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Full Length Research Paper

Gastroprotective Effect of Magnesium on Indomethacin-induced Gastric Ulceration in Normal and Alloxan Induced-Diabetic Rats

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ABSTRACT

Magnesium is reported to reduce acid secretion and possess hypoglycemic properties. However, information about its gastroprotective ability is unknown. The gastroprotective effect of magnesium on indomethacin-induced gastric ulceration in normal and alloxan-induced diabetic rats was investigated. Male rats were divided into 7 groups of 6 animals each. Groups 1,3,5 received normal saline(0.2ml), magnesium(500mg/kg) and omeprazole(20mg/kg) respectively. Group 2,4,6,7 were diabetic animals treated with normal saline, magnesium only, omeprazole only and both magnesium and omeprazole respectively. Diabetes was induced with alloxan (120mg/kg i.p) and ulcer was induced with indomethacin (40mg/kg).Ulcer was scored and stomach samples were stored for histological studies. Ulcer score reduced significantly in normal animals treated with magnesium (2.83±0.55), magnesium treated diabetic group (4.7±0.60) and group treated with both magnesium and omeprazole compared to the controls. Histological assessment of the gastric mucosa and submucosa revealed infiltration by inflammatory cells in normal and diabetic animals whereas the groups pretreated with magnesium did not show any inflammation, edema, and hemorrhage. Animals pretreated with omeprazole only showed mild inflammation while the group pretreated with both omeprazole and magnesium showed scanty inflammation with no signs of edema and hemorrhage. The gastro-protection against indomethacin-induced gastric ulceration observed in this study could have been produced by magnesium pretreatment.

Keywords: Magnesium, *diabetes mellitus*, ulcer, gastrointestinal tract

INTRODUCTION

Gastric ulcer, also known as peptic ulcer, is a localized area of erosion in the stomach lining resulting in abdominal pain and sometimes bleeding. A perforated ulcer is one which has become deep enough to completely penetrate tissue layers. Its therapy has undergone many strides over the past few years and a number of drugs are now available for the treatment. These drugs are broadly classified into two, those that decrease or counter acid/pepsin secretion and those that afford cytoprotection by virtue of their effects on mucosal defensive factors (Goel and Sairam, 2002).

Diabetes mellitus is a syndrome characterized by persistent and sustained hyperglycemia caused by a disorder in the metabolism of carbohydrate, protein and fats which may arise as a result of either partial or total

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insulin deficiency and or insulin insensitivity at the tissue or cellular level.

In addition to well-established risks among diabetic subjects for vascular diseases, renal disease, blindness, and amputations, diabetes is also associated with a poorer outcome from several acute medical conditions, including myocardial infarction and may also be a risk factor for complicated ulcer disease (Engelgau *et al*, 2004; Almdal *et al*, 2004; Thomsen 2006). It has also been observed that patients with diabetes mellitus may have poorer outcomes after peptic ulcer complications due to diabetic angiopathy (Weil *et al*, 2000), delayed ulcer healing (Harsch, 2003), blurring of symptom due to autonomic neuropathy, and increase risk of bacterial sepsis (Shah and Hux, 2003).

In spite of advances in drug management of diabetes, there are still complications and adverse drug reactions, none of them being unequivocally successful in maintaining normal blood glucose level and avoiding complications. Despite all advances in therapeutics, diabetes mellitus still remains a major cause of morbidity and mortality in the world (WHO, 1999).

Magnesium functions as an essential cofactor for more than 300 enzymes. It is essential for all energy-dependent transport systems, glycolysis, oxidative energy metabolism, biosynthetic reactions, normal bone metabolism, neuromuscular activity, electrolyte balance, and cell membrane stabilization (De Valk, 1999; Yeh *et al*, 2003). In diabetes mellitus, it has been reported that magnesium supplementation can prevent cardiovascular disease and has been found to improve insulin sensitivity, insulin secretion, reduce insulin-stimulated glucose uptake and stabilize blood glucose levels (Lal *et al*, 2003; Barbagallo *et al*, 2007). Thus, magnesium supplementation may be an important component of the overall treatment of many diabetics.

