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Research Article

ETHANOLIC LEAF EXTRACT OF *CROTON ZAMBESICUS* INHIBITS GASTRIC LESION IN STREPTOZOTOCINE INDUCED DIABETIC RATS

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ABSTRACT
The aim of this study was to appraise the antiulcerogenic effect of *Croton zambesicus* (*C. zambesicus*) leaf extract in experimentally induced diabetic rats. Thrity-five adult male wistar rats were divided into seven groups (n=5). Group A, control rats; Group B, untreated diabetic rats; Group C, diabetic rats in which *C. zambesicus* therapy started 2 weeks prior to induction of diabetes; Group D and Group E, diabetic rats administered orally with *C. zambesicus leaf* extract for 2 and 4 weeks respectively; Group F, normal rats administered orally with *C. zambesicus*; Group G, diabetic rats administered with glimepiride (2 mg/kg/day).
The leaf extract of *C. zambesicus* significantly ($p<0.05$) reduced the gastric lesion and normalized the integrity of some of the gastric cells compromised in diabetic animals.
In conclusion, this study demonstrated that *C. zambesicus* leaf extract possesses antiulcerogenic activities in streptozotocin (STZ) induced diabetic rats which may be useful in the management of gastric lesion in diabetes mellitus.
KEYWORDS: *C. zambesicus,* gastric lesion, gastric cells, glimepiride, diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is associated with metabolic disorder of the endocrine system [1]. It is characterized by high blood glucose levels, which may result from defects in insulin secretion, or action, or both. DM also known as diabetes was first identified as a disease associated with "sweet urine" [2].
Symptoms of diabetes were first described in ancient times and insulin was first isolated over 50 years ago [3]. Report has it that diabetes is a serious and important health problem involving 2 % of the population of the United States [3]. Yagihashi[3] reported that some of the factors contributing to diabetes are reduced insulin secretion, decreased glucose usage by target tissue, and increased hepatic glucose production. It is worthy of note that metabolic changes associated with diabetes also affect other organs such as stomach, liver, kidney etc, resulting in a large burden on the individual with diabetes, as well as the health system.
So many factors such as reduced amount of normal islet cells, defective biosynthesis of pro-insulin, defective conversion of pro-insulin to insulin, deficient release of insulin into the blood in response to increased blood glucose, production of genetically defective insulin or an abnormally high rate of destruction of insulin are responsible for insulin deficiency [4].
Majorly we have two types of diabetes, called Type 1 and Type 2. Type 1 diabetes is called insulin dependent DM (IDDM), or juvenile onset DM. In this type 1 diabetes, the pancreas undergoes an autoimmune attack by the body itself, and is rendered incapable of making insulin[2]. Type 2 diabetes is also known as non-insulin dependent DM (NIDDM), or adult onset DM (AODM). In this type 2 diabetes, patients' pancreas can still produce insulin, but not as much as is adequate for their body's needs[2]. Insulin release by the endocrine pancreas is affected by a steady decline in beta (β) cell production of insulin in type 2 diabetes that contributes to worsening glucose control. Diabetes is gradually becoming the third 'killer' of mankind, after cancer and cardiovascular diseases, due to high prevalence in morbidity and mortality[5]. Estimate by Kings *et al.,*[6] revealed that at least 150 million people worldwide have diabetes, two-thirds of which resides in developing countries.
Diabetes exerts various influences on gastrointestinal tract, such as gastric lesion, acid secretion and gastric emptying [7]. Herbs are known to act as anti-diabetic agents [8-9], but many of such herbs are yet to be thoroughly investigated such as *C. zambesicus*. For various reasons, in recent years the popularity of complementary medicine has increased. Dietary measures and traditional plant therapies are used often in Nigeria[10].
C. zambesicus is a medicinal plant grown in villages and towns in Nigeria[11]. Some of its medicinal properties and components are reflected in the works of Block *et al.,*[12], Abo *et al.,*[13], Menut *et al.,*[14], Mekkawi[15], Odetola and Bassir[16], and Ryley and Peter [17]. Resent reports confirmed that *C. zambesicus* leaf extract displays antiulcerogenic activity as demonstrated by significant inhibition of the formation of ulcers induced through the three different ulcer models studied[10].
Despite all of these investigations and discoveries, it is yet to be established if the anti-diabetic influence of *C. zambesicus* could ameliorate gastric lesion associated with diabetes mellitus. This study was thus initiated with the aim of evaluating the anti-diabetic effects of ethanolic extract of *C. zambesicus* leaf on gastric lesion associated with streptozotocin-induced diabetes in adult wistar rats.

