Effect of *Gongronema latifolium* Ethanol Leaf Extract on Gastric Acid Secretion and Cytoprotection in Streptozotocin-induced Diabetic Rats

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ABSTRACT

Objectives: *Gongronema latifolium* leaves have been used in folklore medicine to manage diabetes mellitus and alleviate dyspepsia. This study aimed to provide a pharmacological basis to the medicinal use of *Gongronema latifolium* as an antidiabetic and antiulcerogenic agent in diabetes mellitus.

Methods: Ethanol extract from the leaf (200 mg/kg bodyweight) of *Gongronema latifolium* was administered to both streptozotocin-induced diabetic and control groups orally for 14 days. Gastric acid secretion was measured and ulcer was induced using ethanol and four-hour pyloric ligation.

Results: The mean bodyweight was significantly lower ($p < 0.01$), while the mean weight of the stomach, liver and small intestine to bodyweight ratio was increased significantly ($p < 0.05$) in the two diabetic groups compared to control. Extract significantly ($p < 0.01$) reduced the blood glucose level similar to the non-diabetic control.

Keywords: Blood glucose, diabetes mellitus, mucus, organ weight, stomach, ulcer

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Basal and stimulated acid secretion in diabetic control rats was significantly ($p < 0.01$) decreased when compared to control. Extract administration increased the stimulated gastric acid secretion to a level significantly ($p < 0.05$) higher than control while reduction in gastric secretion by ranitidine was similar compared with control. *Gongronema latifolium* treatment significantly ($p < 0.05$) reduced ulcer scores in both ulcer models and increased mucus weight in the diabetic group.

Conclusion: These results suggest that *Gongronema latifolium* antiulcerative activity is due to its prevention of chemical-induced stomach injury.

INTRODUCTION

Gongronema latifolium is a tropical rain forest plant found throughout Nigeria and other tropical countries such as Guinea-Bissau, Western Cameroon and Sierra Leone. It has been used in the traditional system of medicine for various gastrointestinal disorders such as diarrhoea, ulcers and dyspepsia and in the management of diabetes mellitus (1, 2). The leaves has been reported to have a hypoglycaemic effect (3, 4) by decreasing activity of glucokinase enzyme and levels of hepatic glycogen, hepatic and blood glucose. It is rich in fats, proteins, vitamins, minerals and essential amino acids (5). Phytochemical studies of *Gongronema latifolium* show that the root contains polyphenols in abundance, alkaloids, glycosides and reducing sugars (6). Two pregnan ester glycosides have been isolated from its leaves, namely (17β)-marsdenin-12-*O*-acetate 3-*O*-[6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-canaropyranoside (7) and 3-*O*-[β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-canaropyranosyl-11,12-di-*O*-tigloyl-17β-marsdenin-(**2**)(8). The leaves also contain saponins, alkaloids, flavonoids and tannins (9).

The ethanolic extract of *Gongronema latifolium* leaves is reported to possess antioxidant activity by increasing superoxide dismutase and glutathione peroxidase activities (10) and also reduces renal and hepatic oxidative stress, lipid peroxidation and increases the gluthathione/gluthathione disulphide (GSH/GSSG) ratio (3, 11). The ethanolic extract of the root of *Gongronema latifolium* increased white blood cell count and haemoglobin concentration in normal condition (6) while the leaves have a strong modulatory effect against hepatocellular damage induced by carbon tetrachloride (12). The plant also has anti-inflammatory property (13) and also exhibits antimicrobial activities against various microbial pathogens (14). The extract of the leaves may be used to prevent or reduce weight-loss, growth-depression and haematotoxicity in diabetic subjects (15).

Diabetes mellitus and diabetes-related complications have been on the increase despite great strides made in understanding and managing the disease (16). The disease is characterised by hyperglycaemia, depletion of antioxidants and increased generation of reactive oxygen species (17, 18) Diabetes mellitus is associated with an increased prevalence of gastrointestinal tract symptoms (19), and most of the body organs associated with the gastrointestinal tract are compromised. The associated gastrointestinal tract symptoms include altered gastric acid secretion (20−22) and dysmotility of the bowels, upper and lower gastrointestinal tract burns (23, 24).

