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Full-length Research Article

Gastroprotective Activity of Low-Dose Vanadium in Streptozotocin-Induced Diabetic Rats: Roles of Gastric Acid, Mucous Cells and Oxidative Stress

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Summary: Vanadium, a heavy metal with insulin-mimetic properties and gastro-protective potentials, has been reported to protect the stomach of healthy rats against aggressive agents like acetic acid. In this study, we investigated the gastroprotective effects of low-dose sodium metavanadate ($NaVO_3$) in experimental diabetic rats. One hundred male Wistar rats (100-130g) were randomized into two experiments (normal and diseased) with 5 major groups (n=10) each. First experiment included normal (control) and non-diabetic groups treated with varying doses (20mg/kg/p.o, 40mg/kg/p.o, 60mg/kg/p.o and 80mg/kg/p.o) of sodium metavanadate ($NaVO_3$) only. The second experiment included diabetes-induced (65mg/kg/i.p Streptozotocin-STZ) and diabetic groups concomitantly treated with the same doses of sodium metavanadate as in the first experiment. Body weight and blood glucose level (BGL) were measured weekly. After 8 weeks of treatment, gastric acid secretion (GAS) was determined by the continuous perfusion method. Gastric tissue malondialdehyde (MDA), reduced glutathione (GSH), sulfhydryl, nitric oxide levels, Na+/K+ and H^+-K^+-ATPase pump activities were assessed spectrophotometrically. Gastric tissue histological examination and immunohistochemistry expression of gastric MUC5AC were evaluated. Data were analyzed using two-way ANOVA and were significant at $p < 0.05$. The BGL was significantly decreased in 20 and 40 mg/kg $NaVO_3$-treated groups in both non-diabetic and diabetic Wistar groups. Basal GAS significantly decreased in NaVO3-treated diabetic groups. Stimulation with acetylcholine significantly decreased GAS in NaVO3-non-diabetic treated groups. Gastric MDA and GSH were significantly reduced in 60 and 80mg/kg-$NaVO_3$ non-diabetic treated groups. Gastric sulfhydryl and nitric oxide levels were significantly reduced in 20 and 40mg/kg-NaVO3 non-diabetic treated groups. Treatment with $NaVO_3$ in diabetic groups significantly decreased gastric MDA and sulfhydryl but increased GSH and nitric oxide levels. Gastric H^+- K^+ ATPase and Na^+-K^+ ATPase pump activities significantly decreased in diabetic groups treated with 20, 40 and 60mg/kg-$NaVO_3$ compared with the untreated diabetes group. Gastric MUC5AC expression in $NaVO_3$ non-diabetic and $NaVO_3$-treated diabetic groups significantly increased compared with control and diabetes alone, respectively. Sodium metavanadate treatment dose-dependently reduced blood glucose levels and improved body weight in diabetic rats. It also modulated gastric acid secretion via the suppression of H^+/K^+-ATPase and Na^+/K^+-ATPase activities, reduced oxidative stress markers, enhanced antioxidant defences, and increased expression of gastric MUC5AC.

Keywords*:* Diabetes, vanadium, gastric acid, H+/K+ pump activities, MUC5AC.

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INTRODUCTION

The gastrointestinal tract is exposed to various substances as it is a route of entry into the body hence, its functions and secretions can be modulated by these substances. Gastric acid secretion is a major function of the gastrointestinal system that can be affected by external factors (heavy metals, drugs, etc.) through dose and route of administration of such stimulus and the synergistic action of other stimuli (body weight, hormones, neurotransmitters etc.) (Jaishankar *et al*., 2014; Omayone *et al*., 2020; Balali-Mood *et al*., 2021). These factors initiate a response from the nervous and peripheral regulating systems. Acetylcholine and gastrin primarily affect acid secretion by stimulating histamine release from the enterochromaffin-like (ECL) cell of the gastric mucosa to stimulate the parietal cell or by somatostatin, inhibiting histamine release as a response from the regulating systems. These independent pathways converge to activate/deactivate the gastric acid pump "H^+-K^+ - ATPase" responsible for the production of gastric acid (Prinz *et al*., 1992; Kim *et al*., 2021). Sodium potassium ATPase (Na+/K+ ATPase) and H+/K+ ATPase are classified as P-type ATPases because they form a high-

energy phosphorylated intermediate during the catalytic cycle (Morth *et al*., 2009). The possibility of a relationship between the two ATPases is consistent with findings that these pumps can substitute for each other in maintaining intracellular ionic homeostasis (Faraj *et al*., 2021). The potassium ions required for the H+/K+ ATPase to function are supplied by the Na+/K+ ATPase. Gastric acid secretion is facilitated by the H+/K+ ATPase's ability to exchange the potassium that the Na+/K+ ATPase pumps into the cell for hydrogen ions. The Na+/K+ ATPase prepares the way for the H+/K+ ATPase to secrete gastric acids. The high viscosity mucus that forms a protective mucous layer at the stomach lumen-surface epithelium is predominantly made up of mucins and numerous other glycoproteins. The primary gastric mucins in human stomachs are the cell surface mucin, MUC1, and the secreted mucins, MUC5AC and MUC6 (Muthupalani *et al*., 2019). Sulfhydryl groups and nitric oxide (NO) play significant roles in regulating gastric functions, particularly influencing the activity of parietal cells, the process of blood flow, mucus secretion, and response to chemicals/irritants (Nagl *et al*., 2007; Akinade *et al*., 2022).