In this study we investigated the gastroprotective effect of magnesium supplementation on indomethacin-induced gastric ulceration in normal and diabetic rats..

MATERIALS AND METHODS

Magnesium sulphate (Mg_2SO_4) and alloxan were purchased from Sigma Aldrich Chemical Co. (St. Louis, Missouri). Omeprazole and indomethacin were both purchased from Danax Pharmaceuticals, Ibadan.

Animals and Animal Grouping

Forty-two (42) albino rats weighing (200-240g) were used for this study. The rats were housed in cages and acclimatized for two weeks in the animal house of the Department of Physiology, University of Ibadan. They were fed with standard rat chow and allowed free access to drinking water according to guidelines and regulations of the National Institute of Health (NIH) (NIH publication 85–23, 1985) for laboratory animal care and use. The rats were divided into seven groups of 6 animals each. Group 1 was the normal control; group 2, diabetic control; groups 3 and 4 were normal and diabetic animals treated with magnesium (500mg/kg/d) (Dhande *et al*, 2009), groups 5 and 6 were normal and diabetic animals treated with omeprazole (20mg/kg) (Tari *et al*, 1996) prior to ulcer induction, group 7 animals were diabetic animals treated with magnesium and then omeprazole prior to ulcer induction. Diabetes was induced in all diabetic groups with a single intraperitoneal dose of alloxan (120mg/kg) (Szkudelski 2001). Animals were given Magnesium (500mg/kg/day) in their drinking water for a period of 14 days prior to gastric ulcer induction.

Induction of Diabetes Mellitus

Diabetes Mellitus was induced in the experimental animals by a single intraperitoneal dose of alloxan (120mg/kg) (Szkudelski 2001) dissolved in normal saline. The blood glucose of each animal was measured using the glucometer. A drop of blood was obtained from small cuts of the tail unto a glucometer test trip. The glucometer utilized the glucose oxidase principle of glucose analysis. Diabetes was confirmed 48hours after alloxan injection by a sustained blood glucose level between 250 and 360mg/dl.

Experimental protocol

Prior to ulcer induction, animals were fasted for 24 hours, thereafter, Indomethacin (40mg/kg) was administered orally (Elegbe, 1978). Six hours after indomethacin administration, the animals were euthanized by cervical dislocation; stomachs were surgically removed and opened by incision along the lesser curvature. Macroscopic examination of the stomach was carried out with a hand lens at x2 magnification; ulcer was scored using the method of Olaleye and Ajeigbe (2009). The stomach samples for each group were stored in 10% formalin to prevent autolysis. Histological sections of the stomach were made and stained with haematoxylin and eosin dye and then mounted in Canada basalm. Microscopic examination was then carried out under light microscope.

Statistical analysis

Mean ulcer scores were recorded for each group and the standard error of mean was calculated. The student t-test was used to assess the level of statistical significance of

the results obtained between the control group and each of the experimental group. 0.05 was taken as the level of significance.

RESULTS

Ulcer score in Normal control, magnesium treated and omeprazole treated animals (Fig. 1)

There was 63.61% reduction in ulcer score ($p<0.01$) in normal animals treated with magnesium and 53.8% reduction in ulcer score in normal animals treated with omeprazole when compared with normal control animals.

Ulcer score in Diabetic control, magnesium treated and omeprazole treated animals (Fig. 2)

Diabetic animals treated with magnesium only, showed significant reduction ($P<0.01$) in ulcer formation when compared with diabetic control animals. Treatment with both magnesium and omeprazole also caused significant reduction ($P<0.01$) in ulcer formation when compared to diabetic control animals. However, diabetic animals pretreated with omeprazole prior to ulcer induction showed no significant difference in ulcer score in these animals when compared with diabetic control animals.

Comparism of ulcer score in normal and diabetic animals (Fig. 3)

An increase in ulcer score ($p<0.05$) in all diabetic groups was observed when compared with control animals.

