (ABUCAUC/2024/045).

Drugs and chemicals

All the Drugs and chemicals used for the study were of high analytical grades which include 50mg Zinc Gluconate from Sigma Aldrich, St. Louis, MD, (USA), Fluoxetine and Cimetidine were purchased from puritan's pride Inc. (GlaxoSmithKline, United Kingdom**)** and were widely used as antidepressant and antiulcer medication were both were used in this study as a positive control antidepressant and antiulcer drug respectively, magnifying hand lens, 70% ethanol.

Induction of peptic ulcer using chronic restraint stress (CRS)

Peptic ulcer was induced by restraint stress according to the method of Kumar *et al.* (2009). The rats were confined individually and exposed to stress for a period of 6 h a day

for consecutive 21 days. The wire mesh restrained model has a wooden base and stainless-steel wire mesh restrainer hinged to the base (5 cm of diameter and 12 cm of length) with six compartments. The stress procedure was carried out between 7 am and 1 pm. The major advantage of immobilization is that it produces both inescapable physical and psychological stress**.**

Experimental design

Thirty-five (35) Adult Male Wistar rats weighing between (150- 200g) were obtained from the Department of Human Physiology, Ahmadu Bello University Zaria. They were kept in the animal house of the Department in plastic cages and fed with standard rat feed (Pelletised Growers Feed) chow and clean water *ad libitum*. The animals were assigned randomly into seven groups, consisting of five rats each (n = 5). Group I served as the normal control and received 1ml of distilled water, Group II (CRS untreated) served as the negative control and were exposed to CRS with no treatment, Group III were exposed to CRS and administered orally with 20 mg/kg of fluoxetine, Group IV, were exposed to CRS and administered orally with 100 mg/kg of Cimetidine, Group V, VI, and VII were exposed to CRS and administered orally with 50, 100 and 200 mg/kg of Zinc gluconate respectively for 21 days. The treatment was carried out daily immediately after the rats were freed from the restrainer throughout the period of 21 days. Twenty-four hours (24h) after the last treatment was completed the animals were subjected to neurobehavioral assays (forced swim test and Y maze test).

Forced swim test (FST)

The FST is the most commonly used test to assess depression-like behavior in rodents. This test was conducted according to the method of Porsolt *et al.* (1978), except that the water level was deeper. Each animal was forced to swim in a transparent cylindrical polypropylene plastic tube (50 cm height × 20 cm diameter) containing 30 cm of water at 25± 3 ℃, for 6 minutes without the possibility of escaping. The resulting anxiety produces vigorous swimming activity and attempts at escaping by diving or climbing the walls of the cylinder. After an initial 2 min period of vigorous activity, animals ceased all movements, except those necessary for survival (keeping the head above the water). A rat was considered immobile when it floats in an upright position, and made only small movements to keep its head above water. The duration of immobility was measured as recommended by a blind observer (Carbajal *et al.*, 2009). The changes in the duration of immobility of each group were recorded for the last 4 minutes out of the 6 minutes of the experiment. After swimming each animal was towel-dried, then returned to their home cages and were able to access food and water for the remainder of the day.

Y -maze test

The Y-maze is composed of three equally spaced arms Plexiglas (120^0, arm's length 50cm, with 10cm, and wall height 20cm). the floor of each arm is made of Perspex. The Y- Maze is a quick and useful initial test for general cognitive function. This test is based on innate preference of animals to explore an arm that has not been previously explored (Hughes, 2004). The Y Maze test is sensitive to damage in areas concerned with memory functions such as the hippocampus, and is also disrupted by drugs that cause memory loss (Hughes, 2004).

In this version of the experiment, each rat was placed in the Y Maze for 5 minute and the number of arms entered as well as the sequence of entries is recorded and a score is calculated to determine alternation rate. An alternation is defined as the entry into all three arms consecutively (Hughes, 2004). For instance, if an animal makes the following arm entries: A,C,B,C,A,B,C,A,C,A,B,C,A; the animal has made 13 arm entries 8 of which are correct alternations. The number of maximum spontaneous alternations is then the total number of arms entered minus two and the percentage alternation is calculated as (actual alternation/maximum alternation x 100). A high alternation rate is indicative of sustained spatial working memory as the animals must remember which arm was entered last not to re-enter it (Hughes, 2004).

