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Effects of Vitamin B on experimentally induced gastric ulcers in rodents

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ABSTRACT

Background: Peptic ulcer disease (PUD) is associated with water soluble vitamin deficiencies. B group vitamins play vital roles in maintenance of essential body functions. PU patients are found to present with Vitamin B deficiency and high rates of *Helicobacter pyloric* infection. This study aims to investigate the effect of vitamin B (VB) on experimentally induced gastric ulcers in mice.

Method: Gastric ulcers were induced in adult Swiss albino mice using absolute ethanol, a non-steroidal anti-inflammatory agent, diclofenac and acetic acid. Animal were treated for 7 days with vitamin B at 12.5, 25 and 50 mg/kg before ulcer induction with ethanol and diclofenac, while in the acetic acid induced ulcers, treatment (VB 50 mg/kg) was administered for 14 and 28 days post ulcer induction. The degree of gastric ulceration was scored after which tissues subjected to histological analysis.

Results: The results showed that vitamin B ameliorated ulceration caused by absolute ethanol, diclofenac and acetic acid. In the ethanol induce ulcer, pretreatment with VB produced dose-dependent reduction in severity of ulcer when compared to control group. Ulceration of gastric mucosa was inhibited by 3.57%, 23.86% and 55.71% at 12.5, 25 and 50 mg/kg of VB respectively. Omeprazole produced an inhibition of 69.71% at 20mg/kg. VB similarly inhibited diclofenac induced ulceration by 54.31%, 69.05% and 78.94% respectively at tested doses, while omeprazole had 87.79%. For the acetic acid-induced ulcer animals, treatment significantly increased pH of gastric contents when compared with control. Histological analysis revealed dose-dependent (ethanol and diclofenac) and time - dependent (Acetic acid) decrease in severity of damage caused by the ulcerogens on treatment with VB.

Conclusion: These findings suggest that vitamin B possesses potential gastro-protective effects.

1. Introduction

Peptic ulcer disease (PUD) is disruption of mucosal lining which can occur in the stomach or duodenum. The break in mucosal lining occurs as a result of imbalance between the protective and defensive factors of the mucosa[1]. Factors implicated in the development of PUD include infection with *Helicobacter pylori*, frequent or protracted use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol and tobacco consumption, diets with high salt, fat or spice content, stress and genetic predisposition[2]. It has been reported that in one year, about four million people globally are affected by PUD, with an estimated lifetime prevalence of 5−10 % in the general population[3]. PUD causes about 246,700 deaths per year despite the availability of medications[4]. Although the global prevalence of PUD has decreased in the past decades, the incidence of complications from PUD has remained constant[3].While global rates of PUD are decreasing, Africa and Asia still have higher incidences[5] but, the decreasing tendency has leveled-off recently due to changes in risk factors

associated with the etiology of PUD, such as NSAID use and infection with *H. pylori*[6].

About 50% of the world's population is infected with *H. pylori* which inhabits the human gastric mucosa, but only approximately 10 – 20% of those infected, develop the severe gastric diseases like peptic ulcer disease while others may be associated with relatively milder gastrointestinal (GI) diseases like non-ulcer dyspepsia (NUD) and gastritis, which often remains asymptomatic. The propensity for *H. pylori* to cause severe GIT diseases is affected by the contribution from the other aetiological factors of the disease[4,7].

Studies in animals have indicated that deficiencies in water soluble vitamins can affect the development or healing of peptic ulcers in rats and have been shown to be a trigger for the development of peptic ulcers in patients with *H. pylori* infection or NSAIDS users[8]. *H. pylori* have also been implicated as an etiologic factor in vitamin B12 deficiency. Therefore, vitamin B12 deficiency is more pronounced in *H. pylori* infected patient[9]. Diet plays a role in prevention and treatment of PUD thus nutritional based therapy has been advocated for the prevention and treatment of the condition[10,11].

There is a global increase of the use of dietary supplements (DS). The value of DS for disease prevention and treatment is still questionable. There are reports that imply that use of DS in healthy individuals may not lower the risk of developing a chronic disease. However, supplementation in established cases of deficiencies can be beneficial to correct imbalances[2].