MATERIALS AND METHODS

Animal care

Thirty-five adult male albino rats of the Wistar strain were procured and acclimatized for two weeks at the Animal Holdings of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria before the commencement of the research work. Animals were fed with standard rat feed (Capfeeds, Ibadan) and given water liberally.
All the animal experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published

by the National Institutes of Health, USA[18] with ethical clearance number ERC/20090404.

Preparation of plant extract

The leaves of *C. zambesius* MÜll. Arg. (Euphorbiaceae) were collected from Dramatic art garden of the Obafemi Awolowo University, Nigeria, and taken to the herbarium of Botany Department, Obafemi Awolowo University for authentication and identification. Herbarium specimen number of the plant (UHI 16511) was obtained. The fresh leaves of the plant were air dried on a laboratory table for 30 days and reduced to power using squeezing and crushing machine (Daiki Rika Kogyo Co-ltd, Japan). The powder (400 g) was extracted with absolute ethanol (2.8L) for 72 hours. The extract was filtered using a filter paper. The filtrate obtained was concentrated *in vacuo* at 20^0C using a vacuum rotary evaporator (BÜchi Rotavapor R110, Schweiz). The extract obtained was partitioned between dichloromethane and water. The dichloromethane fraction was oven dried at 37^0C. The fraction obtained (13.8 g, 3.5%) was dissolved in 10% tween 80 and administered orally at a dose of 200 mg/kg as the plant extract.

EXPERIMENTAL DESIGN

The animals were divided into seven groups as follows, with five animals in each group.

Group A: Control rats administered intraperitoneally with 0.1 M sodium citrate buffer (pH 4.5)

Group B: Diabetic rats administered orally with 10% tween 80 for 4 weeks after the initial four weeks of diabetic induction.

Group C: Diabetic rats but in which *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% tween 80 therapy started 2 weeks prior to induction and continued throughout the period the experiment lasted (8 weeks).

Group D: Diabetic rats administered orally with *C. zambesicus leaf* extract (200 mg/kg body weight/day/rat) in 10% tween 80 for 2 weeks after the initial four weeks of diabetic induction (Withdrawal group)

Group E: Diabetic rats administered orally with *C. zambesicus leaf* extract (200 mg/kg body weight/day/rat) in 10% tween 80 for 4 weeks after the initial four weeks of diabetic induction.

Group F: Normal rats administered orally with *C. zambesicus leaf* extract (200 mg/kg body weight/day/rat) in 10% tween 80 for four weeks.

Group G: Diabetic rats administered with glimepiride (2 mg/kg body weight/day/rat) in 10% teen 80 solution orally for four weeks[19] after the initial four weeks of diabetic induction.

ASSESSMENT OF GASTRIC MUCOSAL LESIONS

Macroscopic evaluation of gastric damage

The macroscopic evaluation was assessed according to Morini and Grandi[20] which included the following materials and procedures:

Materials

Dissecting board and pins
Stereomicroscope (Leica GZ4)
Transparent plastic 1-mm grids

Procedures

- The stomachs of both the treated and control animals were rapidly removed, open along the greater curvature and rinsed briefly with 0.9% NaCl
- The stomach was pinned flat on a dissecting board with mucosa surface uppermost. Proper care was taking to avoid stretching or distortion of the mucoa.
- The mucosa surface was examined under the stereo microscope
- A transparent gastric grid was placed over the mucosa
- All measurement was done with the plastic paper grid along their greatest length
- Gastric damage was scored as follows:
  - A rating of 0 was assigned when there is no lesion
  - A rating of 1 was assigned to lesion measuring < 1 mm
  - A rating of 2 was assigned to lesion measuring 1 to 2 mm
  - Rating was subsequently assigned according to their length in millimeters to lesion measuring > 2 mm
  - All the length of the lesion was summed up and overall total obtained which was designated as the lesion index for each stomach.

Microscopic evaluation of gastric damage

The light microscopic evaluation of gastric mucosa damage was measured according to the method of Morini and Grandi[20]. Samples of the stomach of the sacrificed animals were fixed in 10% formol saline and processed for light microscopic study. Stained slides from each rat were subjected to microscopic evaluation of gastric damage.