Sample Collection

The rats were fasted for 18 hours for the collection of gastric juice according to the method of Shay *et al.* (1954). The rats were anaesthetized with intraperitoneal injection of combine dose of 70 mg/kg of ketamine and 5 mg/kg diazepam (Molina *et al.*, 2015). The abdomen of the rats was shaved and a midline incision was made extending 2 cm downwards from the xyphoid. The junction between the pylorus and the duodenum was identified, gently picked up and a pyloric ligature applied using silk thread, and closed by interrupted sutures. Four hours later, the abdomen was opened. The stomach was harvested and washed in physiological saline and dried. An opening was then made along the greater curvature and the gastric juice was collected using test tube and centrifuged at 2000 x g for 10 minutes, the supernatant was collected for the analysis of gastric volume, pH, total acidity, pepsin concentration and gastric wall mucus contents. Also, Gastric mucosal damage was examined by evaluating the degree of ulceration, which was expressed as ulcer index (UI), and percentage inhibition (PI). The stomach tissues were then harvested for oxidative stress analysis.

Determination of Peptic Ulcer Parameters

Determination of gastric mucosal damage

The ulcer index (UI was determined according to the method described by Abubakar *et al.* (2021). The stomach was removed and opened along the greater curvature, rinsed slowly under a running water, and stretched out as much as possible on Whitman's filter paper and mount on a ceiling board. The ulcerated stomach of each animal was measured with a transparent millimetre scale ruler and result for each group were expressed as ulcer index (UI) in millimetre of mean ulcer± SE (standard error). The preventive index percentage (PI), which is the degree of protection offered by a treatment against ulcer-causing agent, was calculated using the formula as described by Hano *et al.* (1976).

P.I (%) = [(MUI untreated control - MUI treated)/MUI untreated control] x 100 ……. equation 1

Where MUI = mean ulcer index

Determination of gastric juice volume and *p*H

The gastric juice collected was centrifuged for 10 min at 2000 rpm and the volume of the supernatant was measured an expressed as ml/4hr. An aliquot of 1 ml of the gastric content was diluted with 1 ml of distilled water and pH of the solution was measured using pH meter (Adwa AD8000) (Dashputre & Naikwade, 2011).

Determination of total acidity

The acidity was measured by the method of Hawk *et al.* (2002) as modified by Abebaw *et al.*, (2017), An aliquot of 1 ml of the gastric content was diluted with 1 ml of distilled water and was taken into a 50 ml conical flask and 2-3 drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH used was noted. The total acidity was expressed as mEq/L and calculated by the following formula:

Acidity = (Volume of NaOH × actual normality of NaOH × 100) /0.1 mEq/L……...equation 2

Determination of pepsin concentration

Pepsin concentration which is the major factor involved in the proteolytic activity of gastric secretion was determined in terms of the amount of protease enzymes produced after incubation of the substrate for 30 minutes with pepsin. It was determined by the spectrophotometric method devised by Hawk *et al.* (2002) at 280nm wavelength and was expressed as mg/L.

Determination of gastric wall tissue mucus

The barrier mucus of gastric tissue was estimated by the method of Corne *et al.*, (1974). The dissected stomach was soaked for 2 h in 0.1% Alcian blue. Dye complexed with mucus was diluted by immersion in 10 ml aliquots of 0.5 M $MgCl_2$ for 2 h. The resulting blue solution was shaken with equal volume of diethyl ether and optical density of aqueous phase was measured at 605 nm. The mucus content was expressed in terms of µg of Alcian blue/g of glandular tissue.

Data Analysis

The results were presented as mean + standard error of mean (SEM) and were analyzed using one-way Analysis of Variance (ANOVA). *Tukey's post-hoc* test was used to compare the level of significance between the groups, $p < 0.05$ was considered statistically significant. All analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) package program.