Vanadium compounds have been a source of interest to researchers because of their potential as therapeutic agents for the treatment of various health issues, including diabetes, atherosclerosis, and cancer (Treviño *et al*., 2019; Gilbert *et al*., 2023). Vanadium has been reported to maintain mucosa integrity by decreasing gastric acid output and enhancing mucus activity in the pyloric ligation ulcer model (Suthar *et al*., 2007). It is also suggested to have protective activities against gastric ulceration by acting as a proton pump inhibitor, enhancing antioxidant enzyme activities as well as mucosal blood flow via increased NO mechanism (Omayone *et al*., 2016; 2020). The disturbances of the gastrointestinal tract caused by autonomic gastrointestinal neuropathy, which is a consequence of diabetes mellitus complications, manifest as gastroparesis, enteropathy, and cholecystoparesis. This is a major reason for the focus on the functional state of the stomach for early diagnosis of digestive changes that can aggravate the clinical course of the existing pathology (Sirchak *et al*., 2021).

There is a dearth of information on acid and mucus secretory responses in diabetic rats during vanadium treatment. This study investigated the gastric acid and mucus secretory responses of nondiabetic and diabetic rats treated with varying doses of sodium metavanadate.

MATERIALS AND METHODS

Animals: One hundred Male Wistar rats (110-130g) were used in the study. All rats received pellet chow and water *ad libitum*.

Induction of experimental diabetes: A single intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg was used to induce diabetes (Junod *et al*. 1969). Animal with a fasting blood glucose concentration above 200 mg/dL was considered diabetic after 72 hours of induction.

Experimental design: The experimental protocol was reviewed and approved by the Animal Care and Use regulation Comittee of the University of Ibadan with Ethical approval number: UI-ACUREC/19/0025. The rats were randomly divided into 5 groups of 10 rats in 2 experiments.
Experiment 1 (non-diabetic): Group I- Control (normal rats); Group II- administered 20mg/kg/p.o $NaVO_3$; Group III- administered 40mg/kg/p.o $NaVO_3$; Group IV- administered 60mg/kg/p.o $NaVO_3$ and Group V- administered 80mg/kg/p.o $NaVO_3$.
Experiment 2 (diabetic): Group I- Diabetes alone (Diabetic untreated rats); Group II- Diabetic treated with 20mg/kg/p.o $NaVO_3$; Group III- Diabetic treated with 40mg/kg/p.o $NaVO_3$; Group IV- Diabetic treated with 60mg/kg/p.o $NaVO_3$ and Group V- Diabetic treated with 80mg/kg/p.o $NaVO_3$. Sodium metavanadate ($NaVO_3$) was administered daily by gavage for 8 weeks.
The body weight and fasting blood glucose level (BGL) of rats was measured daily. At the last day of the experiment, rats were fasted for 24 hours, gastric acid secretion was determined using the continuous perfusion method of Ghosh and Schild (1958), modified by Amure and Ginsburg (1964).
A midline laparotomy was made to expose the stomach and duodenum after the rats had been anaesthetized with pentobarbital (35 mg/kg i.p). 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. The stomach was gently rinsed with isotonic saline (pH 7.0, 37^0C) that was introduced through an orogastric cannula until gastric effluent was clear. Thereafter, the animal was perfused at a rate of 1mL/minute and gastric acid was collected via the pyloric cannula at 10 minutes intervals. Gastric acid secretion was allowed to stabilize for about 50 minutes and the mean acidity of three gastric secretions was termed basal acid output. After the basal output collection, Acetylcholine was administered (0.5 mg/kg i.m) for the stimulated acid secretory response (Skliarov, 1995). To determine acidity, 10 mls of the stomach perfusate was titrated against 0.025M sodium hydroxide (NaOH) solution with phenolphthalein as indicator (Salami *et al*., 2017).

Biochemical assays: After STZ injections, blood samples from the rats were collected from the tail of both control and diabetic animals for evaluation of blood glucose levels using the glucose oxidase method. After the 8 weeks, stomach tissues were collected and stored in 4 volumes of ice-cold 0.1M phosphate buffer, pH 7.4 and homogenized. The resulting homogenates were centrifuged at 10,000g at 4^0C for 10 minutes and the supernatant was collected and processed for biochemical estimations of; lipid peroxidation (MDA) using the method of Varshney and Kale (1990), reduced glutathione (GSH) using the method of Beutler *et al* (1963), total sulfhydryl group using the method of Sedlak and Lindsay (1968), nitrite levels using the method of Ignarro *et al* (1987), gastric Na^+/K^+ and Proton-Pump ATPse activities using the method of Ronner *et al* (1977).

Histology of the Gastric tissue: A small section of the stomach was cut and fixed in phosphate buffer formalin. Hematoxylin and eosin (H&E) staining was done after tissue processing and slide mounting. The stained sections underwent morphological evaluation, and a microphotograph was taken to reveal any pathology or microscopic alterations (Salami *et al.,* 2018).

Immunohistochemistry method: Immunohistochemistry procedure as described by Salami *et al* (2024) for gastric MUC5AC with slight modification using 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution (Catalog number: E-IR-R217 from Elabscience Biotechnology®, China). Briefly, the processed histology waxed gastric samples were subsequently dewaxed using absolute xylene for and afterward, hydration in different progressing ethanol concentrations with 70% as the lowest. Antigen retrieval was performed on the hydrated slides with citrate buffer solution (pH 6.0) Endogenous peroxide was according to manufacturer's instructions on the kit (E-AB-51447) and incubated in a humidifying chamber. The slides were rinsed afterwards while Goat serum (E-1R-R217A) was added onto the slides to prevent nonspecific binding and returned into humidifying chamber and the gastric tissues were probed with primary antibodies [Gastric MUC5AC antibody (E-AB-40037)]. They were then incubated for 2 hours at room temperature and with a secondary antibody (E-1R-R217B) with drops of substrate diaminobenzidine (DAB) was added at room temperature in the dark. The slides were rinsed with deionized water and slides were immersed in haematoxylin before ethanol and then xylene. The dried slides had a DPX mountant applied on it before a cover slip was affixed on it. Sections were observed with a light microscope (Leica LAS-EZ®) using Leica software application suite version 3.4 equipped with a digital camera.