Measurement and Scoring for degree of mucosa damage

- 0 was assigned for mucosa with no damage
- 1 was assigned for mucosa surface with damage not exceeding 25% of mucosa depth
- 2 was assigned for mucosa surface with damage more than 25% of the mucosa depth but not exceeding 75% of the mucosa depth
- 3 was assigned for mucosa surface with damage reaching up to 75% of mucosa depth

The mean score representing the degree of mucosa damage of gastric mucosa examined was calculated for each group as lesion index (Morini and Grandi[20], Takeuchi *et al.,*[7].

STATISTICAL ANALYSIS

Data were expressed as Mean ± Standard Error of Mean (S.E.M). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 17.0 (SPSS, Cary, NC, USA) with Duncan's Multiple Range Test (DMRT) option. A value of $p<0.05$ was considered to indicate a significant difference between groups.

RESULTS

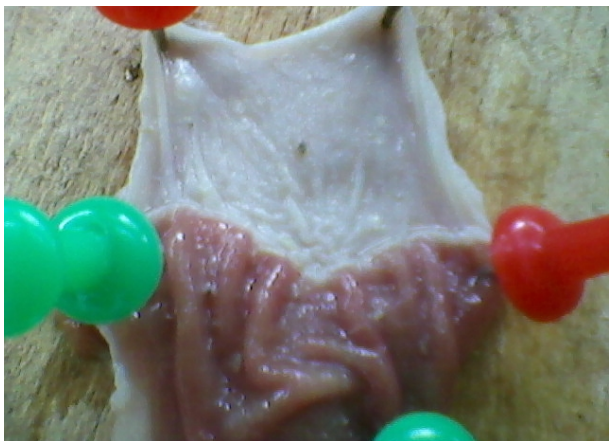
GASTRIC MUCOSA LESION

Macroscopic evaluation of gastric lesion

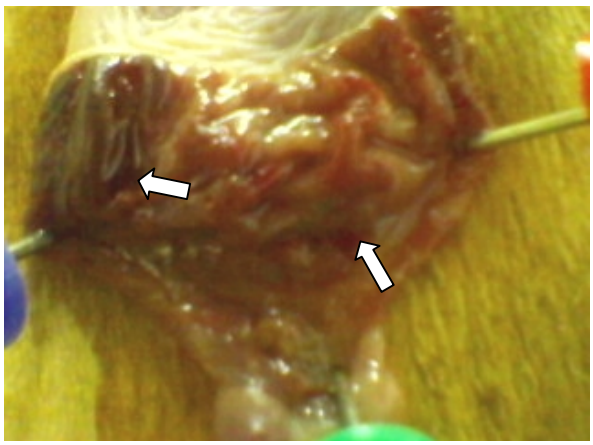
The macroscopic assessment of the gastric lesion was examined in all animal groups. The gastric lesion in the untreated diabetic group showed the highest gastric damage which was characterized by numerous hemorrhagic patches on the mucosa surface (Table 1, Fig. 1) when compared with the control animals. With early commencement of extract administration two weeks prior to STZ induction and administration of the extract for four weeks after four weeks of diabetic stabilization, there were few heamoragic patches on the mucosa surface when compared with the untreated

diabetic group (Table 1, Fig 1). Withdrawal of the extract administration for two weeks mildly increased the gastric lesion as evident by increased number of heamoragic patches when compared with the control and the group E (group which was administered with extract for four weeks after four weeks of diabetic stabilization) (Fig. 1). Group administered with glimepiride also showed similar reduction in gastric lesion (Table 1) when compared with the group which was administered with extract for four weeks after four weeks of diabetic stabilization (group E). The group administered with extract alone did not present any gastric lesion vis-à-vis untreated diabetic group (group B).

Figure 1: Showing the macroscopic view of the stomach in all the animal groups.

