

Effects of Selenium Treatment on Healing of Acetic Acid Induced Gastric Ulcer in Albino Wistar Rats

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Abstract The effects of selenium on healing of acetic acid induced gastric ulcer investigated in albino Wistar rats and the results were compared with that of a standard drug omeprazole. Animals were anesthetized with 50mg/kg sodium thiopental, laparotomy was performed and gastric ulcer was induced by application of 80% acetic acid to the serosal surface of stomach. Animals were then divided into three groups. Group 1 was treated with 1 ml/kg/day normal saline (NS) (p.o), group 2; 100µg/kg/day selenium (p.o) and group 3; 20mg/kg/day omeprazole (p.o). Treatment period was 10 days post ulcer induction. Assessment of ulcer healing was done on days 3, 7 and 10 respectively by measurement of ulcer area, lipid peroxidation, catalase activity and mucus secretion. Result showed that by day 7 and 10, the reduction in ulcer area was more significant in selenium and omeprazole treated ($p < 0.05$, 0.05) respectively. By day 7 and 10, lipid peroxidation was significantly lower in selenium and omeprazole treated as compared with NS treated ($p < 0.05$, 0.05). Furthermore by day 3, 7 and 10, catalase activity was significantly higher ($p < 0.05$) in selenium treated as compared with NS treated. Result also showed that mucus secretion was significantly higher in selenium and omeprazole treated ($p < 0.001$, 0.001) compared with NS by day 7. However, by day 10, secretion in both selenium and omeprazole treated had started to decline. In conclusion, selenium accelerated ulcer healing by facilitating mucosal regeneration, reducing lipid peroxidation, increasing antioxidant activity and by altering mucus secretion response.

Keywords: selenium, gastric ulcer, healing, rats

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1. Introduction

Gastric ulcer is a deep defect in the gastric (stomach) wall penetrating the entire mucosal thickness and the muscularis mucosa [1]. It is the most prevalent gastrointestinal disorder ever known, accounting for an estimated 15 mortality out of every 15,000 complications yearly in the world [2,3]. The prevalence of gastric ulcer is 2.4% in the Western population and in certain regions of Mainland China, the prevalence is as high as 6.07% in the general population [4,5,6]. Furthermore, the recurrence rate of ulcer is as high as 60% [7]. Current therapeutic regimens largely rely on Western medicine, however, their side effects are often inevitable [8,9]. Numerous studies have also demonstrated that herbal medicines can effectively treat gastric ulcer in human [10,11].

The trace mineral, selenium (Se), is an essential nutrient of fundamental importance to human biology. This has become increasingly obvious in recent years as new research has revealed a hitherto unsuspected role for this element and it has been reported to possess many medicinal effects. Selenium is needed for the proper functioning of the immune system [12,13], reduction of cancer mortality, and preventing heart disease [14].

Selenium is an important component of antioxidant enzymes, such as glutathione peroxidase, thioredoxin reductase and iodothyronine deiodinases and several selenoproteins or selenoenzymes [15,16]. These play a significant role in protecting cells against oxidative damage from reactive oxygen species (ROS) and reactive nitrogen species [17,18].

Previous studies revealed that selenium accelerated wound healing in diabetic conditions [19]. Furthermore, selenium had been reported to be gastroprotective against gastric mucosal damage induced by water immersion restraint stress in Wistar rats [20]. Kim et al. [16] also reported that selenium has curative effect on gastric ulcer, however the model of ulcer used in their study was indomethacin induced and their period of study was 3 days. Thus their study was more of gastroprotective effect of selenium and not healing. Gastric ulcer in animal and man is a chronic disease, which is characterized by repeated episodes of healing and re-exacerbation, a phenomenon which is problematic to both patient and clinician. Most experimental ulcerative lesions heal quickly in a few days without scar formation and do not re-ulcerate spontaneously. However, Takagi et al. [21] developed a model for inducing chronic gastric ulcer in rats by means of submucosal injection of acetic acid and reported on the healing process of lesions for extended

intervals after the ulcer induction. The experimental gastric ulcer was termed chronic because it persisted for a long time and resembled human chronic ulcer both grossly and histologically. Since its development in 1969, modifications were made to acetic acid induced ulcer. Therefore, the aim of this study was to investigate the effects of selenium on healing of acetic acid induced gastric ulcer in rats, considering its effects on ulcer area, oxidative stress, antioxidant status and mucus secretion.