With paucity of data regarding the effect of *Gongronema latifolium* leaf extract on gastric acid secretion and cytoprotection in the diabetic condition, the present study seeks to provide pharmacological basis for some of the medicinal uses of this plant in gastrointestinal disorders with specific attention on gastric acid secretion and cytoprotection in streptozotocin-induced diabetic rat model.

SUBJECTS AND METHODS

Preparation of plant extract

Fresh and uninfected leaves of *Gongronema latifolium* were collected in April from the botanical garden of the University of Calabar, Calabar, Nigeria. The botanical identification of the plant leaf was done by Dr Markson at the Department of Botany, University of Calabar, Calabar, Nigeria, where voucher samples were kept for reference (GL-A-05). The leaves collected were rinsed with distilled water and dried under shade. The dry leaves were ground into coarse powder after which 1200 g of the coarse powder were suspended in 2 L of ethanol and allowed to percolate for 24 hours at room temperature. This was filtered with Whatman No.1 filter paper. The filtrate was then evaporated by hot air oven (Amstel Hearson Oven, England) treatment at 40−45 $^{\circ}$C to a thick, dark brown gummy crude extract with a yield of 11.1% (66.6 g) This was stored at -4 $^{\circ}$C for future use. The crude extract was reconstituted in distilled water to an appropriate concentration before administration to experimental animals.

Experimental animals

Male albino rats of Wistar strain weighing between 170−200 g were used for the study after approval by the College Ethical Committee. The animals were kept under environmentally controlled conditions (constant temperature 26−28 $^{\circ}$C, with 12 hours light/dark cycle) for one week prior to starting the experiments. All rats were kept in plastic cages and they were provided with tap water and commercial diets.

Experimental design

The animals were divided into four groups of twelve rats each:

Group 1: Normal control rats fed on normal feed and tap water. Group 2: Diabetic control rats fed on normal feed and tap water. Group 3: Diabetic rats administered with extract at a dose of 200 mg/kg bodyweight for two weeks. Group 4: Normal control rats administered with extract at a dose of 200 mg/kg bodyweight for two weeks. The dose of 200 mg/kg was chosen based on a previous study that extracts from the *Gongronema latifolium* leaves have LD50 of 1050 mg/kg and was efficacious at this dose (9). Groups 3 and 4 were treated with the extract by gavage in conformity with folkore practice.

Induction of diabetes mellitus

Diabetes mellitus was induced in two groups of rats by a single intraperitoneal injection of 65 mg/kg bodyweight streptozotocin (STZ) dissolved in citrate buffer (pH 4.5). Age-matched control rats were injected with the vehicle. Bodyweight and basal blood glucose levels were measured just prior to STZ injection using animal balance and an automated glucose analyser (glucometer Acucheck mini plus, Roche, Germany), respectively. A drop of blood sample was collected by a prick on the tail vein. Diabetes mellitus was confirmed 48 hours after STZ injection in animals by the presence of blood glucose above 10 mmol/L (25). The diabetic animals were experimented upon after four weeks of induction of diabetes mellitus.

Gastric acid secretion study

Gastric acid secretion was measured in all groups of animals by a continuous perfusion method as previously reported (26). Briefly, after an overnight fast, anaesthesia was induced using 25% urethane at a dose of 6 ml/kg bodyweight intraperitoneally. A cannula was inserted into the trachea to maintain airflow while an oesophageal cannula for infusion of

saline was passed through the mouth to the stomach. Another cannula was introduced into the stomach through an incision in the duodenum and was ligated at 0.5 cm from the pylorus. The stomach was firstly flushed using 10 ml saline at room temperature through the oesophageal cannula and then flushed again with normal saline (pH: 7.0) at 37 $^{\circ}$C at the rate of 1 ml/minute using an infusion pump (Harvard apparatus, MA, USA). The stomach perfusate was collected every 10 minutes and acid output was measured by the titration of the perfusate with 0.01 N NaOH to a pH 7.0 using phenolphthalein as indicator. When a stable acid secretion was obtained, carbachol (a secretagogue) and ranitidine (histamine H_2-receptor antagonist) were administered and acid output determined every 10 minutes using the method described above.