B vitamins, also known as B-complex vitamins consist of thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_7), folate (B_9), and cobalamin (B_{12}). B vitamins act as coenzymes in several enzymatic processes that support every aspect of cellular physiological functioning[13]. B group of vitamins play vital roles in maintenance of essential body functions. They facilitate energy production,[14] improve microcirculation[15,16] and promote wound healing. Vitamin B deficiencies indirectly affect the wound healing process by impairing antibody production and white blood cell function, which in turn increase the risk of infectious complications[17]. This study was designed to investigate the effect of Vitamin B (VB) on induced ulcers mice.

2. Material and methods

2.1 Materials

Omeprazole (Fidson Healthcare Plc. NAFDAC No: 04-3823, expiry date: 08/2023), Vitamin B complex was obtained from MASON vitamin INC Miami, USA, zylazine (Bioveta, Czech Republic), Ketamin (Laborate pharmaceutical India) and acetic acid (Flukka, Brazil).

2.2 Experimental Animals

Adult Swiss albino mice (29 – 34g) and Wistar Albino rats 180 – 220g of both sexes were used for this study. They were obtained from the Animal facility Center of the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja. These were fed on standard rodent pellets and clean drinking water which was given *ad libitum*. They were housed under ambient conditions and kept in plastic cages with wood shavings as bedding. The animals were allowed a duration of 5 days to acclimate to the laboratory conditions before commencement of experiment. Ethical approval (NIPRD/05:03:05-18) given for the study protocol was obtained from the Animal Care and Ethics committee of the Department of Pharmacology and Toxicology, NIPRD, Abuja Nigeria.

2.3 Studies on acute gastric ulcers induced with ethanol

The animals were weighed, labeled and randomized into five groups of 6 animals each. Animals were orally administered water (10 ml/kg), vitamin B (12.5, 25 and 50 mg/kg) respectively or omeprazole (20 mg/kg). Treatment was given for 7 consecutive days. One hour after the last dose, gastric ulcers were induced in overnight fasted mice by oral administration of absolute ethanol (10 ml/kg) to all mice. Sixty minutes after ethanol was given, the animals were euthanized by inhalation of diethyl ether. The stomachs were isolated, opened along the greater curvature and gently rinsed with normal saline. Each stomach was flattened between two petri dishes and digital pictures were taken. Ulcers formed on the glandular potion of the stomach were assessed and measured using a hand lens, divider and ruler. The ulcers were then scored[18,19].

0.0 = normal colored stomach
0.5 = red coloration
1.0 = spot ulcers
1.5 = hemorrhagic streaks
2.0 = ulcers with area >3 but ≤5 mm2

The stomachs were then placed in 10 % formal saline after which the tissues were processed for histological analysis. The sum of the score of ulceration in each group of mice divided by the number of animals was expressed as the

mean ulcer score and the percentage protection was calculated using equation 1

$$\text{Percentage protection} = \frac{(\text{Control mean ulcer score} - \text{Test mean ulcer score})}{\text{Control mean ulcer score}} \times 100 \quad \text{.....Equation 1}$$

2.4 Diclofenac Induced Gastric Ulceration.

Animals were randomly grouped (n=6) and treated as follows. Group 1 received distilled water (negative control), groups 2 - 4 were treated with vitamin B at 12.5, 25 and 50 mg/kg respectively, while group 5 was administered omeprazole 20 mg/kg. Treatment was given orally as daily single dose for seven consecutive days. Animals were fasted (food 18 h, water 2 h) before day 7 treatment. Sixty minutes after the last dose, all mice received 80 mg/kg of diclofenac and animals were subsequently euthanized after six hours. The stomachs were then isolated, opened and rinsed gently with normal saline and scored as reported earlier, after which the tissues were placed in 10 % formal saline and further processed for histological analysis. The sum of the total severity of scores in each group of mice divided by the number of animals was expressed as the mean ulcer score and the percentage protection was calculated.

