Research Article

Gastroprotective effect of vanadium in rats - the roles of gastric acid and nitric oxide

T.P. Omayone, A.T. Salami, F.S. Oluwole and S.B. Olaleye

Gastrointestinal Secretion and Inflammatory Research Unit, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

ABSTRACT

Vanadium (various forms) has been proven to be beneficial in the treatment of certain diseases, especially diabetes. Reports have it that vanadium may protect the stomach from gastric ulcerogens such as ethanol and acid. This study was designed to investigate the probable mechanism Vanadium exerts its' gastroprotective activities. **Methods:** Sodium metavanadate (0, 5, 10 and 20 mg/kg. p.o) was administered to 20 male Wistar rats weighing (150±20g) for two weeks. Gastric ulcer was induced using ethanol after which animals were sacrificed 2 hours later. In study two, 30 male Wistar rats (114±10g) were administered sodium metavanadate at (0, 50 and 200 ppm) in drinking water for 10weeks after which ulcer was induced using pylorus ligation method. Basal and histamine (10mg/kg i.m) stimulated gastric secretions were determined through continuous perfusion technique afterwards in un-ulcerated animals. Ulcer score, mucin content, gastric nitrite level, anti-oxidant and $H^+K^+ATPase$ activities were investigated in gastric homogenate samples. **Results:** Sodium metavanadate significantly reduced ulcer index and MDA levels while enhancing NO concentrations, catalase and SOD activities in both method of gastric ulceration. Mean basal gastric output was not significantly different in control compared with 50 and 200 ppm V group. Stimulation with histamine caused significant increases in gastric output by 187.72%, 57.40% and 78.69% in control, 50 and 200 ppm V respectively and was significantly reduced in the vanadium treated groups. A significant decrease in H^+K^+ ATPase (proton) pump activities of the vanadium exposed groups compared with control in the pylorus ligation ulcer model was observed. **Conclusion:** Vanadium may be suggested to have protective activities against gastric ulceration by acting as a proton pump inhibitor, enhancing anti-oxidant enzyme activities as well as mucosal blood flow via increased NO mechanism.

INTRODUCTION

Gastric ulcers occur as a result of increased gastro aggressive factors over gastro protective factors (Hoogerwerf and Pasricha, 2001). However, recent treatment of gastric ulcer focuses on producing agents that can reduce or curb the aggressive factors (gastric acid, abnormal motility, pepsin, bile salts e.t.c) as well as enhancing gastric mucosal protection (mucous/bicarbonate, prostaglandin synthesis and local mucosa blood flow) (Tulassay and Herszenyi, 2010). However, various exogenous factors such as stress, smoking, improper use of Non-Steroidal Anti Inflammatory Drugs (NSAIDs), poor dieting and alcohol consumption have also been documented as predisposing (risk) factors to peptic ulcer development. Alcohol (a risk factor) infiltrates the gastric mucosa thus breaching its' protective mucous layer hereby exposing the mucosa to aggressive factors. It also causes imbalance(s) in the body antioxidant system due to increased reactive oxygen species like malondialdehyde (MDA - a marker of lipid peroxidation), superoxide anions and hydroxyl free radicals while decreasing antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GSH), (Galvin and Szabo, 1992; Marotta *et al*., 1999). Gastric acid secretion had been a main focus in the production of ulcer healing drugs as

Address for correspondence:
E-mail: sbolaleye@gmail.com
Tel.: +2348023255893

its reduction has been proven to help enhance the healing rate of gastric ulceration (Aihara *et al*., 2003; Tuorkey and Karolin, 2009). Pharmaceuticals had engaged in the production of histamine H_2 receptors antagonists and H^+K^+ ATPase (acid pumps) inhibitors in order to facilitate the treatment of acid-base peptic ulcer diseases (Black *et al*., 1972).

Vanadium a trace element is fast becoming of great interest today owing to certain characteristic features distinguishing it as a very important mineral. It is highly reactive with viable therapeutic effect on diabetes owing to its' insulin mimetic properties. It also inhibits the activity of phosphotyrosine phosphatase (PTP-1β) as well as activating PκB/Akt leading to increased uptake of glucose by the GLUT4 transporter (Vardatsikos *et al*,. 2009). Suther *et al.,* 2007 reported that vanadium maintains mucosa integrity by decreasing gastric acid output and enhancing mucus activity during pyloric ligation ulcer model while Kemeir, 2013 reported that vanadium significantly inhibits lipid peroxidation while enhancing the antioxidant systems (SOD, CAT and GSH) during ethanol induced ulcer formation. However, the role played by vanadium in gastrointestinal functions during ulcer formation is not fully unraveled. This study sought to investigate and compare the probable mechanism(s) action by which Vanadium exerts its gastroprotective activities both in ethanol and pylorus ligation induced ulcer model.

MATERIALS AND METHODS
Drugs and Chemicals
Sodium Metavanadate, Thiobarbituric Acid, Trichloroacetic Acid and Adrenaline, where purchased from BDH Chemicals Ltd Poole England while N-1-Naphthyl Ethylene Diamine Dihydrochloride (NED), Sulphanilic Acid and Sodium Nitrite were purchased from Loba Chemie Pvt. Ltd. India

Gastric Acid secretion
15 Male Wistar rats (114±10g) were divided into 3 groups of 5 rats each and receive Sodium Metavanadate (NaVO$_3$) in drinking water for 10 weeks. Group 1 (control- 0ppm), Group 2 (50ppm) and Group 3 (200ppm). After 24hr fast, gastric acid secretion was measured using the continuous perfusion method of Ghosh and Schild (1958), modified by Amure and Ginsburg (1964).