Statistical analysis: The data were expressed as Mean ± SEM and were analyzed using a Two-Way ANOVA by means of Graphpad prism version 8.0 (GraphPad software, San Diego, CA). Differences were considered significant at $p < 0.05$.

RESULTS

Effect of sodium metavanadate on the Body Weight of Normal and Diabetic Rats: Sodium metavanadate treatment in normal rats decreased percentage body weight compared with control (Figure 1). Diabetes caused a significant reduction in percentage increase in body weight but were significantly increased by sodium metavanadate treatment (Figure 2).

Effect of sodium metavanadate on the Blood Glucose Levels of Normal and Diabetic Rats: Blood glucose levels significantly decreased in the non-diabetic 20mg/kg and 40mg/kg sodium metavanadate treated groups (83.31±0.52, 82.22±0.47) compared with control (87.29±0.64) after 8 weeks of experimentation in the healthy rats (Figure 3). The 20 and 40mg/kg sodium metavanadate treated diabetic groups had a significantly lower blood glucose level (372.7±43.63, 374±44.52) while there was no significant difference in the blood glucose level of the 60 and 80mg/kg sodium metavanadate treated diabetic groups (465±57.89, 390.2±48.64) compared with Diabetes alone group (452.3±52.85) (Figure 4).

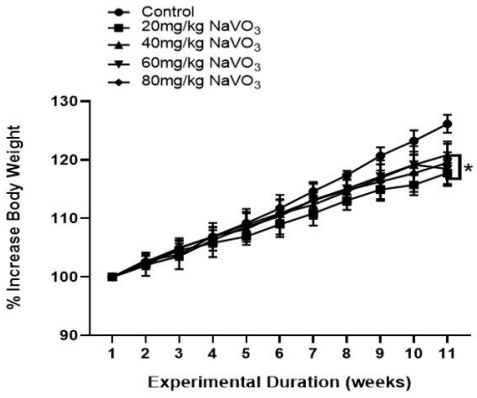


Figure 1
Effect of sodium metavanadate on percentage increase in body weight of normal (not-diabetic) rats. Values are represented as Mean ± SEM, *significant difference compared with control.

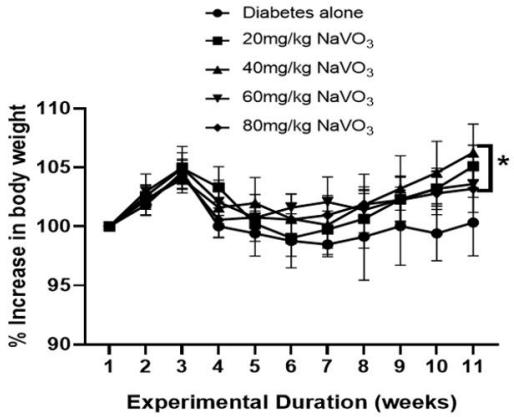


Figure 2
Effect of sodium metavanadate on percentage increase in body weight of diabetic rats. Values are represented as Mean ± SEM, *significant difference compared with diabetes alone. p=0.05

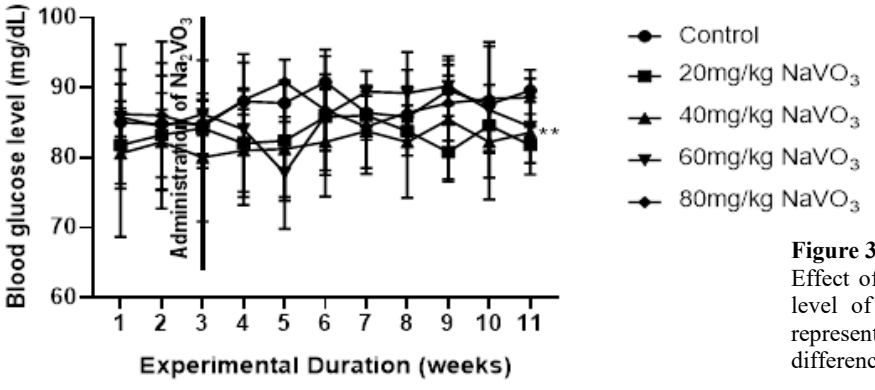


Figure 3:
Effect of sodium metavanadate on blood glucose level of normal (non-diabetic) rats. Values are represented as Mean ± SEM, *significant difference compared with control. p=0.05

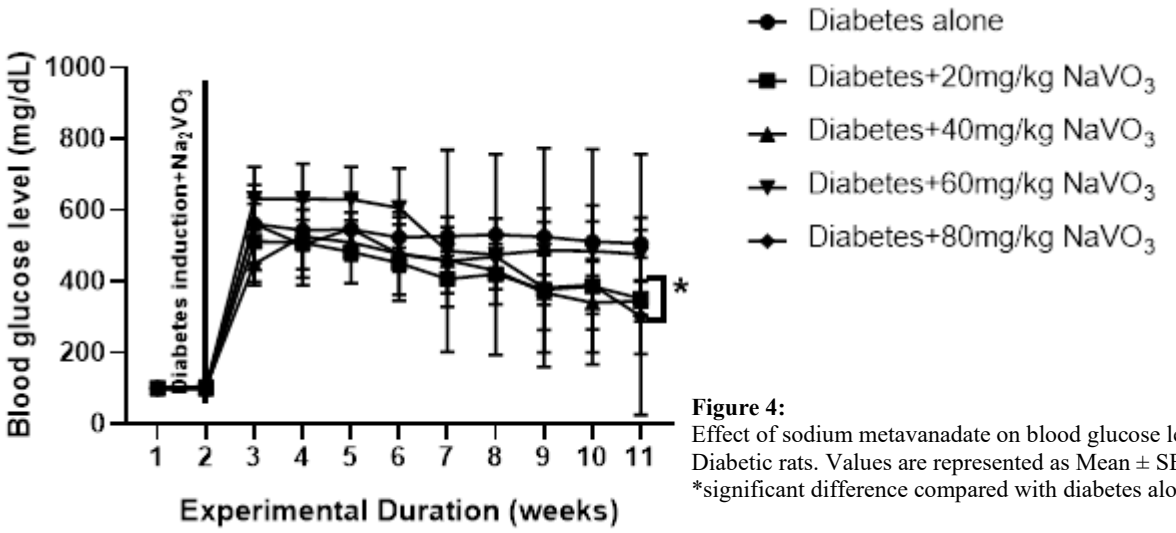


Figure 4:
Effect of sodium metavanadate on blood glucose level of Diabetic rats. Values are represented as Mean ± SEM, *significant difference compared with diabetes alone p=0.05