2.5 Acetic acid induced gastric ulceration

The method of Takagi *et al.,* [20] as described by Tanaka *et al.,* [21] was modified to induce gastric ulcers in adult rats (n=6). The method was modified by application of ketamine and diazepam (50/10 kg/kg) anesthetization and 0.03 ml of undiluted acetic acid. This was injected using a micro syringe into the sub-mucosal layer of the glandular potion of the stomach. After the surgical procedure, animals were allowed to recover and then returned to their home cages with food and water. Twenty-four hours after the surgery, the animals were treated with normal saline, Vitamin B (50 mg/kg) or omeprazole (20 mg/kg) for 14 days or 28 days. Another group served as the sham group, where no ulcer was induced and no treatment was administered. Twenty-four hours after the last drug administration, rats were euthanized by inhalation using diethyl-ether. The stomachs were rapidly removed and the contents collected into a centrifuge tube after which the stomach was opened along the greater curvature. The stomachs were placed in 10 % formal saline for histological analysis. The gastric contents were subsequently centrifuged at 1000 rpm for 10min. The volume of gastric juice was measured after which 1 ml of gastric juice was taken out to which 1 ml of distilled water was added. The pH was then determined using a digital pH meter[22].

2.6 Histological evaluation

The stomach tissues were placed in formal saline (10 %) for fixation then dehydrated using graded concentrations of ethanol and cleared with xylene. These then infiltrated and embedded in paraffin wax. Subsequently, sections of the tissues (4-6 µm) were made with a rotary microtome and stained with hematoxylin-eosin. The prepared slides were examined under a light microscope. The tissues were assessed for distortion of the mucosal cyto-architecture, necrosis and ulceration[23].

2.7 Statistical Analysis

Group data were presented as Mean ± SEM. The Data were analyzed by ANOVA followed by a post hoc Dunnet's test using Graphpad PRISM version 6.02. The level for statistical significance was set at $p<0.05$.

1. RESULTS

3.1 Effects on Ethanol Induced Gastric Ulcers

Intragastric administration of absolute ethanol produced gastric ulceration in all mice; however, severity of ulcers in the glandular portion of treated animals was decreased by administration of Vitamin B. The severity was decreased from 7.00 ± 1.16 by water, to 6.75 ± 0.86, 5.33 ± 0.89 and 3.10 ± 0.91 by 12.5, 25 and 50 mg/kg of vitamin B respectively while omeprazole decreased the ulcer score to 2.12 ± 0.27. The corresponding percentage protection for gastric ulcerations is 3.57, 23.86 and 55.71% for the respective graded doses of Vitamin B while omeprazole exhibited 69.71% protection from the ulcerogen ethanol (Table 1). This reduction was significant at 50 mg/kg doses of Vitamin B and omeprazole.

Table 1: Effect of vitamin B on ethanol induced gastric ulcers in mice

Treatment	Dose (mg/kg)	Ulcer score	% Protection
Water	-	7.00 ± 1.16	-
VB	12.5	6.75 ± 0.86	3.57
VB	25	5.33 ± 0.89	23.86
VB	50	3.10 ± 0.91[a]	55.71
Omep	20	2.12 ± 0.27[b]	69.71

VB = Vitamin B; Omep = omeprazole. Values are presented as mean ± SEM (n = 6), Significance: compared to control [a]$p<0.05$, [b]$p<0.01$ groups (One-way ANOVA)

3.2 Effects on diclofenac-induced ulcers

Administration of graded doses of vitamin B decreased the severity of ulcers induced from 4.75 ± 1.12 in control group to 2.17 ± 0.70, 1.41 ± 0.34 and 1.00 ± 0.34 respectively. Omeprazole group showed ulcer score of 0.58 ± 0.20. The reduction in ulcer score was statistically significant in groups treated with 25 and 50 mg/kg of vitamin B. The percentage protection observed in treatment groups were 54.31, 69.05 and 78.95 % for while omeprazole produced 87.79 % protection (Table 2).