3. Results

The effect of Zinc gluconate on immobility time in chronic restraint stress-induced peptic ulcer in male Wistar rats is presented in Figure 1. The result showed that there was a significant increase ($p < 0.05$) in immobility time in the CRS untreated group (106.00 ± 0.89 sec), CRS + 20mg/kg Fluoxetine group (41.60 ± 0.93 sec); CRS + 100mg/kg Cimetidine group (65.00 ± 1.39 sec), CRS + Zinc gluconate groups 50 mg/kg (55.20 ± 1.28); and 100 mg/kg (39.40 ± 0.87) and a significant ($p < 0.05$) decrease in CRS + Zinc gluconate group 200 mg/kg (25.40 ± 1.03 sec) when compared to the normal control group (32.40 ± 1.03 sec). While, a significant decrease ($p < 0.05$) was observed in the immobility time of CRS + 20mg/kg Fluoxetine group (41.60 ± 0.93 sec); CRS + 100mg/kg Cimetidine group (65.00 ± 1.39 sec), CRS + Zinc gluconate groups 50 mg/kg (55.20 ± 1.28); 100 mg/kg (39.40 ± 0.87); and 200 mg/kg (25.40 ± 1.03 sec) when compared to CRS untreated group (106.00 ± 0.89 sec). The decrease in immobility time in the CRS + Zinc gluconate-treated groups was dose-dependent when compared to CRS untreated. Moreover, 200 mg/kg of Zinc gluconate (25.40 ± 1.03 sec) caused a significant decrease ($p < 0.05$) in immobility time of CRS rats when compared to all the group.

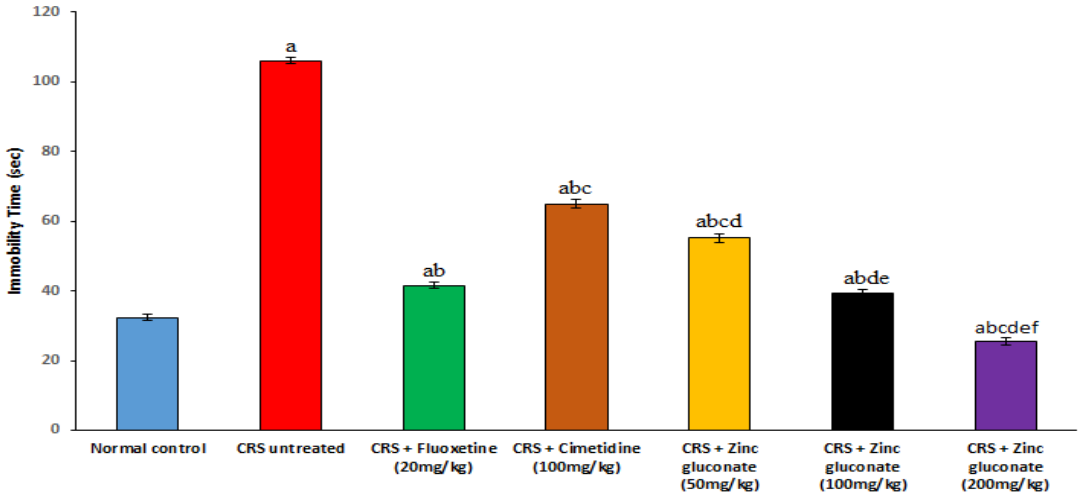

Figure 1: Effect of Zinc gluconate on immobility time of chronic restraint stress-induced peptic ulcer in male Wistar rats. n = 5; data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. Values with superscripts $^{a, b, c, d, e,}$ and ^{f} are significantly different ($p < 0.05$) when compared to normal control, CRS untreated, 20mg/kg Fluoxetine, 100mg/kg Cimetidine, 50 and 100 mg/kg of zinc gluconate respectively. CRS: Chronic restraint stress.

The effect of Zinc gluconate on total entries into all arms and spontaneous alternation in chronic restraint stress-induced peptic ulcer in male Wistar rats is presented in Table 1. The result revealed that there was a significant increase ($p < 0.05$)

in the total entry into the arms in CRS + 200mg/kg Zinc gluconate group (15.40 ± 0.40) when compared to CRS + 20mg/kg Fluoxetine treated group (12.60 ± 0.24), CRS +100mg/kg Cimetidine treated group (12.80 ± 0.37) and CRS + 50mg/kg Zinc gluconate (12.80 ± 0.58). For the spontaneous alternation ratio, there was a significant increase ($p < 0.05$) in the percentage alternation of the normal control group (77.40 ± 1.12%), CRS +20mg/kg Fluoxetine group (70.00 ± 1.64%); CRS + 100mg/kg Cimetidine group (53.40 ± 1.54%), CRS + Zinc gluconate groups 50 mg/kg (50.00 ± 1.27%); 100 mg/kg (72.00 ± 1.67%); and 200 mg/kg (83.60 ± 1.17%)] when compared to the CRS untreated group (13.20 ± 1.39%).