Histological studies

Histological examination of the stomach indicate that in normal control animals there was multi infiltration of the mucosa and submucosa by inflammatory cells including plasma cells, eosinophils, lymphocytes and neutrophils. Minimal infiltration of the surface epithelium was also observed (Plate A- left). In normal magnesium treated animals (Plate A- right) histological examination of the stomach indicated that the gastric mucosa was free of inflammation, edema and hemorrhage. In diabetic control animals (Plate B - left), histological examination revealed moderate to severe infiltration of the gastric mucosa and submucosa by eosinophils. Limited surface mucin production of mucous epithelial cells was also observed. In the magnesium treated diabetic animals (Plate B - right), the mucosa and submucosa of the stomach were free of inflammatory cells as well as hemorrhage. In normal control animals treated with omeprazole just prior to ulcer induction(Plate C – top left), mild focal submucosal and laminal propia infiltration by eosinophil polymorphs was observed while in the diabetic animals treated with omeprazole, (Plate C – top right) gastric submucosa and epithelial infiltration by inflammatory cells was observed. In diabetic animals treated with omeprazole and magnesium, the gastric mucosa showedscanty infiltration of eosinophils in the lamina propia and the submucosa showed no signs of hemorrhage or edema (Plate C – bottom left).

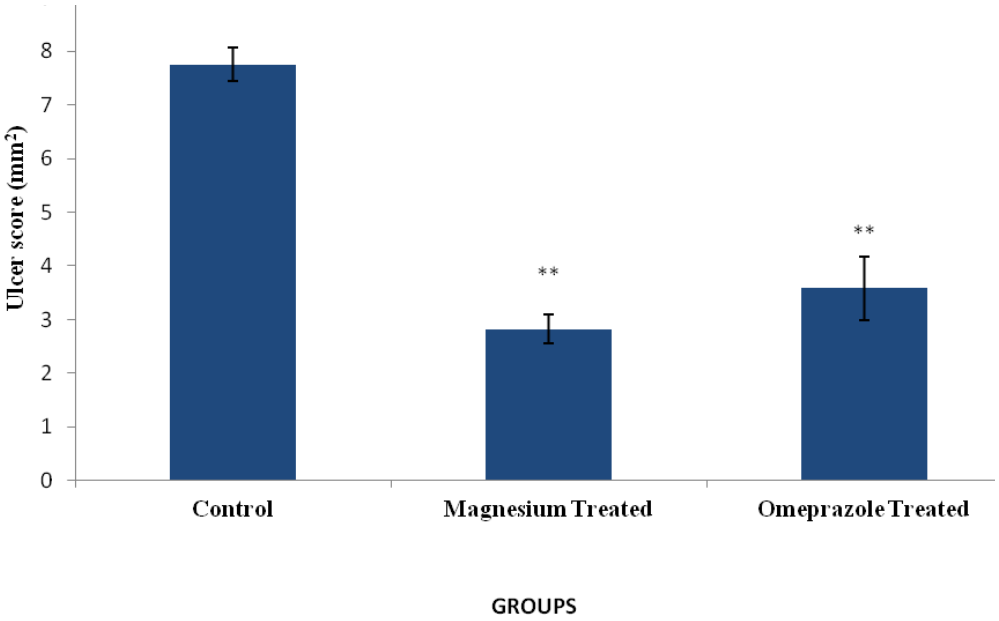


Figure 1

Ulcer score (mm^2) in Control, Magnesium treated and Omeprazole treated Normal animals. Values represent Mean ± SEM. ** indicate value significantly different ($P<0.01$) from normal control values.