Table 2: Effect of vitamin B on diclofenac induced gastric ulcers in mice

Treatment	Dose (mg/kg)	Ulcer score	% Protection
Water	-	4.75 ± 1.12	-
VB	12.5	2.17 ± 0.70[a]	54.31
VB	25	1.47 ± 0.34[b]	69.05
VB	50	1.00 ± 0.34[b]	78.95
Omep	20	0.58 ± 0.20[c]	87.79

VB = Vitamin B; omep = Omeprazole. Values are presented as mean ± SEM (n = 5), Significance [a]p<0.05, [b]0.01,[c]0.001 treatment vs control groups (One-way ANOVA)

3.3 Effect of Vitamin B on acetic acid induced ulcers

Oral administration of vitamin B significantly (p<0.05) increased pH of gastric contents from 2.10 ± 0.28 to 4.39 ± 0.34 after 14 days of treatment, this increase was sustained up to day 28 with the pH being raised from 2.13 ± 0.11 to 5.00 ± 0.23. This effect was similar to omeprazole which significantly raised pH to 3.50 ± 0.37 (day 14) and 4.08 ± 0.11 (day 28). There was no significant difference in the volume of gastric contents after 14 and 28 days of treatment (Table 3).

Table 3: Effect of vitamin B on Acetic acid-induced gastric ulcers in rats

Treatment	Dose (mg/kg)	Volume of gastric contents(ml)		pH of gastric contents	
		D 14	D 28	D 14	D 28
Sham	-	1.21 ± 0.12	1.26 ± 0.17	3.84 ± 0.23	3.36 ± 0.36
Water	10 ml/kg	1.53 ± 0.29	1.61 ± 0.24	2.10 ± 0.28	2.13± 0.11
VB	50	1.30 ± 0.19	1.33 ± 0.31	4.39 ± 0.34[d]	5.00 ± 0.23[d]
Omep	20	1.13 ± 0.13	1.10 ± 0.12	3.50 ± 0.37[a]	4.08 ± 0.11[d]

VB = Vitamin B, omep = omeprazole; Values are presented as mean ± SEM (n = 5), Significance p<0.05 treatment vs control groups (One-way ANOVA)

Histological analysis

Histological analysis showed that administration of ethanol, diclofenac and instillation of acetic acid caused damage to gastric mucosal cell. Deep lesions were observed in negative control animals. Pre-treatment of animals with vitamin B ameliorated the damage. The intensity of the lesions was reduced from severe to moderate or slight ulceration when compared to control group. The effect of gastric tissue protection vitamin B was dose-dependent for ethanol and diclofenac induced ulcers (Figure 1 and Figure 2) and time-dependent for acetic acid induced ulcers (Figure 3).