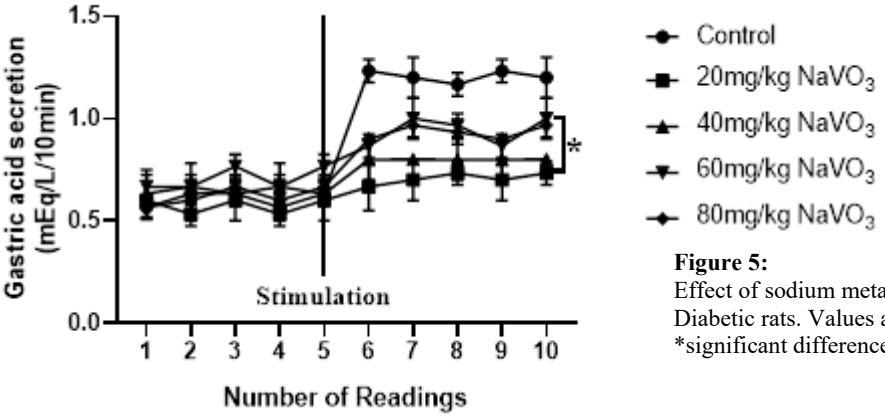


Figure 5:
Effect of sodium metavanadate on blood glucose level of Diabetic rats. Values are represented as Mean ± SEM, *significant difference compared with diabetes alone p=0.05

Figure 5:
Time-course of basal and acetylcholine-stimulated gastric acid secretion in Sodium metavanadate treated normal (not-diabetic) rats at 8 weeks**.** Values are represented as Mean ± SEM, *significant difference compared with control. p=0.05

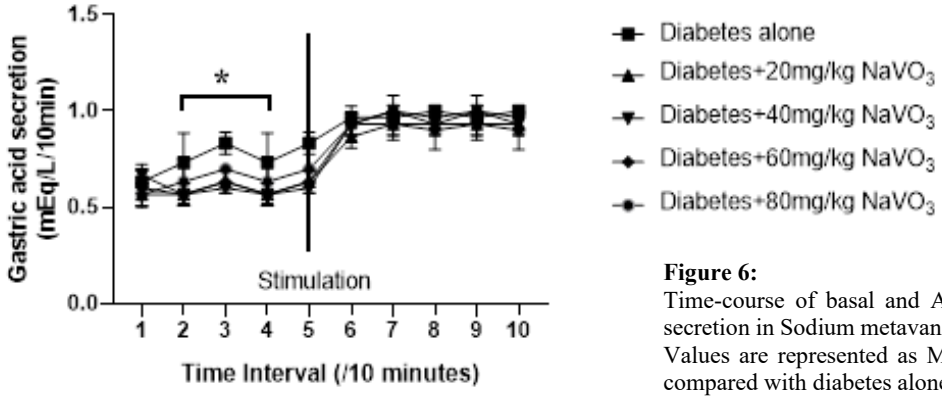


Figure 6:
Time-course of basal and Acetylcholine-stimulated gastric acid secretion in Sodium metavanadate treated diabetic rats at 8 weeks. Values are represented as Mean ± SEM, *significant difference compared with diabetes alone. p=0.05

Effect of sodium metavanadate on Basal/Stimulated Gastric Acid Secretion in Normal and Diabetic Rats: The action of sodium metavanadate on both basal and acetylcholine-stimulated gastric acid secretion in healthy rats is illustrated in Figure 5. There was no significant difference in the basal gastric acid secretion across the groups while stimulation with acetylcholine resulted in significant increase in gastric acid secretion. This was significantly decreased in groups administered sodium metavanadate (0.64±0.02, 0.72±0.03, 0.82±0.04, 0.78±0.05) compared with the Control group (0.91±0.10) (Figure 5).

Figure 6 shows the action of sodium metavanadate in diabetic rats on both basal and acetylcholine-stimulated gastric acid secretion. There was significant decrease in the basal gastric acid secretion of the groups treated with sodium metavanadate while stimulation with acetylcholine caused no significant difference in gastric acid secretion in groups treated with sodium metavanadate compared with the diabetic alone group (Figure. 6).

Effect of sodium metavanadate on Gastric MDA, GSH, Sulfhydryl and NO in Normal and Diabetic Rats: Gastric MDA significantly increased only in the 60 and 80mg/kg $NaVO_3$ treated non-diabetic groups compared to control. Gastric GSH activity significantly increased in the 20mg/kg $NaVO_3$ and decreased in the 60 and 80mg/kg $NaVO_3$ treated non-diabetic groups compared with control. Gastric sulfhydryl and nitrite levels significantly reduced in the 20 and 40mg/kg $NaVO_3$ treated non-diabetic groups compared with control (Table 1).

The 40, 60 and 80mg/kg $NaVO_3$ treated diabetic rats had a significantly lower MDA with a corresponding increase in GSH levels. Decrease in sulfhydryl levels and increase in nitrite levels of the 40, 60 and 80mg/kg $NaVO_3$ treated diabetic rats compared with diabetes alone (Table 1).