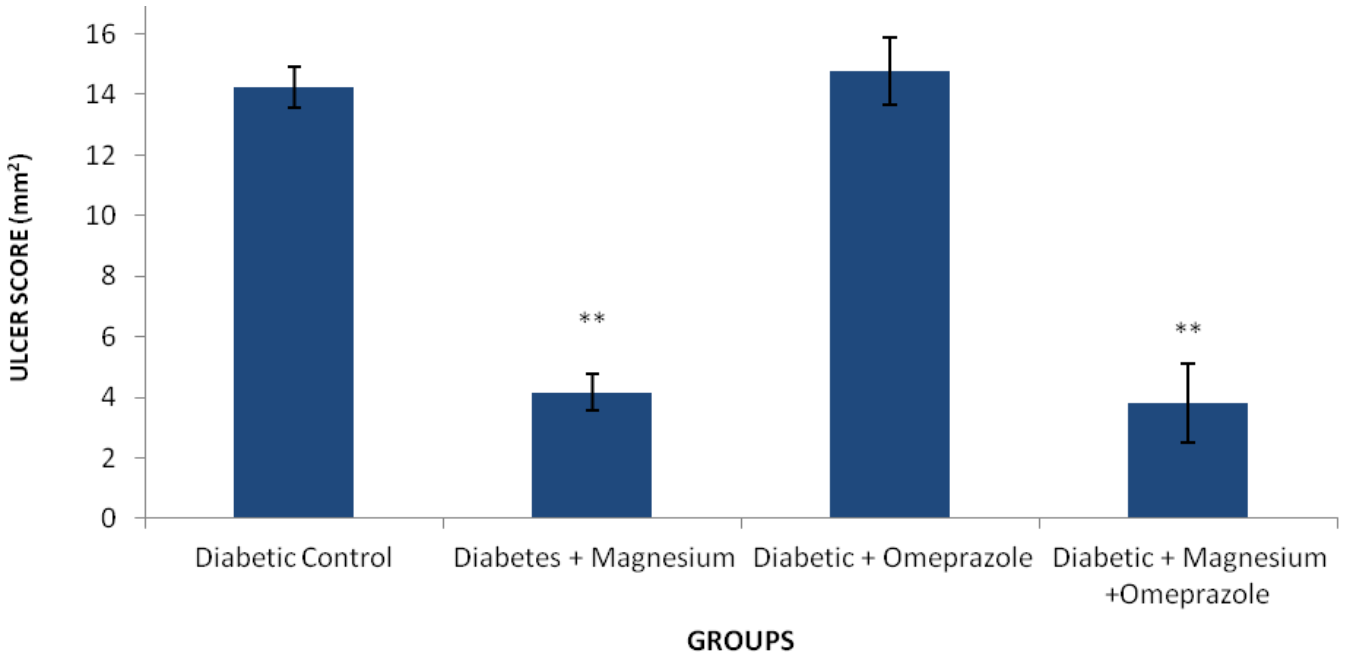


Figure 2
Ulcer score (mm2) in diabetic control, diabetic magnesium treated, diabetic omeprazole treated and diabetic magnesium and omeprazole treated animals. Values represent Mean ± SEM. ** indicate value significantly different (P<0.01) from diabetic control values.