2. Materials and Methods

2.1. Animals

Male albino Wistar rats weighing between 150-200g were used for the study. They were kept in the Animal House, College of Health Sciences, Kogi State University, Anyigba. They were housed under standard conditions of temperature ($23 \pm 2^\circ\text{C}$); humidity ($55 \pm 15\%$) and 12 h light (7.00 am 7.00 pm). They were kept in wire meshed cages and fed with standard commercial rat pellets. The care and use of the animals and the experimental protocol of this study were in accordance with Experimental Animal Care and Use Regulation of Kogi State University, Nigeria, which were also in accordance with the internationally accepted principles for laboratory animal use (EEC Directive of 1986; 86/609/EEC).

2.2. Ulcer Induction

Gastric ulcer was induced in the stomach of all rats by the method of Wang et al. with slight modifications [22]. Rats were fasted for 18 hours; they were then anesthetized using 50mg/kg sodium thiopental. Laparotomy was performed and stomach was exposed. Acetic acid (0.5 ml, 80% vol/vol) was applied to the serosal surface of glandular portion of the stomach for 60 seconds, through a 2ml syringe barrel that has been cut and smoothed. The syringe containing the acid was removed from the stomach and the area of stomach that had contact with acid was cleaned with cotton wool soaked in normal saline. The abdomen was sutured. Thereafter, the animals were returned to standard diet of laboratory chow and tap water.

2.3. Animal Grouping and Treatment

Animals were randomly divided into 3 groups and treatment commenced 24 hours after ulcer induction. The grouping and treatment are as shown in Table 1. The total period of treatment was 10 days post ulcer induction, however, measurement of ulcer area and other parameters related to ulcer healing were done on day 3, 7 and 10 using five animals for each investigation from the different groups.

Table 1. Animal grouping and treatment

S/NO	Groups	Treatment
1	Normal saline	Rats with ulcer and treated with 1ml/kg normal saline (p.o) per day
2	Selenium	Rats with ulcer and treated with 100µg/kg /day (p.o)
3	Omeprazole	Rats with ulcer and treated with 20mg/kg /day (p.o)

20 rats per group.

2.4. Measurement of Ulcer Dimension

Ulcer areas in animals were measured on days 3, 7 and 10 after ulcer induction. Five animals were randomly picked from each group, they were killed by cervical dislocation and the stomach was removed, opened along greater curvature, rinsed with normal saline and pinned on a wax block. Transparent paper was placed over ulcer area and the ulcer area was traced out. The area of ulceration was converted to units of square millimeters using 1mm by 1mm paper grid. The percentage area of ulcer healed was calculated as:

Percentage of area healed on day 7

$$= \frac{\text{Area of ulcer on day 3} - \text{area of ulcer on day 7}}{\text{Area of ulcer on day 3}} \times 100$$

Percentage of area healed on day 10

$$= \frac{\text{Area of ulcer on day 3} - \text{area of ulcer on day 10}}{\text{Area of ulcer on day 3}} \times 100.$$

2.5. Assessment of Lipid Peroxidation

Assessment of lipid peroxidation was carried out following the procedure described by Varshney and Kale [23]. It is based on the reaction of malondialdehyde (MDA) produced during lipid peroxidation with thiobarbituric acid (TBA) forming a pink coloured MDA-TBA adduct that absorbs strongly at 532nm.

2.6. Determination of Catalase Activity

Activity of catalase in gastric mucosa was determined according to the procedure of Sinha [24]. This method is based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of H_2O_2 , with the formation of perchromic acid as an unstable intermediate. The chromic acetate so produced is measured calorimetrically at 530 nm.