Ulcer studies

Two experimental rat gastric ulcer models, namely, pyloric ligation and ethanol-induced ulcers were used. Six rats from each group were used for either pyloric ligation or ethanol-induced ulcer studies.

Pyloric ligation: The method described by Joshi *et al* (27) was followed. Another set of animals were fasted for 18 hours and were anaesthetized with pentobarbitone sodium (35 mg/kg, intraperitoneally). The abdomen was opened and ligation of the pylorus was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen closed in layers. The animals, having recovered from anaesthesia, were deprived of water during the postoperative period and were sacrificed using pentobarbitone sodium anaesthesia after four hours. Stomachs were dissected out and the gastric juice collected and centrifuged for five minutes at 2000 rpm. The supernatant obtained was used for estimation of total acid. The stomach of the animal was opened through the greater curvature and was spread on a sheet of cork so as to have a clear macroscopic view of the gastric mucosa. It was

examined macroscopically for the presence of ulceration. Ulcer index was calculated based on a 0−6 point scale (28). The scores were as below: 0 = no lesion, 1 = 1−3 small lesions; 2 = 1−3 large lesions, 3 = 1−3 thick lesions, 4 = more than 3 small lesions, 5 = more than 3 large lesions, 6 = more than 3 thick lesions. The macroscopic assessment of ulcer scores was performed by two independent examiners who were blinded to the treatment protocol so as to prevent possible experimental bias.

Ethanol-induced ulceration: Another group of both diabetic and control rats were used for the study. Ulceration was induced according to the method of Joshi *et al* (27) by intragastric instillation of ethanol (95%, 1 ml/200 g bodyweight). One hour after, the rats were anaesthetized using pentobarbitone sodium and the stomachs were removed and opened along the greater curvature of the stomach to macroscopically examine any ulcerative lesions as previously described.

Adherent mucus

Adherent mucus weight was determined by the method of Tan *et al* (29). Briefly, the mucus covering the stomach wall of both diabetic and control animals was carefully scraped using a glass slide into a small sample tube containing 1 ml of water whose weight was predetermined. The weight of the container and mucus was taken using a digital electronic balance and the difference taken as the weight of the mucus.

Drugs

Streptozotocin, carbachol, ranitidine and thiopentone sodium were obtained from Sigma Chemical Company, Poole, United Kingdom. All drugs were dissolved in distilled water except for streptozotocin which was dissolved in citrate buffer.

Statistical analysis

The results are expressed as mean ± standard error of mean (SEM). The results were analysed using GraphPad Prism software version 5 (GraphPad Software, San Diego, California, USA). One way analysis of variance (ANOVA) was used to compare means followed by Bonferroni's multiple comparison test where *p*-values were significant. *P*-value of 0.05 was considered statistically significant.

RESULTS

Effect of Gongronema latifolium on fasting blood glucose level and bodyweight

The mean fasting blood glucose levels were comparable in all groups of rats at the start of experiment. The blood glucose levels in diabetic control and the extract-treated groups were raised significantly ($p < 0.05$) following diabetic induction (Table 1). Extract treatment after two weeks of diabetic condition significantly ($p < 0.05$) reduced the fasting blood glucose level in the diabetic treated group when compared with control. Administration of *Gongronema latifolium* extract to non-diabetic rats did not significantly alter the blood glucose level.

Table 1 also shows the effect of STZ-induced diabetes on bodyweight of the diabetic rats. The weight of the control rats (n = 6) was 195.0 ± 3.1 g whereas the control group treated with *Gongronema latifolium* extract was 197.1 ± 2.1 g. The mean bodyweight of diabetic control rats (n = 6) was 154.3 ± 3.2 g, and the diabetic treated group was 162.1 ± 3.4 g. The difference was highly significant ($p < 0.01$) in the diabetic groups when compared with control. Extract administration did not significantly change bodyweights between diabetic control and diabetic plus extract groups.