Effect of sodium metavanadate on Gastric Hydrogen Potassium ATPase (H^+/K^+ATPase) Pump Normal and Diabetic Rats: There was significant decrease in the activity of gastric H^+/K^+ATPase pump in groups administered sodium metavanadate (2103±8.73, 2511±32.19, 2252±55.15, 2357±43) compared with the control group (2747±41.12) at 8 weeks (Figure. 7). Gastric H^+/K^+ATPase pump activity in groups administered 20, 40 and 60mg/kg sodium metavanadate has a significant decrease compared with the Diabetic alone group while H^+/K^+ATPase pump activity was significantly increased in the 80mg/kg sodium metavanadate group (Figure. 8).

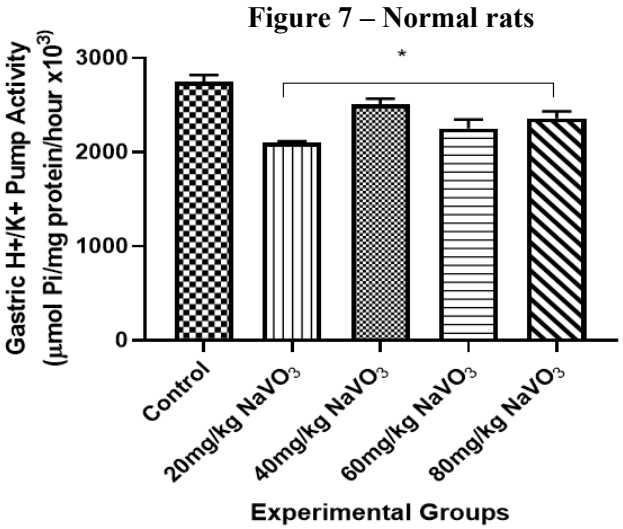
Effect of sodium metavanadate on Gastric Sodium Potassium ATPase (Na^+/K^+ATPase) Pump Normal and Diabetic Rats: There was no significant diifference in the activity of gastric Na^+/K^+ATPase pump in groups administered sodium metavanadate compared with the control group (Figure. 9). Gastric Na^+/K^+ATPase pump activity in groups administered 20, 40 and 60mg/kg sodium metavanadate has a significant decrease compared with the diabetic alone group while Na^+/K^+ATPase pump activity was significantly increased in the 80mg/kg sodium metavanadate group (Figure. 10).

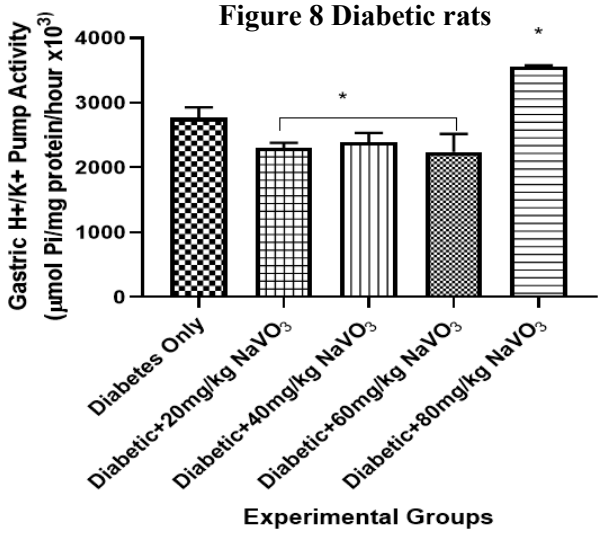
Effect of sodium metavanadate on gastric tissue histology in normal and diabetic rats: Mild sloughing off of the surface mucous cells was observed in the 60mg/kg $NaVO_3$ exposed group compared with the control (Plate 1). The histological architecture of the diabetic group showed degenerative damage compared with the NaVO3-treated groups.

Table 1:
Gastric MDA, GSH, Sulfhydryl and NO in Normal and Diabetic Rats treated with varying doses of Sodium metavandate.

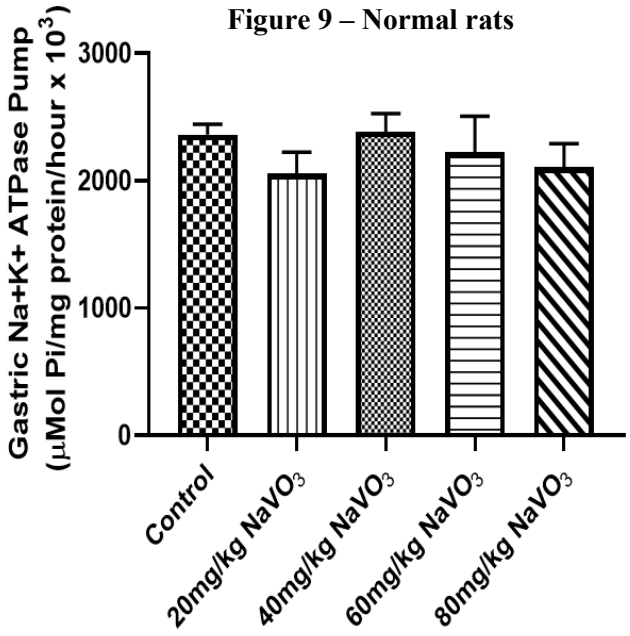
Healthy Rats	**Experimental Groups**				
Biochemical Parameters	Control	20mg/kg $NaVO_3$	40mg/kg $NaVO_3$	60mg/kg $NaVO_3$	80mg/kg $NaVO_3$
MDA (µmol/L)	1.17±0.08	1.33±0.11	1.34±0.04	1.96±0.14*	1.75±0.12*
GSH (mmol/L)	2.84±0.05	3.14±0.07*	2.70±0.04	1.95±0.07*	1.8±0.01*
Sulfhydryl (nM)	0.14±0.01	0.06±0.01*	0.07±0.01*	0.10±0.02	0.17±0.01
Nitrite (µg/g tissue)	197.8±4.17	122.3±13.19*	129.8±8.19*	192.3±5.28	169.8±5.28
Diabetic Rats	**Experimental Groups**				
Biochemical Parameters	Diabetes alone	Diabetes+20 mg/kg $NaVO_3$	Diabetes+40mg/kg $NaVO_3$	Diabetes+60mg/kg $NaVO_3$	Diabetes+80mg/kg $NaVO_3$
MDA (µmol/L)	5.97±0.41	5.15±0.25	1.89±0.25#	2.69±0.11#	1.97±0.25#
GSH (mmol/L)	1.98±0.01	2.66±0.03#	2.85±0.02#	2.78±0.06#	2.73±0.11#
Sulfhydryl (nM)	0.08±0.00	0.12±0.01#	0.06±0.00#	0.05±0.00#	0.04±0.00#
Nitrite (µg/g tissue)	79.64±1.81	115.6±6.94#	114.4±3.19#	178.5±6.11#	172.6±12.64#