Animals where anaesthetized with pentobarbital (35 mg/kg i.p) and a midline laparotomy was made to expose the stomach and duodenum. A semi-transection was made at the junction of the pylorus with the duodenum where a pyloric cannula was inserted and ligated to collect gastric contents. An orogastric cannula was inserted for perfusion of isotonic saline (pH 7, 37^{0}C), the stomach was gently rinsed with the isotonic saline until gastric effluent was clear. Thereafter, using a perfusion pump, the animal was perfused at a rate of 1ml/minute. Gastric acid was collected via the pyloric cannula at 10 minutes intervals. In order to determine acidity, 10 mLs of the stomach perfusate was titrated against 0.025M sodium hydroxide (NaOH) solution with phenolphthalein as indicator. Titrable acidity was expressed in mmol/10mins after calculation in each sample.

After surgical operation, gastric acid secretion was allowed to stabilize for about 50 minutes and the mean acidity of three gastric secretions was termed basal acid output. At the 50th minutes, histamine was administered (10 mg/kg i.m) for the stimulated acid secretory response.

Ethanol-induced gastric ulceration
20 Male Wistar rats (150±20g) were divided into four (4) groups of 5 rats each and were administered Sodium Metavanadate (NaVO$_3$) at doses 5, 10 and 20 mg/kg (Group 2, 3 and 4, respectively) orally for 2 weeks. Group 1(Control) was administered with distilled water. After 24hr fast, gastric ulceration was then induced by the administration of 1ml/200g absolute Ethanol and animals were sacrificed 2hrs later (Suthar *et al.,* 2007). The stomachs were removed, opened along the greater curvature, rinsed in iced cold normal saline and ulcer indices were determined according to Rasika *et al*., 2010. The glandular portion of stomach was prepared for Histopathological observation and measurement of Total protein (TP), Malondialdehyde (MDA), Catalase levels (CAT), Superoxide Dismutase (SOD) and Nitric Oxide (NO) concentration.

Induction of Pylorus Ligation Ulcer
15 Male Wistar rats (114±10g) were divided into 3 groups of 5 rats each and receive Sodium Metavanadate (NaVO3) in drinking water for 10 weeks. Group 1 (control- 0ppm) animals were fasted for 24 hours prior to pylorus ligation. Under light anesthesia cocktail [ketamine (60mg/kg) and xylazene (10mg/kg)], the abdomen was opened and pylorus end ligated. The abdomen was then sutured and after 8 hours of ligation, the animals were sacrificed. The stomach was dissected out, gastric juice cumulated was drained into a test-tube, centrifuge at 3000rpm for 5 minutes and the volume was noted. The gastric content was subjected to analysis for free acidity, total acidity and mucin content. The stomachs were then rinsed in ice-cold phosphate buffered saline and scored for ulcer severity according to Rasika *et al*., 2010.

Determination of free acidity
To 0.5ml of gastric juice pipetted into a 25ml conical flask, 2 drops of topfer's reagent was added and titrated

with 0.01N sodium hydroxide (NaOH) until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of NaOH added was noted.

Determination of total acidity

To 0.5ml of gastric juice pipetted into a 25ml conical flask, 2 drops of phenolphthalein solution was added and titrated with 0.01 NaOH until a purple colour appears. The volume of NaOH added was also noted. Acidity was calculated by using the formula below:

Acidity= Volume of NaOH x Normalty of NaOH x mEq/L/100g x 0.1

Determination of Mucin content

Mucin content was determined according to the method of Winzle 1995. The principle is based on the determination of the hexose component of the mucin. It depends on the reaction of carbohydrate in concentrated sulfuric acid with orcinol (5-methyl resorcinol) to give a coloured product that can be measured colorimetrically.

Procedure

0.25ml of 1:20 diluted sample was added to an equal volume of 1.6% orcinol and 2ml of 60% sulfuric acid. The mixture was boiled in a water bath for 10 minutes and then cooled on ice. The optical density was measured at 425nm. Results expressed as mg hexose/dl. The total hexose content was determined from the standard curve of galactose-mannose

Ulcer scoring

The number of ulcers and the length of each ulcer were measured. Ulcer index was calculated using severity scores and average number of ulcers per animal. Severity was scored as below
0 - Normal stomach
0.5 - Red coloration
1 - Spot ulcers
1.5 - Haemorrhagic streaks
2 - Ulcer > 3 mm but ‹ 5 mm
3 - Ulcers > 5 mm

Ulcer index (UI) was calculated using the formula:
$UI = UN + US + UP \times 10^{-1}$
Where UN = Average of number of ulcer per animal;
US = Average of severity score and
UP- Percentage of animal with ulcer
Percentage inhibition was also calculated using the formula

Percentage inhibition = $\frac{UI\ control - UI\ Pretreated}{UI\ control} \times 100$

(Rasika *et al.,* 2010)

Biochemical Analysis:

Lipid peroxidation was determined by measuring the Thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation according to the method of Varshney and Kale (1990). Catalase activity was determined according to the method of Sinha, 1971. The superoxide dismutase (SOD) activity was determined based on the ability of superoxide dismutase to inhibit the autoxidation of epinephrine according to Misra and Fridovich, 1972. Nitric Oxide was determined as Nitrite concentration using Griess reagent (Ignarro *et al.*, 1987).