Gastric acid secretion

The result for continuous acid secretion in diabetic and control rats is presented in Fig. 1. The mean basal gastric output in control was 2.6 ± 0.1 μmol/10 minutes, and 2.7 ± 0.5 μmol/10 minutes in diabetic control group. The mean basal gastric output in the control group treated with the extract was 4.2 ± 0.2 μmol/10 minutes while it was 3.6 ± 0.2 μmol/10 minutes in diabetic rats treated with *Gongronema latifolium* extract. The mean basal acid output was significantly ($p < 0.05$) lower in the diabetic control group when compared to the extract-treated diabetic group. Extract administration for 14 days significantly ($p < 0.05$)

increased basal gastric secretion in the diabetic group compared with the control group administered with the extract. Carbachol administration increased mean gastric acid output in the diabetic treated group which was significantly ($p < 0.05$) higher compared with diabetic and control groups. Ranitidine administration significantly ($p < 0.05$) reduced gastric acid secretion in all groups of rats to a level lower than the basal output.

Gastric ulcer studies

Diabetic rats showed increased tendency to ulceration in both models of rat gastric ulcers when compared with control. Ethanol had a higher potency in inducing ulcer scores than pyloric ligation. In ethanol-induced ulcer and four-hour pyloric ulcer models, ulcer scores were significantly ($p < 0.05$) higher in the diabetic group when compared to control. *Gongronema latifolium* leaf extract showed significant ($p < 0.01$) ulcer protective activity in diabetic rats in the two ulcer models when compared with control (Fig. 2). *Gongronema latifolium* extract, however, showed anti-ulcerogenic effect in normal rats in both ulcer models.

Effect of Gongronema latifolium extract on gastric juice and mucosal protection

The effect of *Gongronema latifolium* leaf extract administration on total gastric acid output and mucus weight is presented in Table 2. Diabetic rats displayed a decrease in gastric acid output which was significantly ($p < 0.05$) lower than the control. Administration of *Gongronema latifolium* extract significantly ($p < 0.05$) raised the total acid output in diabetic rats to a level significantly ($p < 0.05$) higher than the diabetic control. However, total acid output was significantly ($p < 0.05$) increased in normal rats treated with the extract when compared with control.

The mucus weight was significantly ($p < 0.05$) reduced in diabetic rats when compared with control (Table 2). Extract administration resulted in significant ($p < 0.05$) increase in mucus weight of the stomach in diabetic rats compared with the diabetic control. However, the mucus weight in the two groups of diabetic rats was significantly ($p < 0.05$) lower than the control group. The mucus weight in normal control + extract group was not significantly different when compared with normal control.

Effect of Gongronema latifolium on organ weights

Figure 3 shows the organ weights of the small intestine, liver, stomach and pancreas expressed as kg/kg bodyweight. The intestine, stomach and liver weights were all significantly ($p < 0.01$) increased while there was a significant ($p < 0.05$) decrease in the weight of the pancreas in streptozotocin-treated rats when compared with control. However, the weight of small intestine, liver, stomach and pancreas in normal controls treated with the extract was not statistically different when compared with normal control. The weights of the small intestine, stomach and pancreas in diabetic + extract group were significantly ($p < 0.05$) decreased while the liver was significantly ($p < 0.05$) increased when compared with the diabetic control group.

DISCUSSION

Gastrointestinal symptoms are fairly common in diabetes mellitus and are usually attributed to autonomic neuropathy (19, 30). *Gongronema latifolium* has beneficial effects in diabetes mellitus due to its hypoglycaemic and antioxidant properties (9, 10). Therefore the study investigated the effect of *Gongronema latifolium* leaf extract on gastric acid secretion and ulceration in diabetic rats compared to non-diabetic rats. The results on basal and stimulated acid secretion indicate that gastric acid secretion is suppressed in the diabetic condition when compared to control. This result is in agreement with previous reports that in the diabetic condition, acid secretion in stimulated parietal cells is significantly reduced in both human and animal studies (31−33). The primary stimulus for gastric acid secretion is hypoglycaemia and not insulin (34) and in chronic hyperglycaemia, gastric acid secretion is inhibited due to negative feedback by hyperglycaemia. This might therefore explain the present observation of decreased gastric acid secretion and total gastric output in the diabetic group in this study.