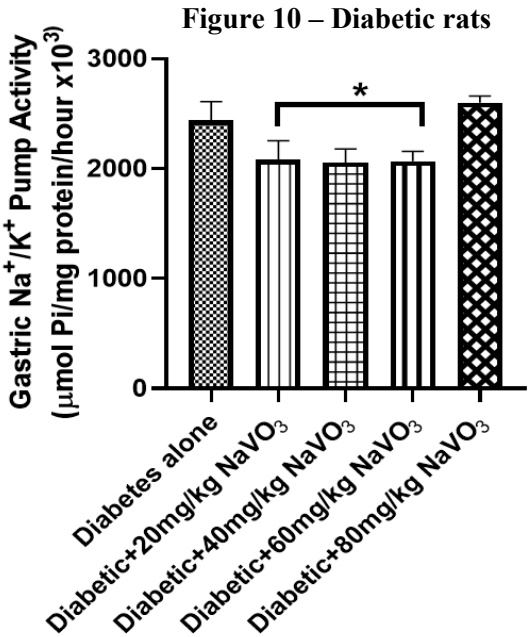
*Values are represented as Mean ± SEM, *significant difference when compared with control, #significant difference when compared with Diabetes alone. p=0.05*





Effect of sodium metavanadate on gastric H^+/K^+ATPase pump activity in normal rats (Figure 7) and Diabetic rats (Figure 8). Values are represented as Mean ± SEM, *significant difference compared with control. p=0.05





Effect of sodium metavanadate on gastric Na^+/K^+ATPase pump activity in normal (Figure 9) and Diabetic rats (Figure 10). Values are represented as Mean ± SEM, *significant difference compared with control. p=0.05

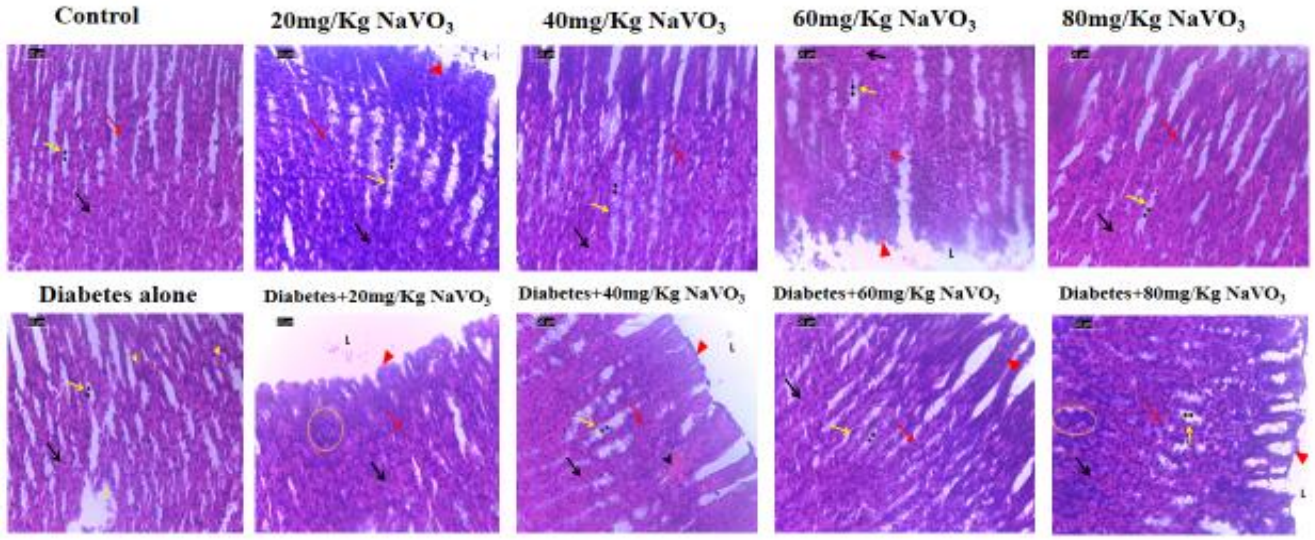


Plate 1
Effect of vanadium on the histology of gastric tissue in normal and diabetic rats
(** = gastric gland; black arrow = parietal cells; red arrow = chief cells; yellow arrow = mucous neck cells; red triangle = epithelial lining; red circle = inflammatory cells). H & E x400

Effect of sodium metavanadate on *Gastric MUC5AC expression* in normal and diabetic rats.

The level of gastric MUC5AC expression in the $NaVO_3$ exposed groups significantly increased compared with control in normal (non-diabetic) rats, as represented in plate 2. Expression of MUC5AC was significantly increased in the $NaVO_3$-treated diabetic groups compared with diabetes alone (Plate 2).