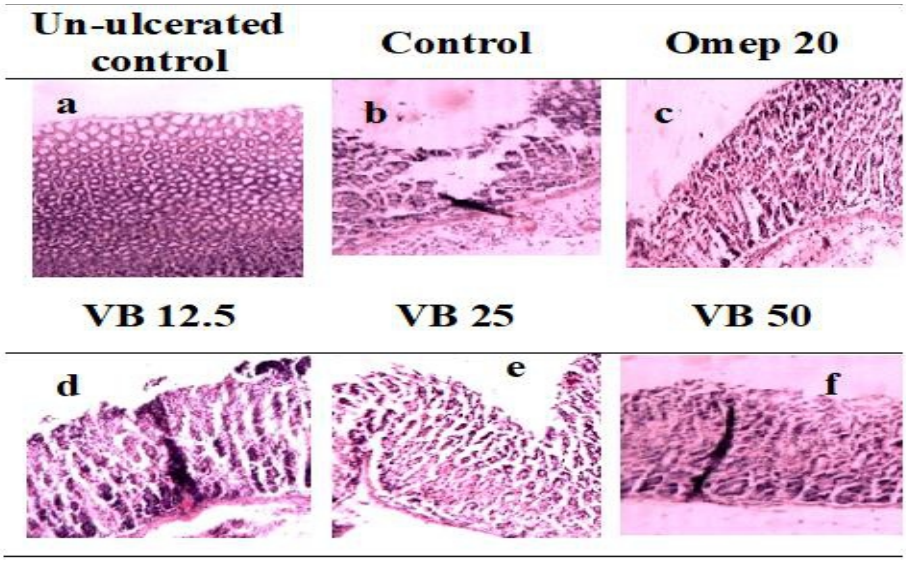

Figure 1: Effect of Vitamin B on ethanol induced ulcer

Representative photomicrographs showing the effect of Vitamin B (VB) mg/kg on gastric ulcers induced by ethanol, (a) normal gastric morphology in sham group, (b) negative control showing deep lesions, necrosis and damaged gastric tissues, (c) slight mucosal ulceration in omeprazole group (d)moderate mucosal ulceration, (e) showing hemorrhage and slight mucosal ulceration, (f) slight mucosal ulceration, (H&E; x100).

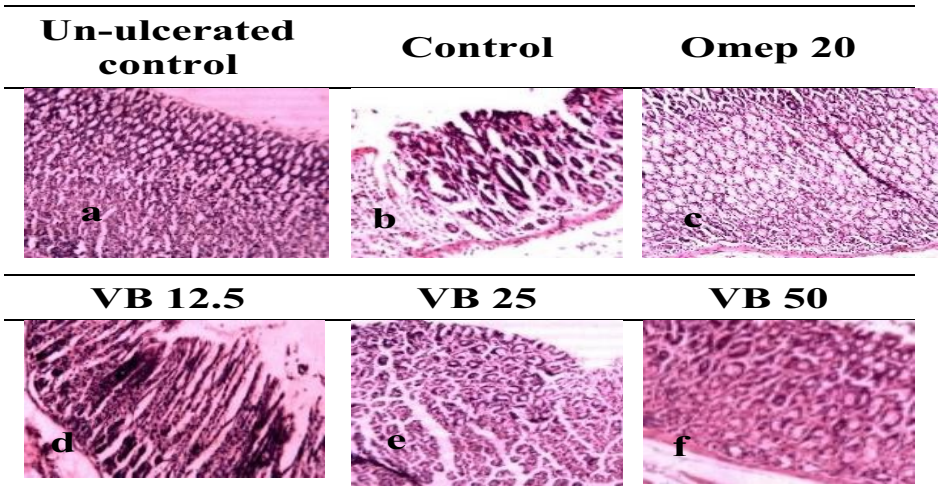

Figure 2: Effect of Vitamin B on diclofenac induced ulcers

Representative photomicrographs showing the effect of Vitamin B (VB) mg/kg on gastric ulcers induced by diclofenac (a) normal structure of mucosal cell, (b) severe mucosal ulceration, (c) necrosis (d)deep mucosal erosions (e)moderate ulceration, (f) slight mucosal ulceration with necrosis (H&E; x100).