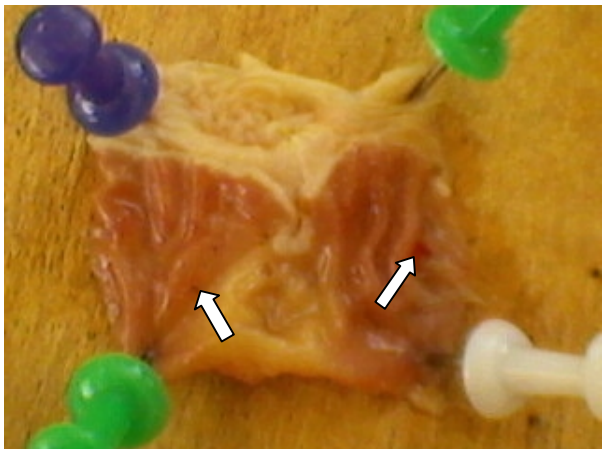


Control group

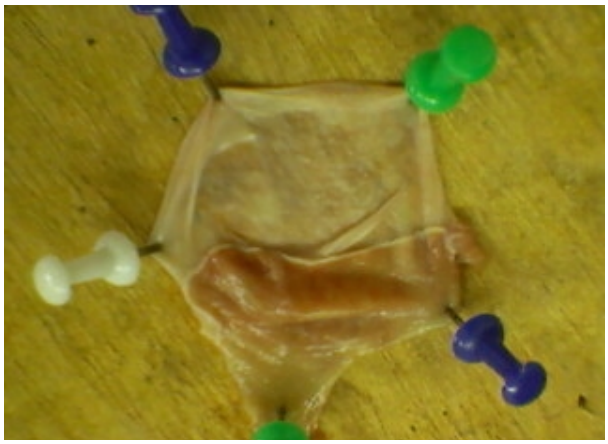


Untreated diabetic group. Note the heamorrhagic patches (white arrow)

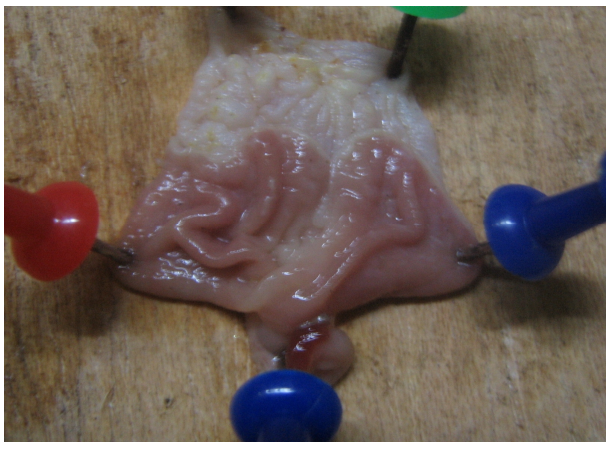
Group pretreated with *C. zambesicus* two weeks before STZ induction



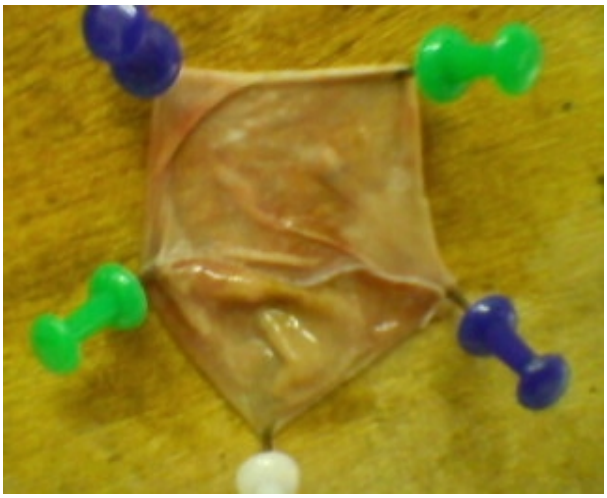
Group treated with *C. zambesicus* for two weeks after four weeks of STZ induction. Note the heamorrhagic patches (white arrow)



Group treated with *C. zambesicus* for four weeks after four weeks of STZ induction



Group treated with *C. zambesicus* only. Note the absence of heamorrhagic patches



Group pretreated with *C. zambesicus* two weeks before STZ induction

The light microscopic evaluation of gastric mucosa damage was measured in all animal groups. Stained slides from each rat were subjected to microscopic evaluation of gastric damage.

The gastric lesion in the untreated diabetic group showed the highest gastric damage which was characterized by ulcerations on the mucosa surface (Table 1) when compared with the control animals. With early commencement of extract administration two weeks prior to STZ induction, there were fewer shallow to moderate ulceration with no deep ulceration on the mucosa surface when compared with the untreated diabetic group (Table 1). Administration of the extract for four weeks after four weeks of diabetic stabilization (group E) drastically reduced the ulcerations on the mucosa surface. Withdrawal of the extract administration for two weeks significantly increased the shallow, moderate and deep ulceration when compared with the control and group E (group which was administered with extract for four weeks after four weeks of diabetic stabilization). Group administered with glimepiride also showed a reduction in gastric lesion when compared with the group which was administered with extract for four weeks after four weeks of diabetic stabilization (group E). The group administered with extract alone did not present any gastric lesion vis-à-vis untreated diabetic group (group B).