DISCUSSION

Vanadium has been a subject of interest for researchers because of the various effects it has, which can be beneficial or adverse. Its role in the management of diabetes mellitus as reported gave it a popularity that has increased the curiosity about this element and its role in the body (Dai *et al.,* 1994; Gilbert *et al.,* 2023). The decrease in body weight of the normal (not-diabetic) $NaVO_3$ treated groups corresponds with the work of Domingo *et al.,* (1996) where sodium metavanadate administered for 64 days decreased body and epididymis weights (Domingo *et al.,* 1996; Dabros *et al.,* 2006). A decrease in body weight gain is frequently observed as an indicator of diabetes mellitus. This weight loss is often associated with the development of diabetes as observed in the diabetic rats (Markholst *et al.,* 1993). However, treatment with $NaVO_3$ increased the body weight of the diabetic rats, indicating the potential therapeutic effect of $NaVO_3$ in mitigating diabetes-induced changes.

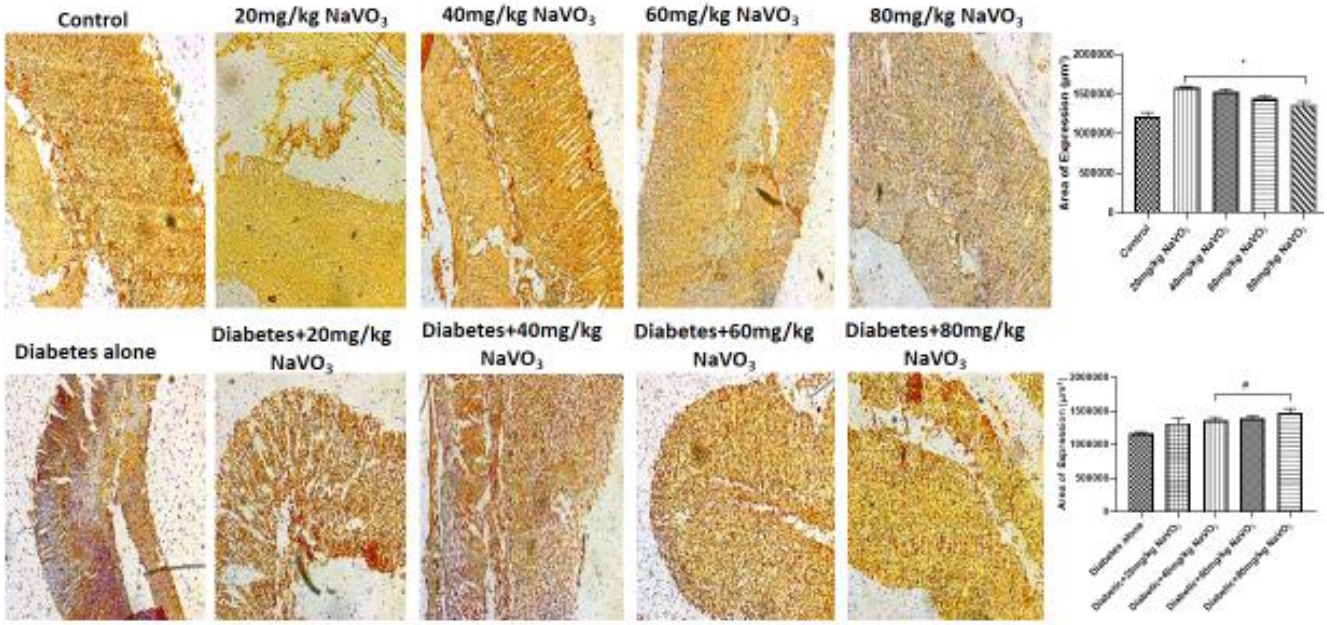


Plate 2
Effect of NaVO3 on expression of gastric MUC5AC in non-diabetic and diabetic rats (x100). The intensity of MUC5AC immunoreactivity is seen within the gastric mucosa and quantified in relation to the area of expression.

Blood glucose level was reduced in 20 and 40 mg/kg sodium metavanadate treated groups to corroborate previous studies on the glucose lowering properties of vanadium (Brichard *et al.,* 1990, Meyerovitch *et al.,* 1991; Domingo and Gomez 2016; Adam *et al.,* 2017; Dayanand *et al.,* 2024).

The secretion of gastric juice is affected by various stimulatory and inhibitory factors arising from the central nervous system and within the gastrointestinal system itself (Yao and Forte, 2003), and one of such stimulatory factors is acetylcholine, which is of the neural pathway; others include histamine and gastrin. Acetylcholine can be released by vagal and intramucosal reflex stimulation and then act directly on the parietal cell (Szelenyi, and Vergin, 1980; Furness *et al.,* 2020). This can be mediated via an increase in cytosolic levels of calcium ions, but strong synergism exists between histamine and either gastrin or acetylcholine through post-receptor interaction between the distinct pathways. All these pathways converge on and modulate the activity of the luminal enzyme, H^+/K^+ ATPase, ultimately responsible for acid secretion. (Schubert and Shamburek, 1990). Gastric H^+/K^+ ATPase is known to transport H^+ against the concentration gradient and it's the final step of acid secretion, which suggests that an inhibition of the pump will be more effective in suppressing gastric acid secretion than the receptor antagonist (Schubert and Peura, 2008). However, gastric Na^+/K^+ ATPase establishes the electrochemical gradient that is necessary for H^+/K^+ ATPase to function properly (Morth *et al.,* 2009). Hence, the decrease in the activity of gastric Na^+/K^+ ATPase in the NaVO3-treated diabetic groups explains the observed modulated reduction of gastric H^+/K^+ ATPase, unlike the diabetic untreated group, leading to the reduction in gastric acid secretion observed in this study. Disturbances to the gastrointestinal tract functions are common in diabetes mellitus. Streptozotocin (STZ) induced diabetes exhibits different levels of acid output, which are obviously dependent on the time interval after diabetes induction in rat studies (Takeuchi *et al.,* 1994). Administration of acetylcholine increased gastric acid secretion in non-diabetic experimental animals in this study; however, exposure to sodium metavanadate inhibited this increase. Activity of the gastric H^+/K^+ ATPase pump was reduced as an indication of inhibition of the activity of the cholinergic pathways and its direct effect on the parietal cells. Omayone *et al.* (2016) reported that vanadium reduced basal and histamine-stimulated gastric acid secretion in pylorus ligated animals through its probable role as a proton pump inhibitor. Basal gastric acid secretion possesses a complex, multifactorial control system. Adrenergic agonists, serotonin, secretin and somatostatin are the potent endogenous inhibitors of gastric secretion (Bech, 1986), therefore, the reduction in basal gastric acid secretion of $NaVO_3$ treated diabetic groups might probably be due to the effect of sodium metavanadate in modulating any of these pathway (Ozcelikay *et al.,* 1993; Adewoye *et al.,* 2007). Tashima *et al*. (2000) explained that vagus nerve stimulation diminished rather than increased the acid output among diabetic rats. Acetylcholine stimulation of gastric acid secretion in diabetic animals treated with sodium metavanadate resulted in no response as observed in the secretion of gastric acid which suggests the dysfunction of the cholinergic system via the vagal nerve due to the diabetic vagal neuropathy (Chang *et al.,* 2002). Burghen *et al*. (1992) pointed out that diabetic children or adolescents, especially uncontrolled patients, were at risk of developing peptic ulcer diseases. Sodium metavanadate action as a potential therapeutic agent for the management of diabetes mellitus can be coupled with its role as a proton pump inhibitor.