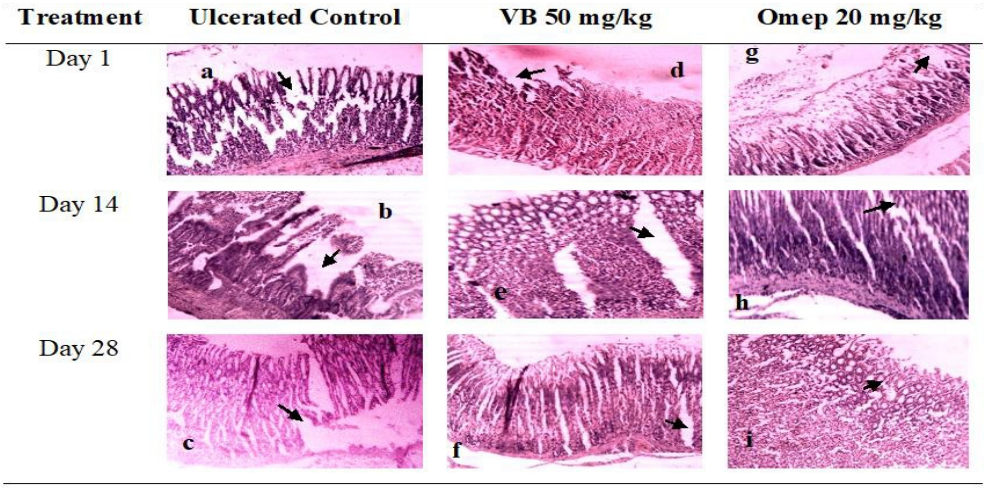

Figure 3: Effect of Vitamin B on Acetic acid-induced ulcers

Representative photomicrographs showing the effect of Vitamin B (VB) mg/kg on gastric ulcers induced by Acetic acid. (a), (b)and (c)show severe ulceration with disruption of mucosal cellular architecture on Day 1 up to Day 28. (b), (e) and (f) show deep mucosal erosion on mucosal cells on day 1 and 14 of VB treatment but severity of damage decreased by day 28 of treatment. (c), (h) and (i) omeprazole treated group showing moderate (day 14) and slight ulceration (day 28) x100; H&E

4. Discussion

The present study was designed to investigate the effect of vitamin B on gastric ulcers using commonly applied laboratory models. This study added more information regarding the potential gastro-protective effects of the B-Complex vitamins against agents which are frequently implicated in the pathogenesis of gastric ulcers. Previous studies have shown the protective effects of Vitamin C and Vitamin E[24], but this is the first study to show that treatment with vitamin B decreases the severity of gastric mucosal damage induced by the ulcerogens; absolute ethanol, diclofenac and acetic acid.

Ethanol has direct necrotizing effect on gastric mucosa. It digests the mucosal layer and exposes the mucosa to the proteolytic and hydrolytic actions of gastric juices which consist of hydrochloric acid and pepsin[25].This action by ethanol causes necrotic damage and subsequent inflammatory cell infiltration with reduced secretion of bicarbonate, gastric mucus, and nitric oxide. Ethanol also reduces gastric blood flow and causes microvascular injuries in addition to the induction of oxidative stress by increasing the production of reactive oxygen species (ROS) and inducing pro-inflammatory cytokines (e.g., TNF-α and IL-6) thereby reducing the cellular antioxidant level. This leads to a high number of infiltrating immune cells which eventually leads to gastritis and erosion of mucosal cells[26,27]. In a manner similar to ethanol, pro-inflammatory, oxidative and vascular mechanisms have been implication in the pathogenesis of NSAID-induced gastric lesions[28].The mechanisms through which NSAIDs produce damage in the gastric mucosa can be by topical or systemic actions. The topical actions of NSAIDs on the gastric epithelium may involve several mechanisms that include the irritant effects of the drugs on the epithelium with epithelial damage, reduced mucous and bicarbonate secretion which impairs the barrier properties of the mucosa. These effects are in addition to reduced mucosal blood flow, impaired platelet aggregation, reduced angiogenesis and leucocyte activation. While the most important of the systemic effects of NSAIDs in inducing gastric ulceration is inhibition of cyclooxygenase (COX) which leads to the depletion of endogenous prostaglandins[29,30]. The tissue necrosis is caused by vascular and microvascular injury, resulting in mucosal ischemia, hypoxia, cessation of nutrient and oxygen delivery, along with free radical formation. The production of free radicals in gastric mucosa is important in gastric injury due to NSAIDs like diclofenac. Tissue necrosis and the release of leukotriene B attract leukocytes and macrophages, which phagocytize necrotic tissue and release pro-inflammatory cytokines, e.g., TNFα, IL-lα, and IL-lβ[31]. In addition to pro-inflammatory cytokines, the accumulation and activation of neutrophils, contributes to microcirculation disturbances. Mucosal pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α,

interleukin (IL)-1β, and IL-8 are considered to be key factors of gastric injury induced by NSAIDs[30,32].