2.7. Measurement of Mucus Secretion

Adherent gastric glandular mucous was measured by the method of Corne et al. [25]. The excised stomachs were soaked for 2 hours in 0.1% Alcian blue dissolved in buffer solution containing 0.1 M sucrose and 0.05 M sodium acetate (pH adjusted to 5.8 with hydrochloric acid). After washing the stomach twice in 0.25 M sucrose (15 and 45 min), the dye complexed with mucous was eluted by immersion in 10 mL aliquots of 0.5 M MgCl_2 for 2 hours. The resulting blue solution was shaken with equal volumes of diethyl ether and the optical density of the aqueous phase measured at 605 nm using a spectrophotometer.

Using a standard curve, the absorbance of each solution was then used to calculate the various concentration of the dye and the weight of dye (expressed in mg). The weight of the dye was then expressed over the weight of the stomach.

2.8. Statistical Analysis

Results were expressed as mean \pm SEM. Student t-test was used to assess the statistical difference of results obtained between the two groups. Confidence interval of

95% was taken as statistically significant using SPSS version 17 statistical package.

3. Results

3.1. Measurement of Ulcer Dimension

On day 3 after ulcer induction, there was no significant difference ($p > 0.05$) in the ulcer areas between the normal saline, omeprazole and selenium treated groups respectively. Ulcer area reduced in all groups of animals by day 7. However, the reduction was more significant in selenium treated ($p < 0.05$) and omeprazole treated ($p < 0.05$) compared with normal saline treated animals. Likewise by day 10, there was a significant reduction in ulcer area in selenium treated ($p < 0.05$) and omeprazole treated ($p < 0.05$), when compared with saline treated. But, there was no significant difference in the ulcer areas between animals treated with selenium and omeprazole on

day 7 and day 10 ($p > 0.05$) respectively as shown in Table 2.

Table 2 Effect of selenium, omeprazole and normal saline on ulcer area after induction

Groups	Day 3 (mm ²)	Day 7 (mm ²)	Day 10 (mm ²)
Normal saline	29.6±0.7	24.8±0.7	20.6±1.12
Selenium	29.4±1.62	20.6±0.8 _{ax}	16.0±0.9 _{ax}
Omeprazole	31.0 ±0.48	21.6±1.03 _{ax}	15.2±0.9 _{ax}

Values are represented are MEAN ± SEM, N=5, _a= significant compared with animals in same group at day 3 $p < 0.05$, _x= significant compared with animals treated with normal saline at $p < 0.05$.

Figure 1 showed that by day 7, the percentage reduction in ulcer area in normal saline treated animals was significantly lower ($p < 0.05$, 0.05) than that in selenium treated and omeprazole treated. Furthermore by day 10, the percentage reduction in area of ulcer was significantly higher in selenium (44.7 ± 5.2%) and omeprazole (51.08 ± 3.7%) treated ($p < 0.05$, 0.05) respectively compared with saline treated animals (30.4 ± 3.49%).