Heavy metals can activate lipid peroxidation in the gastric tissue, especially concerning the dose and duration of exposure (Balali-Mood *et al.,* 2021). This occurs through various mechanisms, including disrupting the gastric mucosal barrier, promoting inflammation, and generating reactive oxygen species (ROS), leading to oxidative damage

and potentially contributing to gastric tissue damage (Fernandes *et al.,* 2012). Vanadium at the lower doses (20 and 40mg/kg $NaVO_3$) posed no threat to lipids, unlike the higher doses (60 and 80mg/kg $NaVO_3$) that increased the levels of the marker of oxidation (MDA) in the gastric tissues. This might have accounted for the mild sloughing off of the surface mucous cells in the histology of the normal rats. Diabetes mellitus is a disease characterized by hyperglycaemia, depletion of antioxidants (increased generation of reactive oxygen species and decreased antioxidant levels in the body) and alteration in lipid metabolism (Hink *et al*., 2001). This was corroborated by the increase in MDA levels in the diabetic rats, while treatment with vanadium reduced lipid peroxidation. Reduced glutathione (GSH) is a very important antioxidant that protects gastric tissues against oxidative damage, especially lipid peroxidation (Kwiecien *et al.,* 2014). GSH can be oxidized to glutathione disulfide (GSSG) when it reacts with reactive oxygen or nitrogen species, and it can also be involved in the reduction of disulfide bonds (formed when sulfhydryl groups are oxidized) (Fitzpatrick *et al*., 2011). The increased GSH activity explains the protective effect of vanadium on the gastric mucosa at lower doses of vanadium in healthy rats. The diabetic rats had a decrease in GSH activity, but treatment with vanadium at all dosages increased antioxidant activity. This might have caused an improved mucosal protection and reduced damage in mucosal architecture as seen in the histology of treatment groups. To prevent acid, inflammation, and other irritants from damaging the stomach mucosa, sulfhydryl groups are essential. They are antioxidants, aid in mucus secretion, and take part in a number of cellular communication pathways that support the health of the stomach (Komolafe *et al.,* 2025). Nitric Oxide (NO) as a signalling molecule reduces gastric acid secretion by increasing the levels of cGMP in the parietal cells (Berg *et al.,* 2005), reduces inflammation, and influences gastric blood flow and mucus secretion (MacNicol and Pearson, 2021). The oxidative inactivation of NO is often measured as either nitrite (NO^{2-}) or nitrate (NO^{3-}) due to the difficulty of measuring NO levels in biological fluids. The reduction in the Sulfhydryl and NO levels in the groups with lower doses of vanadium (20 and 40 mg/kg $NaVO_3$) corroborates the results seen in the gastric acid secretion and antioxidant studies in the normal rats. This is an indication that NO is being rapidly consumed by molecules (GSH and sulfhydryl) or pathways that is helping in reducing oxidation and inhibiting acid secretion as described by Atakisi *et al.,* (2010).

The increase in the expression of MUC5AC corroborates the rapid use of NO to mediate the increase in gastric mucus release. Diabetes is associated with reduced NO, leading to endothelial dysfunction, disturbances to blood flow, and inflammation. Vanadium as a treatment increased nitrite levels in the diabetic rats, and this resulted in a decrease in gastric acid secretion and an increase in MUC5AC expression, which helped in the preservation of the histological architecture observed in this study.

In conclusion, this study provides compelling evidence for the dual role of sodium metavanadate ($NaVO_3$) in metabolic, gastric regulation and protection, particularly in the context of diabetes mellitus. $NaVO_3$ improved body weight and lowered blood glucose levels in diabetic rats, supporting its insulin-mimetic properties. It also modulated gastric acid secretion by inhibiting H^+/K^+ ATPase activity, potentially through its impact on the Na^+/K^+ ATPase activity, cholinergic pathway and vagal nerve function. Additionally, $NaVO_3$ treatment reduced oxidative damage by lowering malondialdehyde (MDA) levels and enhancing antioxidant markers such as glutathione (GSH), sulfhydryl groups, and nitric oxide (NO). However, higher doses of $NaVO_3$ in non-diabetic rats triggered mild gastric mucosal alterations, indicating possible oxidative stress. Importantly, the upregulation of gastric MUC5AC expression across treated groups suggests enhanced mucosal protection. These findings position $NaVO_3$ as a promising candidate for adjunct diabetes therapy, with added gastroprotective benefits. Nonetheless, its dose-dependent effects highlight the importance of cautious therapeutic application and the need for further studies to explore its long-term safety and mechanistic pathways.

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