Table 1: The effect of Zinc gluconate on total entries into all arms and Spontaneous alternation (%) in chronic restraint stress-induced peptic ulcer in male Wistar rats

Groups	Total entries into all arms	Spontaneous alternation (%)
Normal control	13.40 ± 0.60	77.40 ± 1.12
CRS untreated	13.80 ± 0.80	13.20 ± 1.39[a]
CRS + Fluo (20 mg/kg)	12.60 ± 0.24	70.00 ± 1.64[ab]
CRS + Cim (100 mg/kg)	12.80 ± 0.37	53.40 ± 1.54[abc]
CRS + ZG (50 mg/kg)	12.80 ± 0.58	50.00 ± 1.27 [abc]
CRS + ZG (100 mg/kg)	13.40 ± 0.51	72.00 ± 1.67 [bde]
CRS + ZG (200 mg/kg)	15.40 ± 0.40[cde]	83.60 ± 1.17 [bcdef]

n = 5; data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. Values with superscripts [a, b, c, d, e,] and [f] are significantly different ($p < 0.05$) when compared to normal control, CRS untreated, 20mg/kg Fluoxetine, 100mg/kg Cimetidine, 50 and 100 mg/kg of zinc gluconate respectively. CRS: Chronic restraint stress, ZG: Zinc gluconate, Fluo: Fluoxetine, Cim: Cimetidine.

The effect of Zinc gluconate on gastric mucosal damage in chronic restraint stress-induced peptic ulcer in male Wistar rats was presented in Table 2. For ulcer index, there was a significant increase ($p < 0.05$) in ulcer index in the CRS untreated group (7.40 ± 0.87), CRS + 20mg/kg Fluoxetine group (4.40 ± 0. 60) and CRS + Zinc gluconate groups 50 mg/kg (2.00 ± 0.32) when compared to the normal control group (0 ± 0.0). while, a significant decrease ($p < 0.05$) was observed in the ulcer index of CRS + 20mg/kg Fluoxetine group (4.40 ± 0. 60); CRS +100mg/kg Cimetidine group (0.80 ± 0.37), CRS + Zinc gluconate groups 50 mg/kg (2.00 ± 0.32); 100 mg/kg (1.00 ± 0.32); and 200 mg/kg (0.40 ± 0.26) when compared to the CRS untreated group (7.40 ± 0.87). Furthermore, with regards to percentage inhibition, the CRS + 20mg/kg Fluoxetine group, CRS + 100mg/kg Cimetidine group, CRS + Zinc gluconate groups (50 mg/kg, 100 mg/kg, and 200 mg/kg) had a percentage inhibition of 40.54%, 89.19% 59.46%, 75.67%, and 94.59% respectively, and percentage ulceration of 59.46%, 10.81% 40.54%, 24.33%, and 5.41% respectively.

Table 2: The effect of Zinc gluconate on ulcer indices on chronic restraint stress-induced peptic ulcer in male Wistar rats.

Groups	Ulcer index	Percentage inhibition	Percentage ulceration
Normal control	0 ± 0.0	100	-
CRS untreated	7.40 ± 0.87 [a]	-	100
CRS + Fluoxetine (20 mg/kg)	4.40 ± 0.60 [ab]	40.54	59.46
CRS + Cimetidine (100 mg/kg)	0.80 ± 0.37 [bc]	89.19	10.81
CRS + Zinc gluconate (50 mg/kg)	3.00 ± 0.84 [ab]	59.46	40.54
CRS + Zinc gluconate (100 mg/kg)	1.80 ± 0.76 [b]	75.67	24.33
CRS + Zinc gluconate (200 mg/kg)	0.40 ± 0.26 [bc]	94.59	5.41

n = 5; data analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. Values with superscripts [a, b, c, d, e,] and [f] are significantly different ($p < 0.05$) when compared to normal control, CRS untreated, 20mg/kg Fluoxetine, 100mg/kg Cimetidine, 50 and 100 mg/kg of zinc gluconate respectively. CRS: Chronic restraint stress