RESULTS

Effect of Vanadium on gastric acid secretion in Ethanol induced gastric ulceration

Table 1 shows the effect of sodium metavanadate on gastric acid secretion, both basal gastric output and histamine stimulated gastric acid secretion. There was no significant difference in the basal acid secretion across all groups. The stimulation with histamine resulted in significant increase in gastric acid secretion; however, both sodium metavanadate exposed groups showed significantly decreased histamine stimulated gastric acid secretion when compared with the control group (Fig. 1).

Table 1: Effect of Vanadium on gastric acid secretion after 10 weeks of exposure

Treatment	Basal Acid Output (mEq/L)	Histamine Stimulated (mEq/10ml)	% Change
Control	1.14±0.05	3.28±0.17	187.72
50ppm	1.08±0.06	1.22±0.05***	57.40
200ppm	1.7±0.04	2.18±0.06**	78.69

Values are presented as Mean ± SEM, n=5 Significant at ** $p<0.01$, *** $p<0.001$ when compared with histamine stimulated control.

Effect of Vanadium on Ulcer Index in Ethanol induced gastric ulceration.

Table 2 shows the effect of vanadium on ulcer index and percentage protection of vanadium on ethanol induced gastric ulceration. Vanadium treated groups (5 mg/kg, 10 mg/kg and 20 mg/kg) showed a significant reduction in gastric ulcer index which was dose dependent.

Effect of Vanadium on lipid peroxidation, Antioxidant enzymes and Nitric oxide levels in ethanol induced gastric ulceration.

Table 3 shows the levels of MDA, CAT, SOD and NO respectively. Levels of MDA was significantly reduced

in all vanadium treated group (5 mg/kg, 10 mg/kg and 20 mg/kg) compared with control. All vanadium groups showed significantly increased SOD activities, while CAT activities were significantly increased in the 5 mg/kg and 10 m/kg groups when compared with control. A significantly increased NO concentration was also observed only in the 5mg/kg group compared with control.
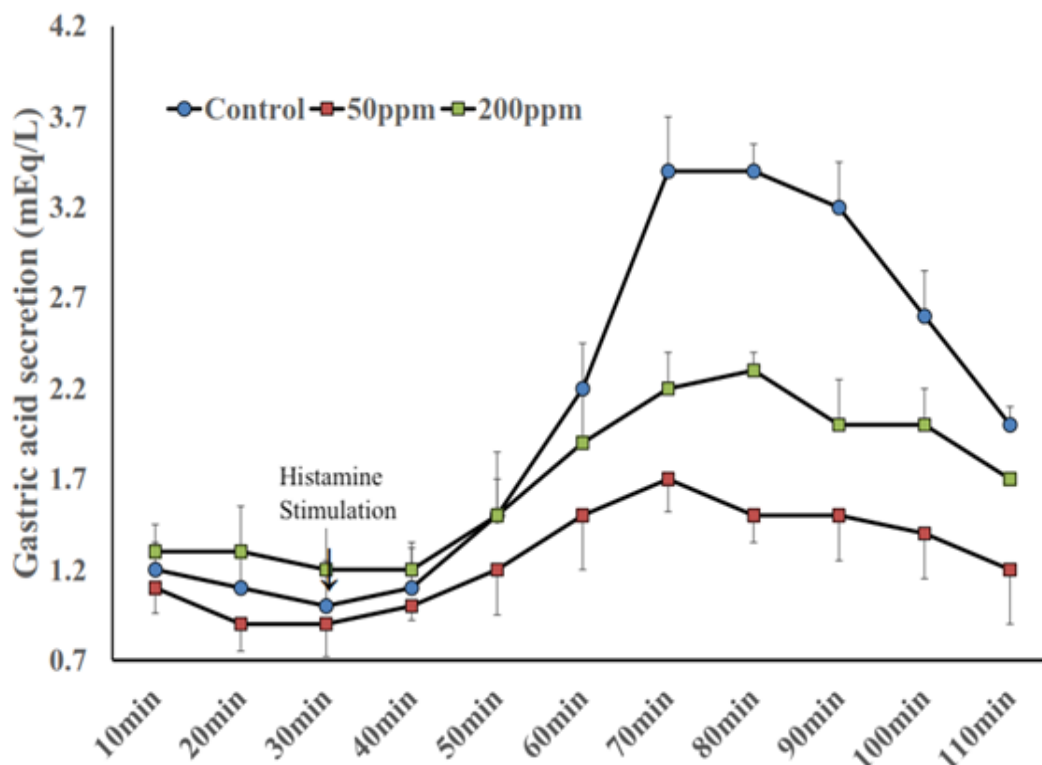

Fig.1: Effect of Vanadium on basal and histamine-induced gastric acid secretion after a 10-week exposure.

Table 2: Ulcer index after ethanol induced gastric ulceration in Vanadium treated animals.

Groups (NaVO$_3$ Treatment)	Ulcer Index (mm^2)	% inhibition
Control (0mg/Kg)	14.28±2.03	-
5 mg/kg	6.38±1.49*	55.32
10 mg/kg	4.28±1.84**	70
20 mg/kg	3.17±0.98**	77.73

Values are presented as Means ± SEM, n=5 Significant at * p<0.05, ** p<0.01, when compared with control.