The present study showed that *Gongronema latifolium* has the propensity to raise gastric acid secretion and paradoxically inhibit acute haemorrhagic ulcers. The evaluation of the ethanolic extract of *Gongronema latifolium* for its antiulcer activity showed a significant antiulcer activity of the extract in the treated diabetic group for both ethanol and pyloric ligation induced ulcer models. The causative factors for peptic ulcer are many and include increase in gastric acid and pepsin secretion, decreased mucosal resistance and mucosal blood flow and increase in free radical generation. Though there was a significant decrease in mucus weight extracted from the diabetic groups compared with normal control, an increase in the mucus weight in the diabetic group treated with *Gongronema latifolium* was noted. There was also no significant difference in the mucus weight extracted from the treated control compared with the untreated normal control. This discrepancy cannot be easily discerned from the present results. The increased mucus in the diabetic group treated with

Gongronema latifolium probably reflects increased ability of the plant extract to protect the mucosa from physical damage as shown by a significant increase in the mucus weight on the surface of the mucosa in the diabetic groups. The mucus layer acts as a barrier against the agents introduced into the stomach or against endogenously formed acid and pepsin in the stomach (27, 35). Thus the overall benefit is probably the balance in favour of defensive factors against ulceration due to correction of blood glucose to near normal value in diabetic condition. This is consistent with the fact that correction of blood glucose level in diabetes mellitus caused a reversal of the diabetes-associated changes in mucosal offensive and defensive factors to protect against peptic ulceration (27).

In addition, the gastric acid secretion of the treated diabetic group was significantly higher than the diabetic control. This could reflect the improvement of the acid-secretory mechanisms due to treatment with *Gongronema latifolium,* thus suggesting that the ulcer protective activity of the plant extract may not be due to its anti-secretory activity but a synergistic effect of antioxidants present in the extract (36). Elevated acid output alone is not the sole causative agent of peptic ulcer (37). Diabetic rats are prone to ulcer by the high ulcer scores exhibited irrespective of the ulcer induction model (38). The high ulcer score could therefore not be attributed to acid output alone but rather to other factors that may mediate ulcerogenesis in the diabetic condition. Such factors include solubilization of mucus constituents (39), increase in proton pump activity and pepsin level, reduction in gastric mucosal blood flow and bicarbonate secretion, alterations in permeability, gastric mucus depletion and increased generation of free radicals (40, 41).

The results also show that diabetic rats lost bodyweight and had a high blood glucose level. This is expected as the diabetic condition is associated with weight loss and hyperglycaemia (42, 43). Treatment with the extract of *Gongronema latifolium* reduced the blood glucose level in the diabetic group. Our present result thus confirms previous

observations where the hypoglycaemic effect of *Gongronema latifolium* has been reported (3, 9). In terms of organ weight, there was an increase in the weight of the liver, small intestine and stomach to bodyweight ratio in diabetic rats. This could be organ compensatory adaptation response to meet the metabolic demands of the oxidative state in diabetes mellitus. This phenomenon was seen in the stomach (33), small intestine (42) and kidneys in rats diabetic (44) for one and eight months.

Administration of ranitidine, a histamine H_2 receptor antagonist, reduced the acid production in both diabetic and non-diabetic groups in the presence of the extract. The ethanol extract caused a reduction in ulcer scores and a significant increase in the mucus weight which shows its protective ability. In the present study, treatment with the leaf extract of *Gongronema latifolium* protected the gastric mucosa against the ulcerogenic actions of ethanol.