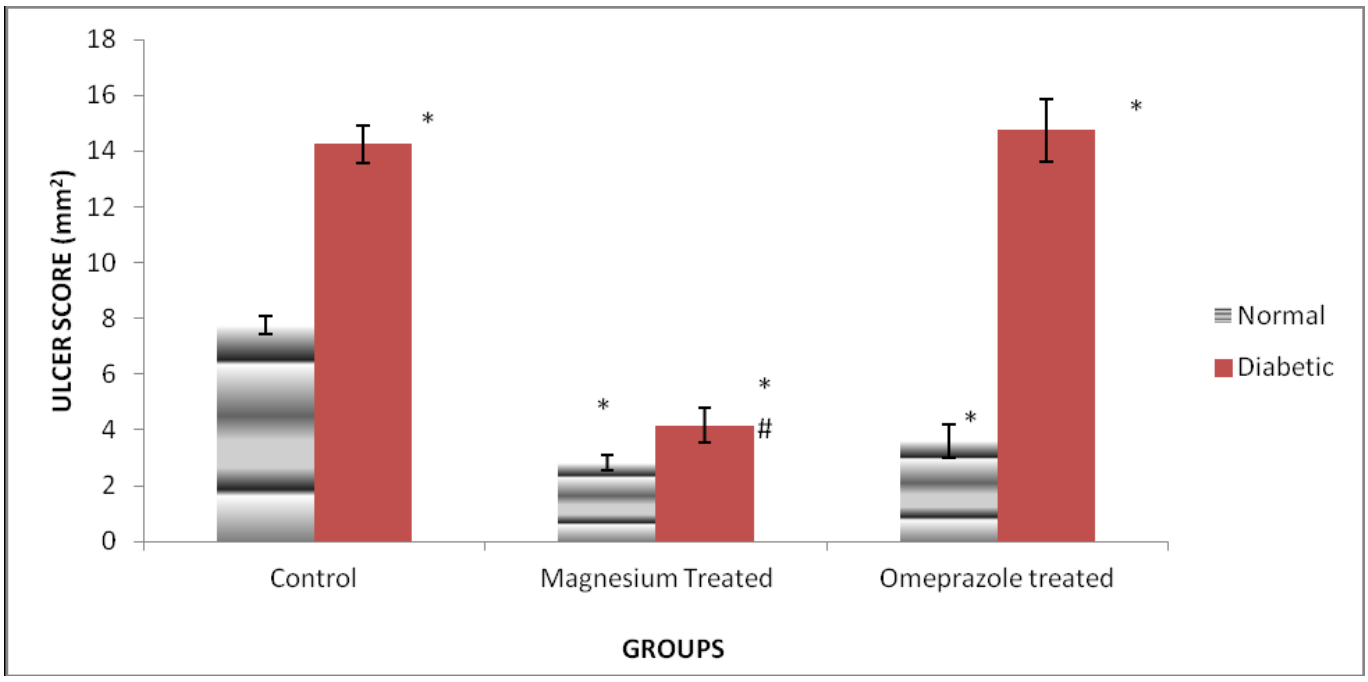


Figure 3
Ulcer score (mm2) in normal and diabetic (untreated, magnesium treated and omeprazole treated) animals. Values represent Mean ± SEM. * indicates value significantly different (P<0.05) from normal control. # indicates values significantly different (P<0.01) from diabetic control values.

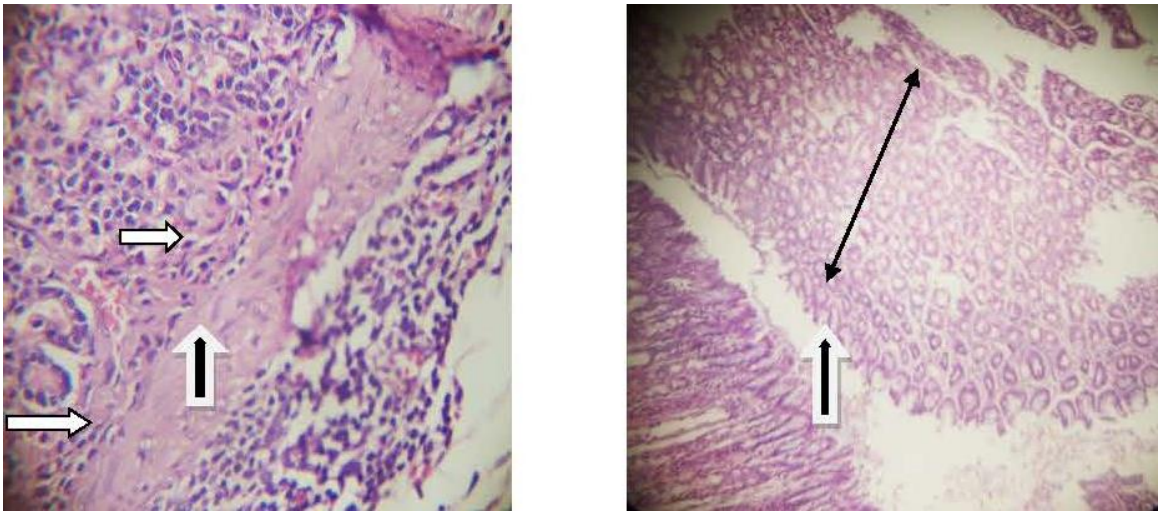


Plate A:
Left: Stomach section of normal untreated animals showing inflammation and infiltration of submucosal layer (black arrow) by inflammatory cells (white arrow). **Right:** Stomach section of normal animals treated with magnesium showing normal architecture of the mucosal layer (spanned) which is free of inflammatory cells (black arrow; surface mucin). x400 H&E