Table 1: Effects of *C. Zambesicus* on Gastric Lesion in STZ induced Diabetic rats.

Groups	Microscopic Lesion Index	Macroscopic Lesion Index	Macroscopic Curative Ratio (%)
Group A	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	100.00
Group B	2.80 ± 0.20^{c}	13.83 ± 0.98^{d}	-
Group C	0.20 ± 0.20^{ab}	5.33 ± 0.66^{b}	61.46
Group D	2.40 ± 0.24^{c}	11.75 ± 1.03^{c}	15.04
Group E	0.80 ± 0.37^{b}	5.20 ± 0.58^{b}	62.40
Group F	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	100.00
Group G	1.00 ± 0.55^{b}	4.80 ± 0.37^{b}	65.29

Values are given as Mean ± SEM for Microscopic lesion index, macroscopic lesion index and ulcer curative ratio. Letters a, b, c, d, ab within a column signifies that means with different letters differs significantly at $p < 0.05$ while means with the same letters does not differ significantly at $p < 0.05$ (using one way ANOVA with Duncan multiple range test).

Cellular density in the stomach

Parietal Cells

The density of parietal cells in the untreated diabetic group showed a significant reduction ($p<0.05$) when compared with the control animals. With early commencement of extract administration two weeks prior to STZ induction, there was significant ($p<0.05$) improvement in the density of parietal cells when compared with untreated diabetic group and control (Table 2). Administration of the extract for four weeks (group E) significantly ($p<0.05$) normalizes the parietal cellular density as compared with the control. Withdrawal of the extract administration for two weeks drastically reduced the density of parietal cells in a significant manner ($p<0.05$) when compared with the control and group E. Group administered with glimepiride also showed significant ($p<0.05$) improvement in the density of parietal cells when compared with the group administered with extract for four weeks after four weeks of diabetic stabilization (group E). The group administered with extract alone also showed a significant ($p<0.05$) improvement in the parietal cellular density vis-à-vis control group (group A).

Zymogenic Cells

C. zambesicus leaf extract did not show any significance in the zymogenic cellular density in all the animal groups as shown in Table 2.

Table 2: Effects of *C. zambesicus* on some gastric cells density in STZ induced Diabetic rats

Groups	Parietal Cells (cells/mm^2)	Zymogenic Cells (cells/mm^2)
Group A	14.00 ± 0.71^{bc}	5.80 ± 0.58^{a}
Group B	8.80 ± 0.86^{a}	5.80 ± 1.07^{a}
Group C	13.20 ± 1.24^{bc}	6.80 ± 0.97^{a}
Group D	9.40 ± 0.40^{a}	5.80 ± 0.96^{a}
Group E	13.00 ± 0.71^{bc}	6.40 ± 0.51^{a}
Group F	14.80 ± 0.73^{c}	6.60 ± 0.75^{a}
Group G	11.00 ± 1.67^{ab}	6.40 ± 0.24^{a}

Values are given as Mean ± SEM for density of parietal and zymogenic cells. Letters a, ab, bc within a column signifies that means with different letters differs significantly at $p < 0.05$ while means with the same letters does not differ significantly at $p < 0.05$ (using one way ANOVA with Duncan multiple range test)

Cellular diameter in the stomach

Parietal Cells

The diameter of parietal cells in the untreated diabetic group showed a significant reduction ($p<0.05$) when compared with the control animals. With early commencement of extract administration two weeks prior to STZ induction, administration of the extract for four weeks after four weeks of diabetic stabilization (Group E), and glimepiride presented a significant ($p<0.05$) improvement in the diameter of parietal cells when compared with the untreated diabetic group and control (Table 3). Withdrawal of the extract administration for two weeks drastically reduced the diameter of parietal cells in a significant manner ($p<0.05$) when compared with the control and group E. The group administered with only extract presented fair correlation in the diameter of parietal cells vis-à-vis control group (group A).

Zymogenic Cells

The diameters of zymogenic cells were examined in all animal groups. There were no significant differences in the zymogenic cell diameter in all the animal groups except groups D and E.