Increased oxidative stress due to hyperglycaemia could contribute to the pathogenesis of gastric ulceration (45). A previous study had reported that removing these oxygen-derived free radicals offer cytoprotection without influencing acid secretion (46). *Gongronema latifolium* has antioxidant effects due to its phytochemical constituents such as tannins, saponin, polyphenols and flavonoids (6, 9, 13) which are known to promote gastric mucus formation, and protect the stomach thereby reducing gastric lesions and ulcers (47). Other components like saponins and tannins are also present in the leaf extract of *Gongronema latifolium* which tend to exhibit some anti-inflammatory and antioxidant properties, thus rendering the stomach mucosa less permeable to chemicals and mechanical injury (48). This may also contribute to its cytoprotective role against peptic ulcers. Irrespective of the cause of ulcer, the net imbalance between the offensive and defensive factors is believed to be the detrimental factor in ulcerogenesis (49).

It is concluded that diabetes mellitus results in reduction of basal and stimulated gastric acid secretion but causes an increase gastric ulceration and increase in weight of liver, stomach and small intestine. Administration of *Gongronema latifolium* leaf extract ameliorates the symptoms of diabetes and reduces ulcerogenic potential while it also stimulates gastric acid secretion in diabetes mellitus. Further studies on the other defensive mechanisms that may provide more insight on the ulcer protective activity of the leaf extract of *Gongronema latifolium* is suggested.

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Table 1: Fasting blood glucose level and bodyweight in rats treated with *Gongronema latifolium* leaf extracts

Groups	**Plasma glucose (mMol/L)**		**Bodyweight (g)**		**Percentage weight gain (%)**
	Initial	**After**	**Initial**	**After**	
Control (n = 6)	3.62 ± 0.91	4.3 ± 0.67	186.7 ± 1.3	195.0 ± 3.1	4.5 ± 2.5
Diabetic control (n = 6)	4.2 ± 0.18	29.6 ± 2.2**	182.8 ± 5.6	154.3 ± 3.2*	-15.6 ± 4.1*
Diabetic + extract (n = 6)	4.2 ± 0.24	6.6 ± 1.1†	186.7 ± 1.2	162.1 ± 3.4#	-13.1 ± 3.0#
Control + extract (n = 6)	3.90 ± 0.23	4.5 ± 0.79	184.3 ± 3.8	197.1 ± 2.1	6.9 ± 4.0

* = $p < 0.05$ diabetic control *vs* control; ** = $p < 0.01$ diabetic control *vs* normal control;

† = $p < 0.01$ diabetic + extract *vs* diabetic control; # = $p < 0.05$ diabetic + extract *vs* diabetic control; n = number of rats.

Table 2: Total gastric acid output and mucus weight in diabetic rats treated with *Gongronema latifolium* leaves extract in rats

Groups	**Total gastric acid output (µEq/4h)**	**Mucus weight (g)**
Normal control (n = 6)	46.3 ± 1.5	1.17 ± 0.04
Diabetic control (n = 6)	34.7 ± 2.8$^{\#}$	0.43 ± 0.02
Diabetic + extract (n = 6)	52.3 ± 2.4*†	0.48 ± 0.03†
Normal control + extract (n = 6)	65.2 ± 3.7**	1.11 ± 0.02

* = $p < 0.05$ diabetic + extract *vs* normal control; ** = $p < 0.01$ control + extract *vs* normal control; # = $p < 0.05$ diabetic control *vs* normal control; † = $p < 0.05$ diabetic + extract *vs* diabetic control