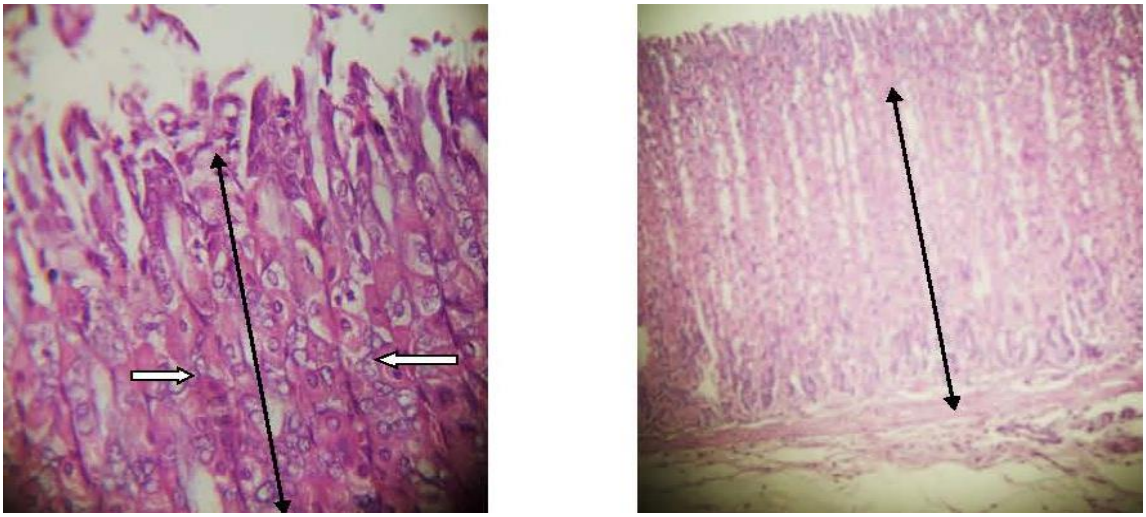
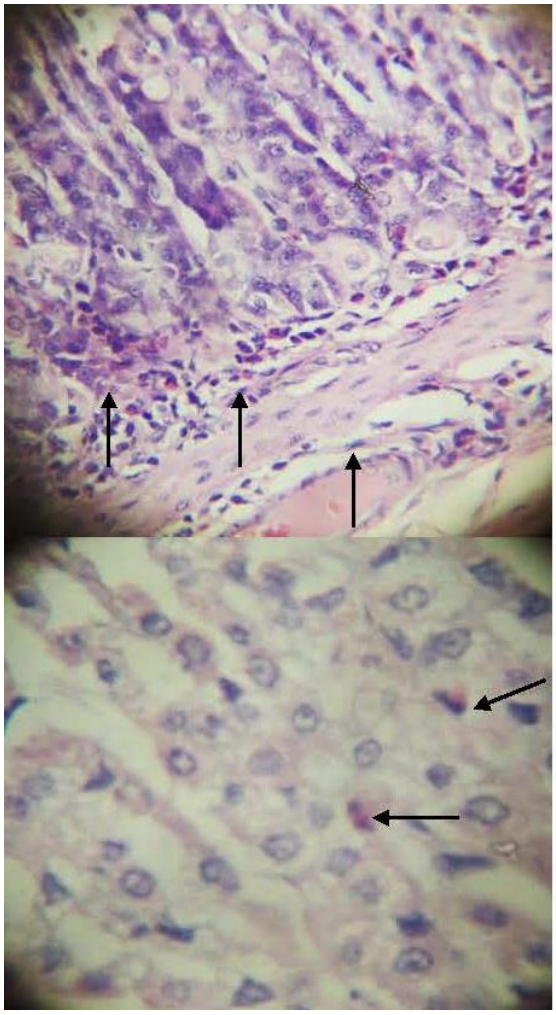