The effect of Zinc gluconate on gastric secretions parameters in chronic restraint stress-induced peptic ulcer in male Wistar rats was presented in Table 3. For the gastric pH, there was a significant decrease ($p < 0.05$) in pH in the CRS untreated group (2.40 ± 0.07), CRS + 20mg/kg Fluoxetine group (3.66 ± 0.09) and CRS + Zinc gluconate groups 50 mg/kg (4.34 ± 0.11) and 100 mg/kg (4.62 ± 0.12) when compared to the normal control group (5.24 ± 0.02). While, there was a significant increase ($p < 0.05$) in the pH of CRS + 20mg/kg Fluoxetine group (3.66 ± 0.09); CRS + 100mg/kg Cimetidine group (4.92 ± 0.19), CRS + Zinc gluconate groups 50 mg/kg (4.34 ± 0.11); 100 mg/kg (4.62 ± 0.12) and 200 mg/kg: (5.10 ± 0.15) when compared to the CRS untreated group (2.40 ± 0.07).

With regards to gastric volume, there was a significant increase ($p < 0.05$) in gastric volume in the CRS untreated group (1.76 ± 0.07 ml) when compared to the normal control group (0.76 ± 0.04 ml). Meanwhile, a significant decrease ($p < 0.05$) was observed in the gastric volume for CRS + 20mg/kg Fluoxetine group (0.92 ± 0.05 ml); CRS +100mg/kg Cimetidine group (0.76 ± 0.07 ml), CRS + Zinc gluconate groups 50 mg/kg (0.92 ± 0.08 ml); 100 mg/kg (0.72 ± 0.05 ml); and 200 mg/kg (0.64 ± 0.04 ml) when compared to the CRS untreated group (1.76 ± 0.07 ml). For total acidity, there was a significant increase ($p < 0.05$) in titratable acidity in the CRS untreated group (86 ± 2.45 mEq/L) and CRS + 20mg/kg Fluoxetine group (70 ± 3.16 mEq/L) when compared to the normal control group (50 ± 3.16 mEq/L). However, a significant decrease ($p < 0.05$) was observed in the acidity for CRS + 20mg/kg Fluoxetine group (70 ± 3.16 mEq/L); CRS + 100mg/kg Cimetidine group (44 ± 2.45 mEq/L), CRS + Zinc gluconate groups 50 mg/kg (60 ± 3.16 mEq/L); 100 mg/kg (52 ± 3.74 mEq/L); and 200

mg/kg (44 ± 2.45 mEq/L)]; when compared to the CRS untreated group (86 ± 2.45 mEq/L).

Regarding pepsin concentration, there was, a significant decrease ($p < 0.05$) for normal control group (4.20 ± 0.23 mg/l), CRS + 20mg/kg Fluoxetine group (4.12 ± 0.12 mg/l); CRS + 100mg/kg Cimetidine group (4.16 ± 0.15 mg/l), CRS + Zinc gluconate groups 50 mg/kg (4.42 ± 0.18 mg/l); 100 mg/kg (4.18 ± 0.19 mg/l); and 200 mg/kg: (3.80 ± 0.16 mg/l) when compared to the CRS untreated group (5.54 ± 0.16 mg/l). When the gastric wall mucus content was compared, there was a significant decrease ($p < 0.05$) in mucus content in the CRS untreated group (7.92 ± 0.25 µg/g), CRS + 20mg/kg Fluoxetine group (10.66 ± 0.09 µg/g) with a significant increase in the CRS + 100mg/kg Cimetidine group (24.78 ± 0.58 µg/g), CRS + Zinc gluconate groups 50 mg/kg (22.82 ± 0.97 µg/g); 100 mg/kg (23.60 ± 0.86 µg/g); and 200 mg/kg (26.04 ± 0.24 µg/g)] when compared to the normal control group (16.24 ± 0.56 µg/g). Meanwhile, a significant increase ($p < 0.05$) was observed in mucus content for CRS + 20mg/kg Fluoxetine group (10.66 ± 0.09 µg/g); CRS + 100mg/kg Cimetidine group (24.78 ± 0.58 µg/g), CRS + Zinc gluconate groups 50 mg/kg (22.82 ± 0.97 µg/g); 100 mg/kg (23.60 ± 0.86 µg/g); and 200 mg/kg (26.04 ± 0.24 µg/g)]; when compared to the CRS untreated group (7.92 ± 0.25 µg/g).