Table 3: Effects of *C. zambesicus* on some gastric cells diameter in STZ induced Diabetic rats

Groups	Parietal Cells (µm)	Zymogenic Cells (µm)
Group A	14.00 ± 0.61^{b}	10.00 ± 0.79^{ab}
Group B	5.50 ± 0.50^{a}	9.50 ± 1.66^{ab}
Group C	11.80 ± 0.92^{b}	11.00 ± 1.00^{ab}
Group D	8.00 ± 1.22^{a}	7.50 ± 1.12^{a}
Group E	12.50 ± 0.79^{b}	12.00 ± 0.94^{b}
Group F	14.00 ± 1.00^{b}	11.20 ± 1.32^{ab}
Group G	11.00 ± 1.27^{b}	11.30 ± 1.34^{ab}

Values are given as Mean ± SEM for diameter of parietal and zymogenic cells. Letters a, b, ab within a column signifies that means with different letters differs significantly at $p < 0.05$ while means with the same letters does not differ significantly at $p < 0.05$ (using one way ANOVA with Duncan multiple range test)

DISCUSSION

Our finding showed that pretreatment of animals with *C. zambesicus* (200 mg/kg/day) two weeks prior to STZ induction (group C) and treatment of diabetic animals with *C. zambesicus* (200 mg/kg/day) for four weeks (group E) had antiulcerogenic effects against gastric ulcer associated with diabetes in rats. Macroscopic and microscopic lesion index showed that *C. zambesicus* was able to protect and ameliorate necrosis and hemorrhagic patches which characterized the mucosa of diabetic animals in this study. Also, the curative ratio of *C. zambesicus* even though slightly lower than the antidiabetic drug, produced a remarkable effect which if developed, could be a potent anti-ulcerogenic agent. Lipid peroxidation and generation of free radicals are the major cause of ulcers in diabetic state[21]. Takeuchi *et al.,*[7] reported that diabetic condition has deleterious effect on the healing of lesions and significantly delayed the reconstruction process of the injured mucosa.

Due to the essential role of flavonoid in wound healing[7], it is expected that the antiulcerogenic effect of *C. zambesicus* may be due to its flavonoid content. Achterrath-Tuckermann *et al.,*[23] reported that an antipeptic action of extracts is always due to their flavonoid constituents. The leaf of *C. zambesicus* has been found to contain flavonoids, terpenes, saponins, alkaloids and cardiac glycosides[10]. Okokon *et al.* [10] in their work on antiulcerogenic activity of ethanolic leaf extract of *C. zambesicus* concluded that the extract displays antiulcerogenic activity as demonstrated by significant inhibition of the formation of ulcers induced through three different ulcer models studied. Flavonoids such as quercetin have been reported to prevent gastric mucosal peptic ulcer disease which is a problem of the gastrointestinal tract implicated in diabetes by scavenging free radicals[10, 23]. One of the major mechanism through which *C. zambesicus* extracts exhibits its anti-ulcer effects may be due to its protective effect on parietal cells, decrease volume of gastric juice, mucus deposition and promotion of prostaglandins synthesis. Prostaglandin has been reported to have strong inhibition for agents capable of causing gastric ulceration by enhancing gastric mucosa resistance through stimulation of mucus and biocarbonate output[24].

The reduction of parietal cell density as seen in the untreated diabetic group may be due to the disruptive processes which characterized the acid secreating cells. Similar study showed that the parietal cell membranes of diabetic rats appear disruptive and lacked suppleness reflecting a disruption of the molecular mechanisms that allow for the essential link between the membranes and F-actin, necessary to the histoarchitecture of the secretory membranes[25]. Bastaki *et al.* [26] observed a reduction in H^{+}-K^{+}-ATPase and canaliculi in parietal cells which explained the reduced acid secretion observed in diabetics. The acid secretory activity of the H^{+}-K^{+}-ATPase is necessary for the viability and normal development of parietal cells[25] as well as zymogenic cells[26-27] which are reduced in DM rats. Evidence from this study has shown that one of the mechanism by which *C. zambesicus* prevent gastric lesion associated with diabetes is by protecting the disruption of gastric cells from the devastating free radicals implicated in diabetes. This observation is consistent with previous study[10].

In conclusion, the result has shown that diabetic conditions can cause gastric lesions over time, and that *C. zambesicus* may be effective in the management of gastric lesions associated with diabetic rats.

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