Plate B:
Left: Stomach section of diabetic untreated animals showing infiltration of the mucosa layer (spanned) by the inflammatory cells. Parietal cells (white arrow) are however normal. **Right:** Stomach section of diabetic animals treated with magnesium showing that the mucosa is free of inflammatory cells. The submucosa and muscularis muscle are also free of inflammation. No hemorrhage seen. x400 H&E

DISCUSSION

Results obtained from this study suggest that in both normal and diabetic animals, treatment with magnesium led to a reduction in gastric ulcer formation. Results obtained also suggest that diabetes mellitus may increase the risk of developing gastric ulcer when compared with normal animals; an observation which is in agreement with the report of Naitoi *et al* (2009) and Chaturvedi *et al* (2009).

Histological observations suggest that indomethacin induced ulceration is characterized by edema, acute necrotic lesion and haemorrhage of the gastric mucosa (Elegbe, 1978; Olaleye and Ajeibe, 2009) and these were observed to be most severe in the untreated (normal and diabetic) groups. However in the magnesium treated groups, histological examination of sections of the stomach showed scanty or no inflammation of their submucosa. The absence of edema and hemorrhage was also noted in these animals as against the normal control and diabetic control animals. These observations further indicate that magnesium treatment may exact a gastro-protective effect on indomethacin induced gastric ulceration.



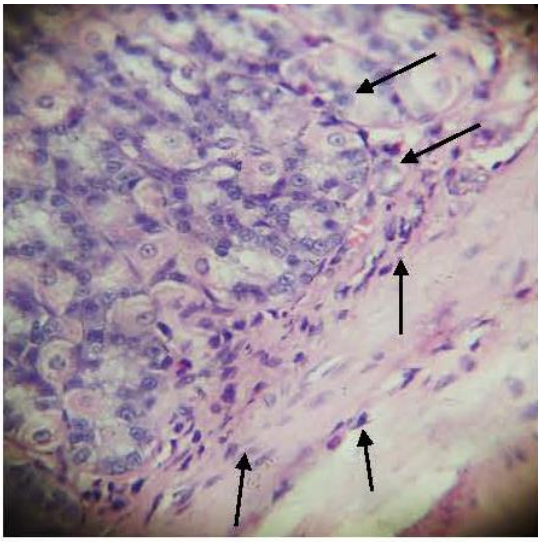


Plate C:
Top left: Stomach section of normal animals treated with Omeprazole showing mild gastric submucosa and epithelial infiltration by inflammatory cells including eosinophyls, neutrophils and lymphocytes (slender arrows). **Top right:** Stomach section of Diabetic animals treated with omeprazole showing mild gastric submucosa and epithelial infiltration by inflammatory cells including eosinophils, neutrophils and lymphocyte (slender arrows) spanning the mucosa layer. **Bottom left:** Stomach section of diabetic animals treated with magnesium and omeprazole showing insignificant inflammatory cells within the mucosa layer (slender arrow showing eosinophils). No submucosa hemorrhage or edema observed. X400 H&E.

Various reports suggest that the drug therapy for treatment of gastric ulceration has been broadly classified into two categories, those that reduce or block acid/pepsin secretion and those that afford cytoprotection by virtue of their effects on mucosal defensive factors (Goel and Sairam, 2002). These drugs act by different mechanisms and most of the commonly used drugs such as H2- blockers (ranitidine, famotidine etc), M1- blockers (pirenzepine, telenzepine etc), proton pump inhibitors (omeprazole, lansaprazole etc), decrease secretion of acid while, drugs like sucralfate and carbenoxolone promote mucosal defenses (Goel and Bhattacharya, 1991; Goel and Sairam, 2002).