Table 3: The effect of Zinc gluconate on gastric secretions parameters in chronic restraint stress-induced peptic ulcer in male Wistar rats

Groups	*p*H	Gastric juice volume (ml/4h)	Total acidity (mEq/L)	Pepsin concentration (mg/l)	Gastric wall Mucus content (µg/g)
Normal control	5.24 ± 0.02	0.76 ± 0.04	50 ± 3.16	4.20 ± 0.23	16.24 ± 0.56
CRS untreated	2.40 ± 0.07 ^{a}	1.76 ± 0.07^{ab}	86 ± 2.45^{a}	5.54 ± 0.16 ^{a}	7.92 ± 0.25 ^{a}
CRS + Flu (20 mg/kg)	3.66 ± 0.09 ^{ab}	0.92 ± 0.05^{b}	70 ± 3.16^{ab}	4.12 ± 0.12 ^{b}	10.66 ± 0.09 ^{ab}
CRS+ Cim (100 mg/kg)	4.92 ± 0.19 ^{bc}	0.76 ± 0.07^{bc}	44 ± 2.45^{bc}	4.16 ± 0.15 ^{b}	24.78 ± 0.58 ^{abc}
CRS + ZG (50 mg/kg)	4.34 ± 0.11 ^{abcd}	0.92 ± 0.08^{bd}	60 ± 3.16^{bd}	4.42 ± 0.18 ^{b}	22.82 ± 0.97 ^{abc}
CRS + ZG (100 mg/kg)	4.62 ± 0.12 ^{abc}	0.72 ± 0.05^{bc}	52 ± 3.74^{bc}	4.18 ± 0.19 ^{b}	23.60 ± 0.86^{abc}
CRS + ZG (200 mg/kg)	5.10 ± 0.15 ^{bce}	0.64 ± 0.04^{bce}	44 ± 2.45^{bce}	3.80 ± 0.16 ^{b}	26.04 ± 0.24 ^{abce}

n = 5; data analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. Values with superscripts $^{a, b, c, d, e,}$ and ^{f} are significantly different ($p < 0.05$) when compared to normal control, CRS untreated, 20mg/kg Fluoxetine, 100mg/kg Cimetidine, 50 and 100 mg/kg of zinc gluconate respectively. CRS: Chronic restraint stress, ZG: Zinc gluconate, Fluo: Fluoxetine, Cim: Cimetidine

4. Discussion

Chronic stress has been reported to induced peptic ulcer which is an acid-peptic disease characterized by the rupture of the protective barrier of the epithelial mucosa lining of the GI Tract (Kuna *et al.*, 2019). A growing body of research has shown that extreme stress raises the risk of depression and peptic ulcer (Lee *et al.*, 2017). This study was aimed to investigate both the antidepressant and gastro-protective effects of zinc gluconate on CRS-induced peptic ulcers in rats.

The current study findings demonstrated that prolonged immobility time in rats exposed to chronic restraint stress (CRS) caused depression, as assessed by the Forced Swim Test. When compared to the CRS untreated group, rats treated with zinc gluconate (ZG) at all dosages had displayed an antidepressant-like effect by reducing the duration of immobility during the Forced Swim Test. In the groups treated with zinc gluconate, the reduction in immobility time was dose-dependent. In addition, CRS rat's immobility period was reduced by 200 mg/kg of zinc gluconate in comparison to all other groups, including the fluoxetine-treated and this indicate the reduced behavioural despair of the rats (a depressive symptom). The current results are consistent with a prior study by Cavalcanti *et al.* (2020), which found that zinc supplementation had an antidepressant effect on diabetic rats in the Forced Swim Test. In patients with depressed symptoms, zinc was also found to considerably lower depression scores in human subject (Yosaee *et al.*, 2020).