Effect of vanadium on gastric juice volume, total acidity and free acidity in Pylorus ligation induced gastric ulceration in rats
Table 4 shows the effects of vanadium on gastric effluent (gastric juice volume, total acidity and free acidity) after pylorus ligation. Gastric juice volume, total acidity and free acidity were also significantly reduced in the 200ppm group compared with the control group, while the 50ppm group showed no significant difference.

Effect of vanadium on relative stomach weight, mean ulcer score, percentage protection, and mucin content in pylorus ligation induced gastric ulceration in rats
A significant decrease in the relative stomach weight was observed in the 200 ppm Vanadium treated group (0.58±0.02) when compared with the control group (0.67±0.02) but there was no significant difference at 50ppm (0.62±0.03). The mean ulcer score of the control group (12.26±1.93) was significantly decreased compared with both vanadium treated groups; 50ppm (6.30±1.20) and 200ppm (4.90±0.87), thereby affording a percentage protection of 48.61% and 60.03% respectively. There was a significant increase in the mucin content of the 200ppm V group (5.16±0.48) compared with control (3.24±0.20), while the 50ppm V (2.40±0.55) showed no significant difference (Table 5).

Effect of vanadium on protein concentration, Malondialdehyde (MDA) level in pylorus ligation induced gastric ulceration in rats

There was no significant difference in the protein concentration of the vanadium treated groups 50ppm (0.68±0.04) and 200ppm (0.70±0.04) compared with the control group (0.76±0.05). However, MDA levels were significantly decreased in both the 50ppm (2.63±0.18) and 200ppm (1.89±0.22) vanadium treated groups compared with control (3.65±0.64) group (Figure 2).

Table 3: Effect of Vanadium on antioxidant status and total nitrite concentration in ethanol induced gastric ulceration.

Group	MDA (U/mg protein)10^{-6})	SOD (U/mg Protein)	CAT (U/mg Protein)	NO (µ/g tissue)
Control (0mg/kg)	13.61±0.42	16.69±0.78	15.78±4.46	23.18±2.40
5mg/kg	8.38±0.99**	19.96±0.14	86.47±13.97**	35.82±0.56**
10mg/kg	9.65±0.79**	18.9±0.60	49.18±7.65**	24.57±1.28*
20mg/kg	9.94±0.61**	18.91±0.44	25.89±4.05	25.32±2.46*

Values are presented as Mean ± SEM, n=5 Significant at * p<0.05, ** p<0.01, when compared with control.
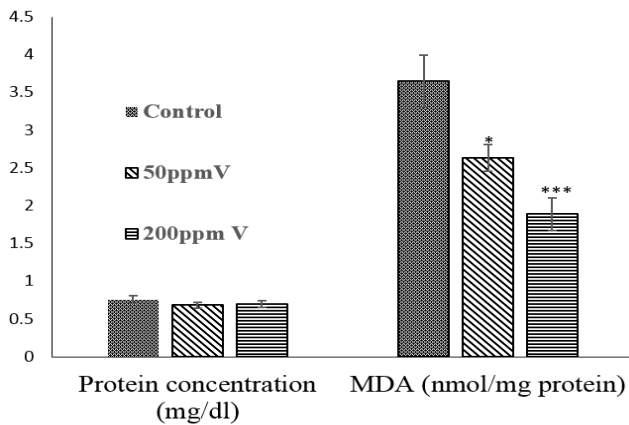

Fig. 2: Effect of vanadium on protein concentration and MDA levels in pylorus ligation gastric ulceration. Values are presented as Means ± SEM, n=5, Significant at * p<0.05, *** p<0.001 when compared to control.

Table 4: Effect of Vanadium on Gastric effluent in pylorus ligation gastric ulceration.

Group	Gastric Juice Volume (ml/100g bw)	Total Acidity (mEq/L)	Free Acidity (µEq/L/100g)
Control	3.15±0.23	1.91±0.12	181.60±15.70
50ppmV	2.01±0.48	1.61±0.23	204.20±15.78
200ppm V	1.42±0.27*	1.01±0.28*	79.95±8.09***

Values are presented as Means ± SEM, n=5, Significant at * p<0.05, ** p<0.01, *** p<0.001 when compared to control.

Effect of vanadium on Antioxidant enzymes and Nitric oxide (nitrite concentration) in pylorus ligation induced gastric ulceration in rats