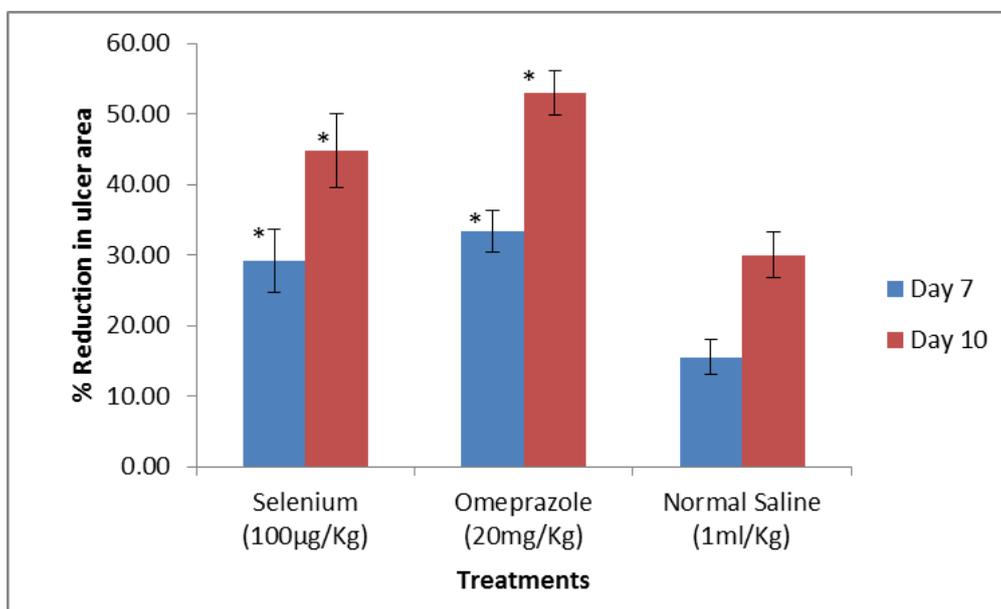


Figure 1. Percentage Ulcer Area Healed In Selenium, Omeprazole and Normal Saline Treated Animals After Induction As Compared With Day 3. N = 5, * = is significant at $p < 0.05$ when compared with normal saline

3.2. Assessment of Lipid Peroxidation

On day 3 after ulcer induction, lipid peroxidation was not significantly different in all groups of the animals ($p > 0.05$). On day 7 and 10, selenium and omeprazole therapy significantly reduced ($p < 0.05$, 0.05) peroxidised lipids respectively, when compared with value in normal saline treated animals as shown in Table 3.

Table 3. Lipid peroxidation in selenium, omeprazole and normal saline treated animals after ulcer induction

Groups	nmol/MDA/mgprotein		
	Day 3	Day 7	Day 10
Normal saline	31.6 ± 3.61	18.4 ± 0.98 _a	6.8 ± 0.67 _a
Selenium	33.2 ± 2.85	11.7 ± 1.54 _{ax}	4.4 ± 0.55 _{ax}
Omeprazole	36.0 ± 2.98	14.4 ± 0.24 _{ax}	5.2 ± 0.10 _{ax}

Values represented are MEAN ± SEM, N=5, _a= significant compared with animals in the same group on day 3 at $p < 0.001$, _x= significant when compared with animals in normal saline group at $p < 0.05$

3.3. Catalase Activity

Table 4 Catalase Activity in Selenium, Omeprazole and Normal Saline Treated Animals After Ulcer Induction

Groups	U/g tissue		
	Day 3	Day 7	Day 10
Normal saline	7.9 ± 0.14	7.2 ± 0.16	6.7 ± 0.16
Selenium	9.1 ± 0.05 _x	8.4 ± 0.13 _{ax}	7.3 ± 0.08 _{ax}
Omeprazole	8.4 ± 0.08 _x	8.4 ± 0.50 _{ax}	7.1 ± 0.13 _{ax}

Values represented MEAN ± SEM, N=5, _a= significant compared with animals in same group on day 3 at $p < 0.05$, _x= significant when compared with animals in normal saline at $p < 0.05$

By day 3 after ulcer induction, catalase activity was significantly higher in selenium treated animals compared with animals treated with omeprazole and normal saline ($p < 0.05$, 0.05) respectively. Moreover, catalase activity was significantly higher ($p < 0.05$) in omeprazole treated animals than in saline treated animals. Furthermore, by

day 7 and 10, catalase activity was significantly higher in selenium and omeprazole treated animals respectively compared with saline treated ($p < 0.05$) as shown in Table 4.

3.4. Measurement of Mucus Secretion

Table 5 showed that by day 3, mucus secretion was lower in animals treated with saline alone, but the value was not significantly different from those obtained from animals treated with selenium and omeprazole respectively ($p > 0.05$). Mucus secretion significantly increased in all groups of animals by day 7, however, mucus secretion was significantly higher in selenium treated and omeprazole treated, when compared with saline treated respectively ($p < 0.001$, 0.001). Mucus secretion was also significantly higher ($p < 0.001$) in omeprazole treated, when compared with selenium treated by day 7. However, by day 10, mucus secretion significantly reduced in omeprazole treated compared with the value on day 7. There was also reduction in mucus secretion in selenium treated animals, but it was not significant, when compared with the value by day 7 ($p > 0.05$). While mucus secretion was significantly higher ($p < 0.05$) in normal saline treated rats by day 10, when compared with secretion by day 7.