A dependable, non-invasive method for assessing cognitive changes in Wistar rats and a gauge of exploratory behavior that represents spatial working memory reliant on hippocampus function is the spontaneous alternation ratio in the Y-maze test (Elhallouty *et al.*, 2022). The results of the current investigation demonstrated that, experimental rats exposed to only CRS had a much lower percentage alternation. The reduced alternation percentage in this study, suggested an impairment with the spatial working memory which is in line with previous study by Akefe *et al.*, (2020). The present study also showed that rats exposed to CRS had less short-term memory impairment after receiving treatments with 50, 100, and 200 mg/kg of zinc gluconate. Stressed rats' short-term memory impairment was effectively reversed by zinc gluconate and fluoxetine (a well-known antidepressant drug). Zinc gluconate at 200 mg/kg, however, outperformed the fluoxetine-treated group indicating that zinc may have a greater impact on cognitive function in the CRS model. Alongside this study, Onaolapo *et al.* (2020) found that dietary zinc supplementation protected rats from methotrexate-induced decreases in spatial working memory

using Y-maze and radial-arm tests, and that it also mitigated against ketamine-induced reductions in working memory. This study findings also support a prior study in which zinc taken orally reduced the decline in working memory in mice by Onaolapo *et al.,* (2017), confirming that zinc can reduced working/short-term memory impairment.

Zinc gluconate, which is a potent antioxidant, exert mitigating effects on impairment in the learning and memory of rats (Onaolapo *et al.,* 2023). The improvements in the spontaneous alternation ratio suggest that zinc gluconate may have neuroprotective and memory-enhancing effects, possibly by mitigating oxidative stress and inflammation that may occur under chronic stress conditions (Onaolapo *et al.*, 2020). Moreover, regulation of working memory occurs via a number of neurotransmitters including acetylcholine, gamma-aminobutyric acid (GABA) and glutamate, whose projections are found in brain regions such as the hippocampus (Onaolapo *et al.,* 2020). Activation of GABAergic impulses impairs spontaneous alternation working memory, while enhanced cholinergic or glutamatergic transmission leads to improvement in working memory (Khakpai *et al.,* 2012). Also, previous studies have confirmed the ability of Zinc to modulate the functions of neuronal acetylcholine receptors and improve cholinergic transmission (Vázquez-Gómez & García-Colunga, 2009). Therefore, Zinc-mediated enhancement of cholinergic transmission may be responsible for the improvement in working/short-term memory in this study.

According to previous researches, the possible mechanism by which zinc shows it antidepressant impact may be via attenuating the glutamatergic system through the suppression of N-methyl-D-aspartate (NMDA) receptor activity (Szewczyk *et al.,* 2011; Mlyniec *et al.,* 2012). Through a complex process that involves pre- and postsynaptic 5-HT1ARs, zinc can also modulate the serotonergic system to produce antidepressant and anxiolytic effects (Satała *et al.,* 2016). Additionally, Solati *et al.* (2015) found that zinc supplementation raised serum levels of brain-derived neurotrophic factor (BDNF), which has been shown to be down-regulated in depressed patients compared to non-depressed matched controls. However, it was shown to increase after zinc supplementation. This suggests that BDNF expression may be another explanation for the antidepressant effect of zinc. Also, the hypothalamus pituitary adrenal (HPA) axis may be modulated, among other potential mechanisms, to mediate zinc's effects on depression (Dallman *et al.*, 2006). According to Swardfager *et al.* (2013) and Al-Dujaili *et al.* (2016), zinc is an anti-inflammatory element that aids in preserving endocrine homeostasis and controlling the cortical and hippocampus glutamatergic circuits, which support affective regulation and cognitive performance.

Lee *et al.,* (20217), reported that Stress or depression can cause neurologic dysfunction, which can lead to increased stomach acid and pepsin release as well as mucosal damage leading to peptic ulcer which is an acid-peptic disease characterized by the rupture of the protective barrier of the epithelial mucosa lining of the GI Tract. By overpowering the gastric mucosa's defenses, stress-induced alterations in the gastrointestinal tract contribute to the pathophysiology of peptic ulcers (Zhang *et al.,* 2012). Stress may alter the equilibrium between defensive and aggressive elements in the stomach environment, which leads to mucosal damage ((Serafim *et al.,* 2019).