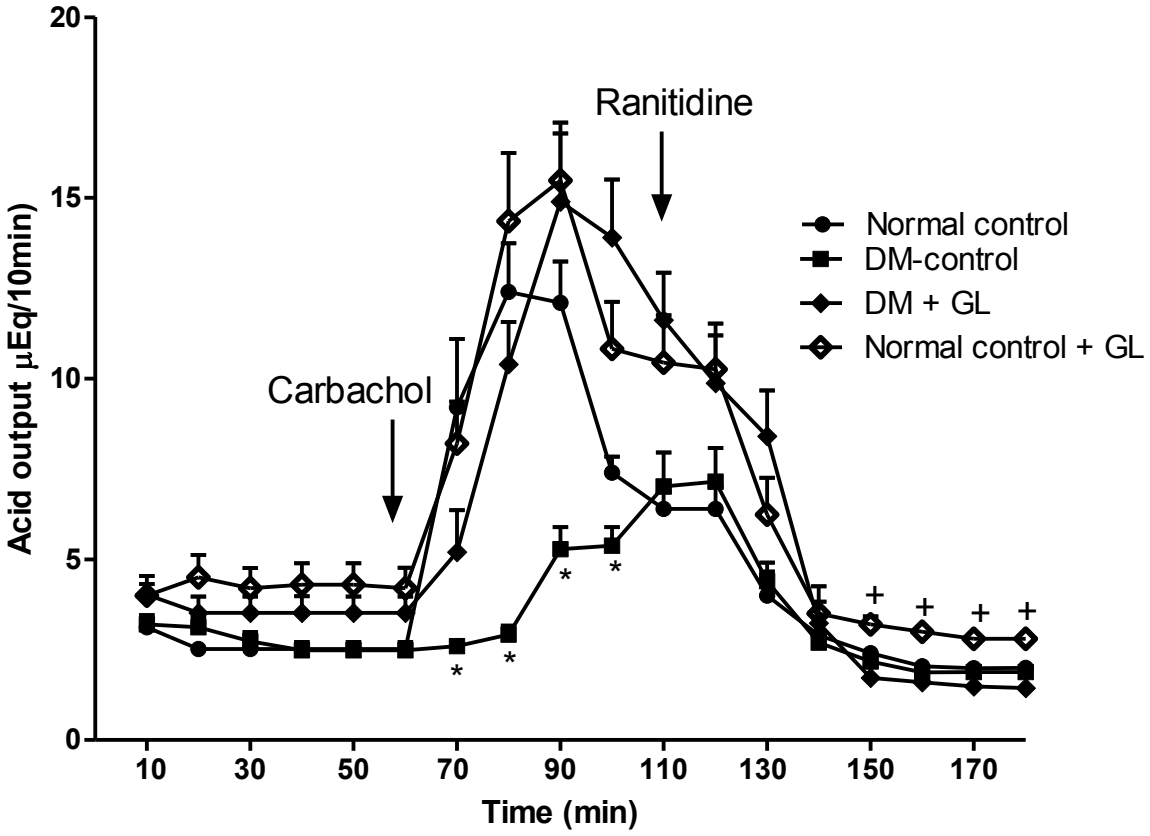

Fig. 1: Effect of *Gongronema latifolium* on gastric acid secretion in diabetic rats.

* = $p < 0.05$ compared with control and control + *Gongronema latifolium* (GL); + = $p < 0.05$ compared with diabetes mellitus + GL and control