The antioxidant enzymes superoxide dismutase activity (SOD), catalase activity (CAT) and glutathione activity (GSH) as well as nitrite concentration (NO) were all significantly higher in the 50ppm V group (79.64±1.82, 72.75±5.14, 69.70±1.84 and 127.70±10.38 respectively) compared with the control group (63.43±4.20,49.68±3.05, 55.71±1.84 and 93.36±1.89 respectively). However, in the 200ppm V group (73.47±4.51, 77.49±6.99, 60.07±2.95, 140.3±7.26) only the CAT and NO were significantly higher compared with the control group at p=0.05. Results are represented in Figure 3.
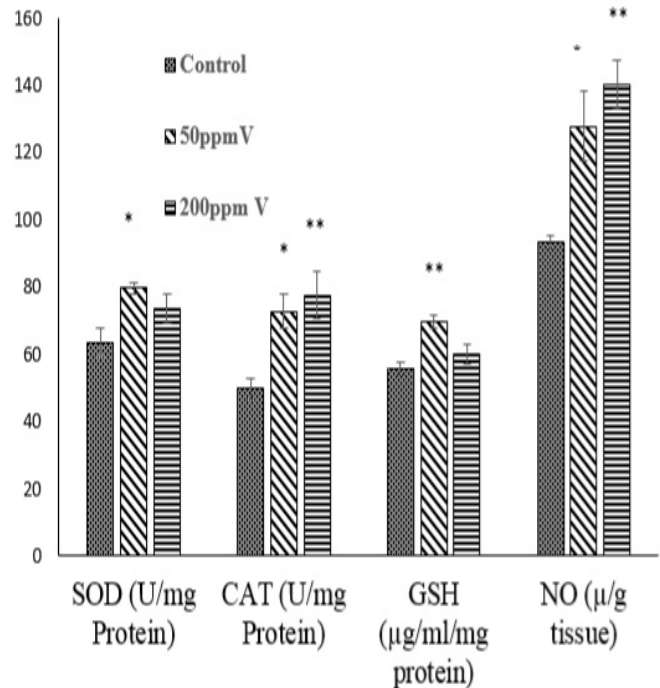

Fig. 3: Effect of vanadium on antioxidant status and total nitrite concentration in pylorus ligation induced gastric ulceration. Values are presented as Mean ± SEM, n=5, Significant difference at *p<0.05, **p< 0.01when compared with control.

Effect of vanadium on H^+K^+ ATPase pump activity in pylorus ligation induced gastric ulceration in experimental rats.

There were significant decreases in the H^+K^+ ATPase pump activity of the vanadium treated groups 50ppm (9.49 ± 0.04) and 200ppm (8.95 ± 0.04) compared with the control group (12.48 ± 0.05). (Figure 4).
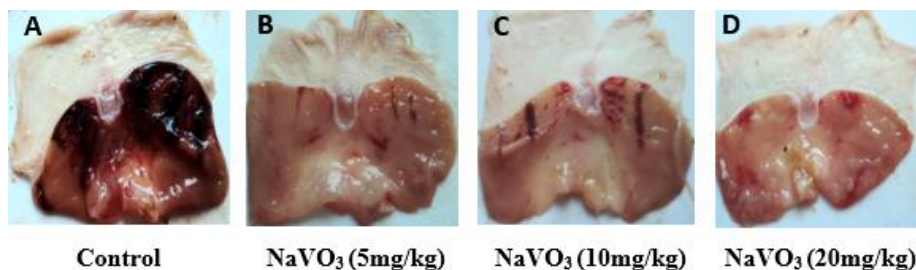

Control   NaVO₃ (5mg/kg)   NaVO₃ (10mg/kg)   NaVO₃ (20mg/kg)

Plate 1:
Gross Morphology of Gastric Mucosa of Rats after Ethanol Administration. (**A**-Control/0mg/kg NaVO₃, **B**-5mg/kg NaVO₃, **C**-10mg/kg NaVO₃ and **D**-20mg/kg NaVO₃). Gastric mucosa was severely eroded in **A** compare to **B**-**D** which showed mild to moderate erosion.
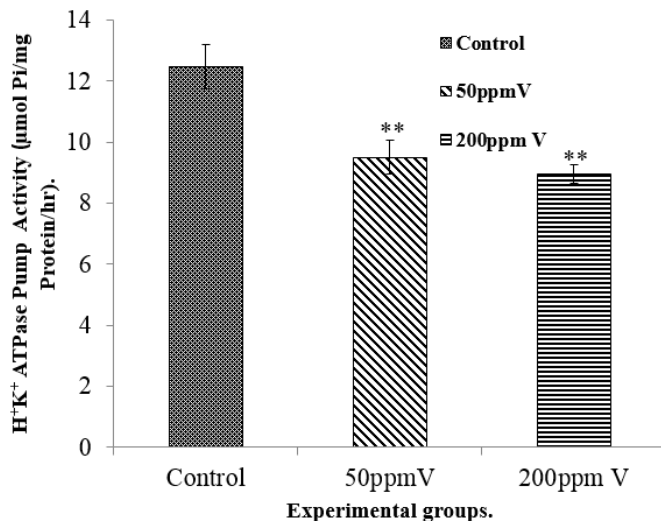

Fig. 4: Effect of vanadium on H^+/K^+ ATPase activity in pylorus ligation gastric ulceration. Values are presented as Means ± SEM, n=5; Significant difference at **p< 0.01 when compared with control.

Effect of Vanadium on Gross and Histological features.
Plates 1 2 and 3 represent gross and histological examinations respectively of gastric tissue for both ethanol and pylorus ligation induced gastric ulcerations. Control groups (1a and 2a) representing gross and histological pictures respectively showed severe mucosal damage and surface epithelial erosion compared to the vanadium treated groups (1b–1d, 2b – 2d and b to c) showing mild to moderate surface epithelial erosions.