According to this study, CRS caused severe stomach tissue damage, which showed up with increased ulcer index and high percentage ulceration ultimately leading to peptic ulcers. In contrast to the CRS untreated group, the zinc gluconate-treated groups showed a substantial decrease in ulcer index along with improved preventive index percentage. It can be reported that significant protection is offered by zinc gluconate, particularly at higher dosages (100 and 200 mg/kg), with the highest dose providing almost total suppression of ulcer formation. Cimetidine also lessen ulcer index, but not as much as zinc gluconate at 200mg/kg. This study is similar to that of Otanwa *et al.* (2015), found that zinc improved stomach mucus and ethanol-induced lesions based on the percentage index determined in ulcerogenic rats. Zinc's antioxidant, capacity to maintain cell membranes, and impact on inflammatory pathways may all contribute to its protective effects on the stomach mucosa (Zhang *et al.*, 2012). The high degree of ulcer protection observed with zinc gluconate at 200 mg/kg suggests that zinc may be an effective and potent agent for preventing stress-induced gastric damage.

Additionally, in terms of gastric secretion, CRS impaired gastric secretion by increasing gastric volume, total acidity, and pepsin concentration while decreasing gastric pH and gastric wall mucus. The groups treated with zinc gluconate exhibit the greatest improvements in all measured parameters (pH, gastric volume, acidity, and gastric wall mucus) against these changes, particularly at higher dosages (100 and 200 mg/kg). This implies that zinc gluconate has a protective effect that is multidimensional and includes mucosal protection and acid neutralization (Otanwa *et al.*, 2015). Cimetidine, also reduces gastric acidity and improves mucosal protection but does not enhance gastric wall mucus to the same extent as zinc gluconate at 200mg/kg as seen in this study. Fluoxetine has a moderate effect on pH, volume, and gastric wall mucus, but it does not fully restore these parameters to normal levels, indicating that its efficacy in protecting the gastric mucosa is limited compared to zinc gluconate or cimetidine. This study is similar to the result of Abubakar *et al.* (2021) who reported that zinc showed a decrease in the volume of gastric juice, titratable acidity, and acid output in ethanol-induced gastric mucosal damage in wistar rats. Zinc gluconate, particularly at higher doses (100 mg/kg and 200 mg/kg), markedly boosted the gastric wall mucus. The protective mucosal barrier may be strengthened by this rise in mucus, reducing its susceptibility to stress-induced gastric injury.

The gastroprotective effects shown by zinc gluconate are possible due to its influence on neurotransmitter systems, such as serotonin and dopamine, and its function in lessening the negative effects of stress on the gut-brain axis (Abubakar *et al.* 2021) or by lowering the activation of the

hypothalamic-pituitary-adrenal (HPA) axis, which is frequently heightened under stressful or depressed circumstances (Zhang *et al.* 2012). Zinc-rich diets may implicitly protect against ulcerogenesis. Red meat, organ meats, and oysters are examples of such foods. Some natural zinc sources include bran, grains, and fodder yeast; other foods include liver, beans, nuts, and shellfish like crab and lobster; whole grains, cereals, almonds, pumpkin seeds, and sunflower seeds (Strnadova *et al.*, 2011). The foods might be gastro-protective, which would lessen the negative consequences of ulcers in people.

5. Conclusion

It can be concluded that Zinc gluconate significantly showed antidepressant-like properties by reducing behavioural despair (a depressive symptom) and improving short-term memory functions in CRS-induced peptic ulcer in adult male Wistar rats. Zinc gluconate also showed gastro-protective effect via reduction in ulcer index, and improved preventive index percentage. Hence, zinc gluconate (100mg/kg and 200mg/kg) is a promising natural treatment for gastric protection and should be considered for stress-related gastric disorders**.** Furthermore, consumption of food rich in zinc should be encouraged to help improve the management of peptic ulcer.

Conflicts of Interest

Authors declare that there is no conflict of interest

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