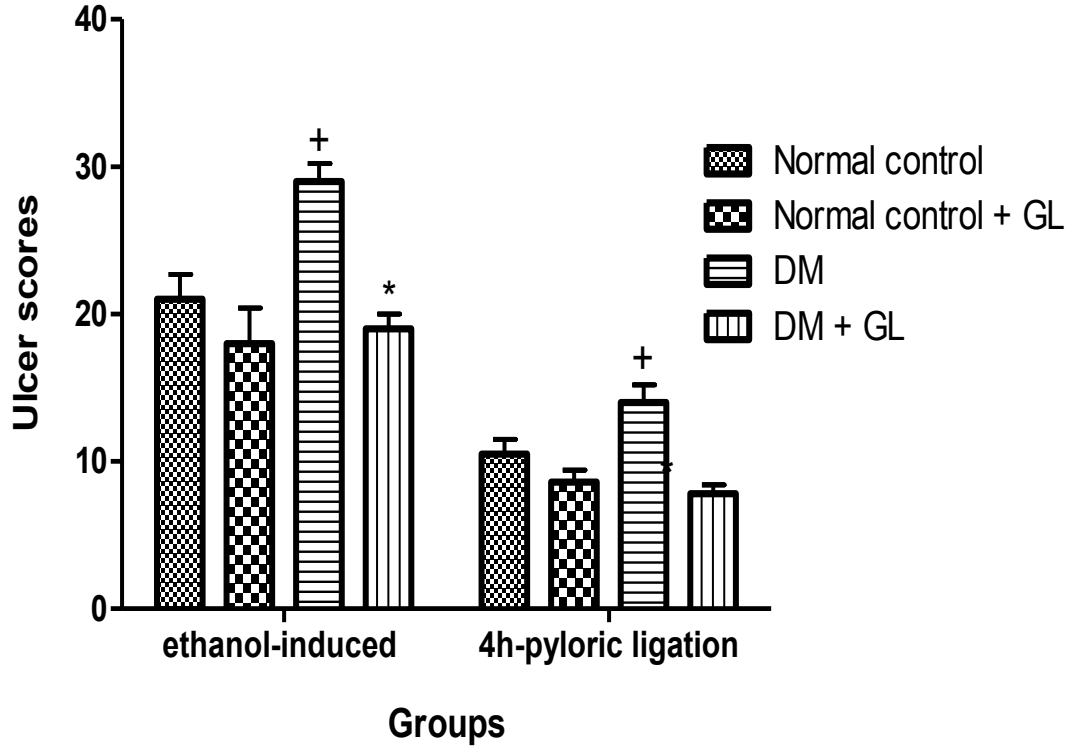

Fig. 2: Ulcer scores in diabetic rats treated with *Gongronema latifolium* leaves extract.

* = $p < 0.05$ compared with diabetes mellitus-control, + = $p < 0.05$ compared with control and control + G*ongronema latifolium*

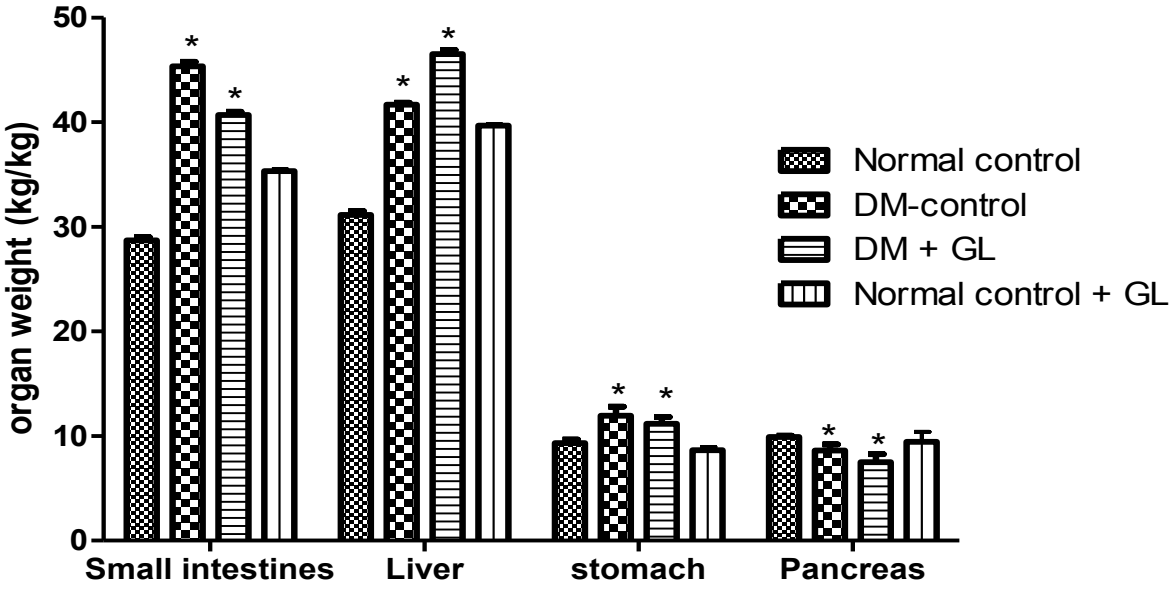

Fig. 3: Organ weights in control and diabetic rats treated with *Gongronema latifolium* leaves extract.

* = $p < 0.05$ compared with control and control + extract; # = $p < 0.05$ compared with diabetes mellitus-control