DISCUSSION

Gastric acid secreted by the stomach is very important for some physiological functions especially in the digestion of proteins and destruction of various unwanted micro-organisms (Martinsen *et al.*, 2005). However, increases in gastric acid secretion and acidity can further digest the stomach wall resulting in formation of gastric ulcers (Goel and Bhattacharga, 1991). Increase in acidity by the stimulation of special receptors (histamine (H_2) receptor) on parietal cell is responsible for acid secretion. In this study, administration of histamine increased basal gastric secretion in all experimental animals however, this increase was not well sustained during vanadium pre-treatment. This finding supports earlier work of Suthur *et al.*, 2007 in which vanadium was reported to reduce total acid output and peptic activity in pylorus ligated
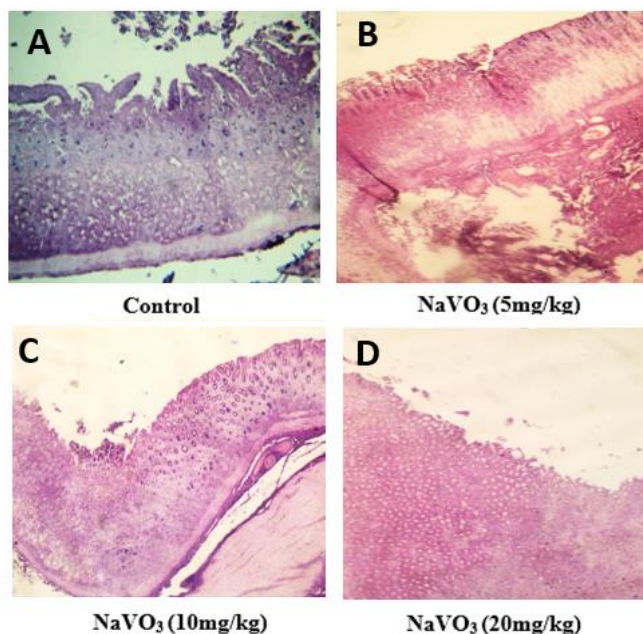

Control   NaVO₃ (5mg/kg)

NaVO₃ (10mg/kg)   NaVO₃ (20mg/kg)

Plate 2:
Histological Sections of Gastric Mucosa of Rats after Ethanol Administration. (**A**-Control/0mg/kg NaVO₃, **B**-5mg/kg NaVO₃, **C**-10mg/kg NaVO₃ and **D**-20mg/kg NaVO₃). Group **A** showed severe surface epithelium with necrosis of mucosal glands. Groups **B-D** showed mild to moderate erosion of surface epithelium with other tunics showing no visible lesion.
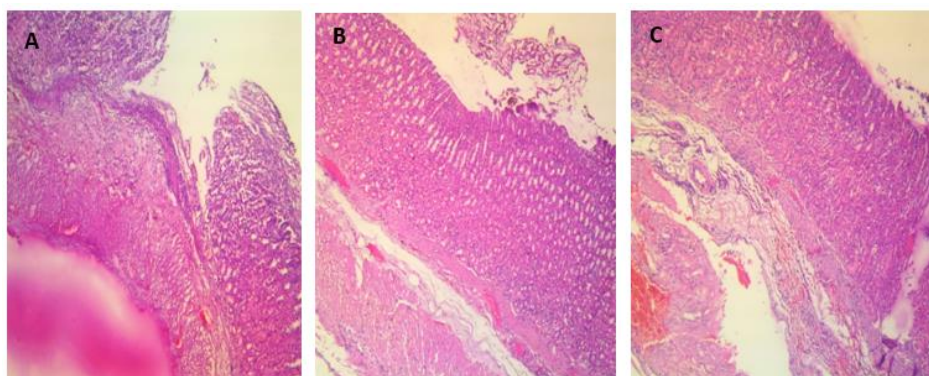
Plate 3: Photomicrograph of gastric tissue section after pylorus ligation gastric ulceration (H&E stain, MAG X100) showing control group (**A**). Multiple foci of moderately deep ulcers of the tunic mucosa with intact *muscularis* mucosa. Moderate expansion of the submucosa with oedema, haemorrhages and congested submucosa vessels. **B - 50 ppmV**: A few foci of mild sloughing off of the epithelial surface, moderate hyperplasia of mucus cells. Other tunics appear normal. **C - 200 ppmV**: Mild necrosis of mucus cells of the crypts, hyperplasia of mucous cells, fairly normal surface epithelium. Mild submucosa haemorrhages and intact *muscularis* external and serosa.

rats; however, on further investigation in this study, vanadium inhibited H^+K^+ ATPase activity in the ligated pylorus ulcer model thus acting as a proton pump inhibitor. This probable might be the mechanism by which vanadium reduces basal and histamine stimulated gastric acid secretion in this study and previous observations. Ethanol coming directly in contact with gastric mucosa produces necrotic lesions and severe erosion of the epithelial surface. It is a method of gastric ulcer induction used in studying the efficacy of potential drugs or agents having cyto-protective and/or antioxidant activities (Adinortey *et al.*, 2013). In accessing the cyto-protective properties of any drug or agent, sizes of induced gastric mucosa lesions are measured both macroscopically and microscopically (Dabo *et al.*, 2014). Macroscopic observation from both studies revealed that vanadium ameliorated gastric ulceration by reducing ulcer index evident by mild erosion of surface epithelium from histological evaluations.