Sandor *et al* (2006) reported that magnesium compounds administered orally are among the most frequently and efficient antacid drugs used. Sandor *et al* (2006) also suggested that the gastro-protective effect of magnesium could be due to its ability to indirectly reduce gastric acid secretion via inhibition of calcium signaling mechanisms on the parietal cells. The observed reduction in ulcer index in both normal and diabetic magnesium treated animals in this study suggests that in addition to its reported ability to reduce gastric acid secretion (Sandor *et al*, 2006), magnesium treatment may also possesses gastro-protective properties against indomethacin-induced gastric ulceration.

It is noteworthy however, that in diabetic animals treated with omeprazole only there was no significant difference in ulcer index when compared with diabetic control animals, whereas treatment with both omeprazole and magnesium caused a significant reduction in ulcer index. This may well be that diabetes mellitus impedes the anti-ulcerogenic properties of omeprazole while its actions (i.e. omeprazole) may be potentiated by magnesium in the diabetic state.

Indomethacin a non-steroidal anti-inflammatory drug (NSAID) used to reduce fever, pain, stiffness and swelling is also used to induce experimental gastric ulceration as it has been reported to cause gastric ulceration by inhibition of prostaglandins (Olaleye and Ajeigbe, 2009). It has also been reported to promote salt and water retention by interfering with the prostaglandin induced inhibition of both chloride reabsorbtion and of the action of anti-diuretic hormone (ADH) (Lieberthal *et al*, 1987).

Prostaglandins particularly PGI_2 and PGE_2 are synthesized by gastric mucosa and are reported to promote cytoprotective mucus production that protects the gastric mucosa against erosion and damage by acting

as an unstirred layer that allows a pH gradient to develop at the mucosal surface of the stomach (Konturek, 1985; Werther, 2000). The adherent mucus gel produced will also present a physical barrier against luminal pepsin and support the surface neutralization of acid by the mucosal bicarbonate (Werther, 2000). Prostaglandins also reported to be involved in the control of gastric mucosal blood flow and protection against potentially noxious agents (Konturek, 1985).

Magnesium has been reported to stimulate the production of prostacylin (Sipes *et al*, 1994) and prostaglandins in vascular cells (Satake *et al*, 2004). Magnesium has been reported to stimulate the production of PGI_2 (Watson *et al*, 1986; Nadler *et al*, 1987; Satake *et al*, 2004). However, its mechanism of prostaglandins production is however unknown (Watson *et al*, 1986; Nadler *et al*, 1987; Satake *et al*, 2004).

Molecular studies on prostaglandin synthesis have shown that both calcium and phospholipase A_2 (PLA_2) are essential in PGI_2 production (Whorton *et al*, 1984; Hassid and Oudinet, 1986; Clark *et al*, 1991). Magnesium, which is sometimes referred to as nature's physiologic Ca^{2+} channel blocker, has the effect of activating various enzymes, being different from synthetic Ca^{2+} channel blockers (Grubbs and Maguire, 1986). Satake *et al* (2004) in their study suggests that magnesium acts on cyclooxgenase and PGI_2 synthetase which converts endogenous arachidonic acid to PGI_2; thus increasing prostaglandins production.

It is not unlikely that magnesium may exert its anti-ulcerogenic effect either due to its blockade of indomethacin action or its ability to stimulate the production of prostaglandins.

In conclusion, this study has shown that magnesium treatment may possess gastro-protective and anti-ulcerogenic properties in both normal and diabetic animals. Its gastro--protective effect may be due to the ability of magnesium to stimulate prostaglandin production which has been reported to exhibit anti-ulcerogenic properties (Wallance, 2008).

Further studies are needed to elucidate the gastroprotective mechanisms involved in the inhibition of gastric ulceration by omeprazole and magnesium in the diabetic condition. In addition to this, studies using other different ulcer models may further elucidate the gastro-protective and anti-ulcerogeinc properties of magnesium.

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