Administration of acetic acid (AA) on the gastric serosa tissues produces mucosal lesions with characteristics similar to human disease in terms of location, chronicity, severity, healing and recurrence process[32]. The healing process of the gastric ulcers is a very complex sequence because it involves the migration and multiplication of epithelial cells that are located at the margin of the ulcer. These developments reestablish the structure of glandular tissue and stimulate angiogenesis at the base of the lesion through the stimulation of granulation tissue. Induction of gastric ulcers by AA is associated with decreased mucous content of the stomach wall, excessive production of free radicals resulting to an increase in the pro-inflammatory interleukins TNF-α, IL-1β, and IL-6, and an increase in neutrophil infiltration into the gastric mucosa[33]. Moreover, acetic acid-induced gastric ulceration leads to chronic oxidative stress. This is a state of imbalance between reactive oxygen species and endogenous antioxidant enzymes (e.g. Superoxide dismutase, glutathione peroxide, catalase) that have protective roles on the gastric mucosa against various damaging agents that cause gastric mucosal injury[34,35].

The healing of gastric ulcers is a regeneration process that includes cell proliferation, migration, re-epithelialization, formation of granulation tissue, angiogenesis, and interactions among various cells and the matrix, all resulting in scar formation and tissue remodeling. All these processes are controlled by growth factors, transcription factors, and cytokines. Re-epithelialization, which is the migration of epithelial cells from the ulcer margin in-order to restore epithelial continuity, is an essential process for cutaneous and gastrointestinal wound/ulcer healing. This process is essential because a continuous epithelial barrier is critical for protecting granulation tissue and newly formed blood vessels from injury by luminal noxious factors[31]. Epithelial Growth Factor (EGF) plays an important role in promoting gastric epithelial cell migration, proliferation and differentiation into the granulation tissue, to cover defects created by the mucosal injury[11]. Epidermal growth factor (EGF) binds to its receptor, EGF-R, and stimulates cell migration, proliferation, and differentiation[31]. Previous studies have shown that the components of B vitamins have accelerated wound healing of other forms of ulcers including oral ulcers[13,36,37,38]. Folic acid (vitamin B_9) and cobalamine (vitamin B_{12}) and have been shown to enhance the activity of EGF[11,39]. Folic acid, a component of vitamin B complex has demonstrated gastroprotective and enhanced gastric mucosal healing effects by antisecretory, anti-inflammatory, improved cell proliferation and angiogenesis actions.[11] The ulcer healing effect of Vitamin B may be attributed to the activity of the components which promotes cell regeneration and regulate growth factors that have a role in healing of ulcers.

Gastric acid plays an important role in the pathogenesis of ulcers and interfere with the repair of superficial injury[40]; therefore, regulation of gastric acidity is important in the management of PUD. Acid suppressants e.g. proton pump inhibitors (PPIs) block acid production in the stomach, providing relief of symptoms and promoting healing[41]. Previous studies using complexed thiamine showed gastro-protective actions of the thiamine complex by reduction of gastric acid secretion[42]. In this test, treatment with vitamin B increased gastric pH which is indicative of acid suppressant actions of its constituent thiamine (Vitamin B_1).

Antioxidants act as scavengers of reactive oxygen species (ROS), prevent lipid peroxidation and neutralize other free radical mediated processes, thereby protecting the human body from some diseases caused by oxidative stress[43]. Injuries of gastric mucosa in ulceration have been related to release of free radicals by ulcerogens[44].Treatment of mice with vitamin B ameliorated the deleterious effect of the ethanol, diclofenac and acetic acid. Ulcer scores were reduced, indicating protection of gastric mucosa against the injurious effect of the ulcerogens used in this study. The appearance of histological features of the gastric mucosa showed decreased damage to the structural integrity of the gastric mucosal cells and tissue protection by administration of Vitamin B. These B complex vitamins have shown antioxidant actions as separate compounds[45,46,47] or when administered in combination as a group of B vitamin compounds[44]. The reduction of severity of ulcers in the animals produced by the administration of vitamin B may be attributed to free radical scavenging actions of the B complex vitamins that include thiamine, pyridoxine and panthotenic acid as contributory to the mechanism of gastro-protective activity.

Data obtained from this study has shown that administration of vitamin B at the doses tested, was more protective of the gastric mucosa when given as preventive treatment rather than when administered as a curative agent. Thus, indicating than vitamin B complex could be employed as an adjunct for the treatment of ulcers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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