Increases in lipid peroxidation as well as a reduction in antioxidant enzymes have also been reported to contribute to mucosal inflammation leading to ulcer (Recknagel *et al.*, 1977). The level of MDA; a derivative of ROS action has been documented to be indicative of oxidative stress (Rio *et al.*, 2005). Vanadium treatment drastically reduced the MDA level implying a decrease in oxidative stress and probably anti-inflammatory activities at the doses administered in both studies. Certain inherent biological enzymes that protect the cells from oxidative damage has been documented – Superoxide dismutase (SOD) and Catalase (CAT) (Kuwar and Ptiyadarsini, 2011). These enzymes help in neutralizing and preventing varied damages caused by reactive oxygen species (ROS) during diseases (Kuwar and Ptiyadarsini, 2011). It was observed that vanadium enhanced the production of CAT and SOD levels especially at a lower concentration. Probably, vanadium helped in inhibiting the levels of ROS and or MDA through increased anti oxidative actions (SOD and CAT production). This might have minimised gastric ulceration formation (and indicative of more beneficial activities) at a lower concentration than at higher concentration.

Mucosal blood flow is important in gastro protection as it aid the provision of oxygen, nutrients, gastrointestinal peptides, hormones and a host of other important materials which are very important in regeneration and re-epithelization of gastric mucosa surface eroded by gastric acid (Abdel-Salam *et al.*, 2001 ;). Researchers have documented a decrease in mucosal blood flow in alcohol induced gastric ulcerations (Repetto and Llesuy, 2002) which is detrimental to gastric ulcer healing. Nitric oxide (NO) which is an endogenous vasodilator is key in the regulation of gastric mucosal blood flow. It is synthesized by the action of the enzyme nitric oxide synthase (NOS) on nitric oxide donor such as L-arginine. Recently, NO has been reported to be beneficial in gastric healing as administration of NO donors enhances healing (Moura Rocha *et al.*, 2010; Dabo *et al.*, 2014) while administration of NO inhibitors such as N(G)-nitro-Larginine methyl ester (L-NAME) showed severe gastric lesions in mice (Silva *et al.*, 2009). In this study NO concentration was seen to be enhanced (especially in the vanadium treated groups) which is indicative of adequate blood supply to the stomach and probably a mechanism by which severity of gastric ulcer formed was reduced.

The effect of vanadium varies depending on the mode of administration, dose and time of exposure to this element (NRC, 2005). Toxicity has been reported at higher doses in the brain (Olopade *et al.*, 2012) and liver (Scibior *et al.*, 2006) cells (but not in gastric

tissues) suggestive of increased oxidative stress as vanadium concentration increases in the biological system. This study reports higher antioxidative activities at lower doses (5 and 10 mg/kg b.w) unlike 20mg/kg b.w vanadium administration. Probably, the 20mg/kg b.w might be ameliorating ulcer formation through another mechanism (may be as a proton pump inhibitor) yet to be unraveled while lower doses might be mediating normal gastrointestinal activities or milieu through increased anti-oxidative actions.

In conclusion, vanadium is protective (at these doses administered) against gastric ulceration by reducing free radicals and gastric acidity, acting as a proton pump inhibitor, enhancing anti-oxidant enzymes activities and mucosal blood flow via nitric-oxide mechanism.

REFERENCES

Abdel-Salam, O.M.; Czimmer, J.; Debreceni, A.; Szolcsányi, J. & Mózsik, G. (2001). Gastric mucosal integrity: gastric mucosal blood flow and microcirculation. An overview. *Journal of Physiology (paris)*. 95 (1-6):105-127.

Adinortey, M.B., Ansah, C., Galyuon, I., and Nyarko, A. (2013). Review Article: In vivo models used for evaluation of potential anti-gastroduodenal ulcer agents. Hindawi Publishing Corporation. Article ID 796405, 12 pp.

Aihara, T., Nakamura, E., Amagase, K., Tomita, K., Fujishita, T., Furutani, K and Okabe, S. (2003): Pharmacological control of gastric acid secretion for the treatment of acid-related peptic disease: past, present and future. *Pharmacol. Ther*. 98:109-127.

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

Black, J.W., Duncan, W.A., Durant, C.J., Ganellin, C.R and Parsons, E.M. (1972): Definition and antagonism of histamine H2 – receptors. *Nature* 236:385-390.

Dabo, U., Oluwole, F.S., and Adedeji, T.G. (2014). Effects of L-arginine and L-citrulline on indomethacin-induced gastric ulceration and gastric pH in male albino rats. *European Journal of Medicinal Plants*. 4(6): 623-640.

El-Demerdash, E., El-Mesallamy, H.O., Abu-Zaid, N.M., Gad, M.Z. (2010). The potential therapeutic effect of nitric oxide modulators in experimentally-induced gastric ulcers. *Drug Discoveries and Therapeutics*. 4(4):276-284.

Galvin, G.B., and Szabo, S. (1992). Experimental gastric mucosal injury: Laboraatory models reveal mechanisms of pathogenesis and new therapeutic strategies. *The FASEB journal*. 6 (3): 825-831.

Goel, R.K., and Bhattacharya, S.K. (1991). Gastroduodenal mucosal defense and protective agents. *Indian Journal of Experimental Biology*. 29: 701–714.

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

Hoogerwerf, W. A., and Pasricha, P. J. (2001). Agents used for control of gastric acidity and treatment of peptic ulcers and gastroesophageal reflux disease." in *The Pharmacological Basis of Therapeutics*, Hardman J.G., Limbird L. E., and Goodman G.A., Eds., pp. 1005–1019, McGraw-Hill, New York, NY, USA, 10th Edition.

Ignarro, L., Buga, G., Wood, K., Byrns, R., and Chaudhuri, G. (1987). Endothelium derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA*. 84**:** 9265–9.