Table 5. Mucus secretion in selenium, omeprazole and normal saline treated animals after ulcer induction

Groups	mg alcian blue/gm glandular tissue		
	Day 3	Day 7	Day 10
Selenium	1.8 ± 0.14	2.7 ± 0.07 _a *	2.6 ± 0.12 _a
Omeprazole	1.9 ± 0.03	3.4 ± 0.02 _{ab} *	2.7 ± 0.22 _a **
Normal saline	1.7 ± 0.09	2.1 ± 0.08*	3.4 ± 0.05**

Values represented MEAN±SEM, N=5, _a= significant compared with animals treated with normal saline on the same day at $p < 0.001$, _b= significant compared with animals treated with selenium on the same day at $p < 0.001$, *= significant compared with value on day 3 in the same group at $p < 0.001$, **= significant compared with value on day 7 in the same group at $p < 0.01$

4. Discussion

The result of this study revealed that selenium accelerated gastric healing in acetic acid induced ulcer and the rate of healing is comparable to the standard drug omeprazole. Acetic acid induced gastric ulceration model was used in this study because: The ulcer induction procedure is quite simple, readily resulting in ulcers of consistent size and severity at an incidence of 100%. The ulcer models highly resemble human ulcers in terms of both pathological features and healing mechanisms. Spontaneous relapse of healed ulcers is frequently observed, just as in peptic ulcer patients and the ulcers respond well to various anti-ulcer drugs [26]. This result is consistent with previous report that selenium accelerated wound healing in diabetic conditions [19].

The result of the present study revealed that lipid peroxidation was significantly reduced in animals treated with selenium, when compared with animals treated with normal saline. This is consistent with reports that increase in activity of reactive oxygen species is involved in the pathogenesis of gastric ulcer [27,28]. Thus selenium was able to reduce lipid peroxidation by increasing the antioxidant status of the animal. Previous research had

reported that selenium protects membrane lipids and macromolecules from oxidative damage produced by peroxides by increasing the activity of antioxidant enzymes [16,29]. Reactive oxygen species are generated through numerous normal metabolic processes and are needed for normal functioning of the organism. Various antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase, control their accumulation [30]. Any imbalance in the activity of these enzymes normally leads to faulty disposal of free radicals and its accumulation. Moreover, radical scavengers stimulate the healing of refractory peptic ulcers [31]. Thus, the antioxidant capacity of drugs and medicinal plant preparations can be used to explain their antiulcer and healing mechanisms of action.

Gastric mucus is the first line of defense of the stomach, which prevents acid and pepsin from destroying the gastric wall [32,33]. Moreover, gastric mucus has been reported to play important role in healing of ulcer [34]. In this study, mucus secretion was significantly higher in all groups of animal by day 7. This is a response of the body to the mucosa injury and it is believed to have a gastroprotective effect and also to promote repair of the damaged tissue. However, mucus secretion was significantly higher in omeprazole treated and selenium treated. Gastric mucus provides a neutral pH environment that promotes epithelial restitution [35] and enhances the binding of epithelial growth factor and other growth factors to their receptors [36]. These would result in increased cell proliferation, leading to granulation tissue formation and re-epithelialization [36]. This response to injury is enhanced in animals treated with omeprazole and selenium. The reduction in mucus secretion in omeprazole and selenium treated group by day 10, might suggest that healing in that groups were rapid. In essence the noxious stimuli had been eradicated and the gastric mucosa is returning to the normal secretory state.

5. Conclusion

This study revealed that acetic acid induced gastric ulcer in Wistar rats. Selenium administration accelerated the rate of healing over period of days by reducing lipid peroxidation, increasing the antioxidant level and rapid increase in mucus secretion in response to mucosa injury. This action of selenium is similar to the actions of the standard drug omeprazole. Thus in addition to the gastroprotective effects of selenium [15,24], selenium accelerates the rate of healing of gastric ulcer.

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Statement of competing interest

The authors have no competing interests.

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