Kemeir, M.E.A. (2013). The protective effect of vanadium sulphate on ethanol-induced gastric ulcer. *Bahrain Medical Bulletin*, Vol. 35 (4) 9 pp.

Kunwar, A. and Priyadarsini, K.I. (2011). Free radicals, oxidative stress and importance of antioxidants in human health. *Journal of Medical and Allied Sciences*. Vol. 1 (2): 53-60.

Marotta, F., Tajiri, H., Safran, P., Fesce, E., and Ideo, G. (1999). Ethanol-related gastric mucosal damage: Evidence of free radical-mediated mechanism and beneficial effect of oral supplementation with bionormalizer, a novel natural antioxidant. *Digestion*. 60(6): 538-543

Martinsen, T.C, Bergh, K. and Waldum, H.M. (2005). Gastric juice: a barrier against infectious diseases. *Basic Clin. Pharmacol Toxicol*, 96: 94-102.

Miranda, K.M., Espey, M.G., Yamada, K., Krishna, M., Ludwick, N., Kim, S., Jourd'heuil, D., Grisham, M.B., Feelisch, M., Fukuto, J.M., and Wink, D.A. (2001). Unique oxidative mechanisms for the reactive nitrogen oxides species, nitroxil anion. *J. Biol. Chem.* 276: 1720-1727.

Misra, H.P., and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *J. Bio. Chem*. 247:3170-3175.

Moura Rocha, N.F., Venâncio, E.T., Moura, B.A., Gomes Silva, M.I., Aquino Neto, M.R., Vasconcelos Rios, E.R., de Sousa, D.P., Mendes Vasconcelos, S.M., de França Fonteles, M.M., and de Sousa, F.C. (2010). Gastroprotection of (-)-alpha-bisabolol on acute gastric mucosal lesions in mice: the possible involved pharmacological mechanisms. *Fundamental and Clinical Pharmacology*. 24 (1): 63-71.

NRC (National Research Council). (2005). Vanadium. Chapter 30. In: Mineral Tolerance of Animals, 2nd revised edition. National Academy Press, Washington, DC, pp. 398-412.

Olopade J.O, Fatola I.O and Olopade F.E (2011). Vertical administration of vanadium through

lactation induces behavioural and neuromorphological changes: protective role of vitamin E. Niger. J. Physiol Sci. 26 (1): 55-60

Rasika, D.B., Mahendra, A.G., Sneha, J.A., Subodh, C.P. (2010). Anti-ulcer activity of ethonolic extract of leaves of s*esbania grandiflora l*inn. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2(4): 206-209.

Recknagel, R.O., Gunde, E.A and Hruszkewycz, A.M (1977). Chemical mechanisms in carbon tetrachloride toxicity. Free radicals in biology, Academic Press, New York, 97.

Repetto M. G. and Llesuy S. F. (2002). "Antioxidant properties of natural compounds used in popularmedicine for gastric ulcers," *Brazilian Journal ofMedical and Biological Research*, vol. 35, no. 5, pp. 523–534.

Rio, D.D., Stewart, A.J. and Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutritional, Metabolism and Cardiovascular Diseases. 15 (4): 316-328.

Scibior, A. (2006). Some selected blood parameters in rats exposed to vanadium and chromium via drinking water. *Trace Element Electrolytes*, 22(1):40-46. Abstract from EMBASE 2005083129

Silva, M.I., Moura, B.A., Neto, M.R., Tomé, A.R., Rocha, N.F., de Carvalho, A.M., Macêdo, D.S., Vasconcelos, S.M., de Sousa, D.P., Viana, G.S., and de Sousa, F.C. (2009a). Gastroprotective activity of isopulegol on experimentally induced gastric lesions in mice: Investigation of possible mechanisms of action. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 380 (3): 233–245. Sinha, A.K. (1972). Colorimetric assay of catalase. *Anal. Biochem*. 47:389-394.

Suthar, P.B., Jain, S.M., Mehta, A.A., and Santani, D.D. (2007). Study of anti-ulcer activity of sodium metavanadate on alcohol and pylorus ligation induced gastric ulcers in rats. *Ind J Pharmaceutical Sciences*. 69(3): 454-6.

Tuorkey, M and Karolin, K. (2009): Anti-ulcer activity of *curcumin* on experimental gastric ulcer in rats and its effect on oxidative stress/antioxidant, IL-6 and enzyme activities. *Biomed. Environ Sci*, 22:488-495.

Tulassay, Z. and Herszényi, L. (2010). Gastric mucosal defense and cytoprotection. *Best practice and Research Clinical Gastroenterology*. 24(2): 99-108.

Vardatsikos, G., Mehdi, M.Z., Srivastava, A.K. (2009). Bis (maltolato)-oxovanadium (IV)-induced phosphorylation of PKB, GSK-3 and FOXO1 contributes to its glucoregulatory responses (review). *Int. J. Mol. Med*. 24:303-309.

Varshney, R., and Kale, R.K. (1990). Effect of calmodulin antagonists on radiation induced Lipid peroxidation in microsomes. *Int. J. Biol*. 158: 733-741.

Vijay Kumar Bansal, Sandeep Kumar Goyal, Dhruba Sankar Goswami, Shiffali Singla, Sandeep Rahar, Suresh Kumar. (2009). Herbal Approach to Peptic Ulcer Disease- Review. J Biosci Tech. Vol 1 (1): 52-58.