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*RESEARCH ARTICLE*

# CYTOPROTECTIVE EFFECT OF *COSTUS SPECIOSUS* ON PANCREATIC B-CELLS IN ALLOXAN-TREATED RATS.

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## *Abstract*

Type I diabetes is one of the most common chronic childhood conditions. It is an autoimmune condition results from partial or complete destruction of β cells. *Costus speciosus* serves as an important source of many therapeutically efficient compounds and it is a well-known as natural antidiabetic agent. This study aimed to investigate the possible protective effect of *costus speciosus* rhizome extract on the structure and ultrastructure of pancreatic β-cells in alloxan treated rats. Hexane rhizome extract was used in low dose of (200mg/kg/day) and high dose of (400mg/kg/day) for treatment of diabetic animals for 4 weeks. At the end of the experiment, pancreatic specimens were taken and prepared for light and electron microscopic examination. The results revealed that the treatment with *costus* rhizome extract significantly ameliorates the histopatholgical changes of the pancreatic islets. There were significant increase in insulin secretion and marked decrease in the serum glucose level. The protective effect of *costus speciosus* was in dependant dose manner. We concluded that *costus speciosus* rhizome extract may possess a strong potential to serve as natural protective agent on pancreatic islets against the toxic agents and it can be used as a possible food additive recommended in human nutrition.



## Introduction:-

Diabetes is a common, chronic and costly disease that is threatening the health of generations of people around the world. Type 1 diabetes (insulin-dependent) affects about 5 percent of individuals with diagnosed diabetes (Shaw, Sicree et al. 2010). It usually diagnosed in children and young adults, but the disease can strike at any age (Daneman 2006). Type 1 diabetes results from an autoimmune process that destroys the insulin-producing β-cells of the pancreas islets (Brownlee 2005). People with type 1 diabetes demand daily insulin administration to regulate their blood glucose as normal levels as possible (Brownlee 2005).

Alloxan is a well-known diabetiogenic agent that induces Type I diabetes in experimental animals (Viana, Medeiros et al. 2004). It is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution. It has been widely accepted that alloxan selectively destroys the pancreatic β-cells. The action of alloxan is preceded by its rapid and selective uptake by pancreatic β-cells. The selective uptake of the compound is due to its structural

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similarity to glucose as well as highly efficient uptake mechanism of the pancreatic β-cells (Daneman 2006, Viswanathaswamy, Koti et al. 2011). The toxic action of alloxan on β-cells involve generation of free radicals, oxidation of essential sulphydryl, inhibition of glucokinase enzyme and disturbances in intracellular calcium homeostasis (Szkudelski 2001, Dhanesha, Joharapurkar et al. 2012).

*Costus speciosus* (Family: *Costaceae*) is a tropical plant referred as spiral ginger or crepe ginger. It is native to India and southeast Asia and used traditionally as food and medicine by the Indians (Janaki Ammal and Nagendra Prasad 1984). *Costus speciosus* serves as an important source of many therapeutically efficient compounds possessing many pharmacological activities. Rhizome extract of this plant showed high antibacterial activity against Gram positive and Gram negative bacteria (Ariharan, Meena Devi et al. 2012, Duraipandiyan, Al-Harbi et al. 2012). Also, it has antifungal (AL-Ameri and Azeez 2014), Antihelmentic (Srivastava, Singh et al. 2011), antiinfammatory (Srivastava, Singh et al. 2013) and significant antioxidant activity (Nehete, Bhatia et al. 2010). The rhizomes of C. speciosus have been reported to possess steroid –diosgenin, which is anti-diabetic in nature (Rani, Sulakshana et al. 2012). The Leaves also possess antihyperglycemic properties and insulin potentiating action (Rajesh, Harish et al. 2009).

The current study was designed to evaluate the possible protective effect of *C. speciosus* rhizome extract on the pancreatic β-cells in alloxan treated rats, as one of the edible natural products that may have more safe and potent therapeutic and protective effects on type I diabetes.

## Materials and Methods:-

**Plant material:-**

Plant Material *C. speciosus* rhizomes were purchased from the local perfumery market in Holy Mecca, Saudi Arabia. The species was identified and authenticated by the Department of Biology, College of science, Taif University. The rhizomes were dried, cut into small pieces and powdered.

**Chemicals:-**

All the chemicals and biochemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA).

**Animals:-**

Forty-eight adult male albino rats with body weights of 200–250 g each, at the beginning of experiment, were used. All the animals were received humane care in compliance with the principles of laboratory animal care. The animals were fed on a standard laboratory food and water *ad libitum*. They were kept under standard conditions of humidity and temperature. The experiment received approval from the ethical committee of College of Medicine, Taif University.

**Preparation of crude extract:-**

The crude extract of *C speciosus* was prepared using the cold percolation method. The powder was dissolved in hexane in a 1:3 ratio. Extract was filtered and evaporated to dryness in a rotary evaporator to yield 1.5% hexane extract. A weighed portion of extract was suspended in 0.5% aqueous carboxymethyl cellulose (CMC) solution in distilled water prior to oral administration to animals (Daisy, Eliza et al. 2008).

**Induction of Diabetes:-**

Hyperglycemia was induced by a single injection of aqueous solution of alloxan monohydrate (170 mg/kg body weight, intraperitoneally) to overnight fasted rats. Control rats receive similar volume of normal saline (2 ml/kg body weight, intraperitoneally) alone. After 48 hrs of alloxan injection, rats with a fasting blood glucose range >200 mg/dl were considered diabetic.

**Experiment Design:-**

Rats were divided into 4 groups, 12 rats each. Group I: control (sham, non-diabetic) received citrate buffer solution (5 ml/kg/day). Group II (diabetic, untreated) included alloxan-induced diabetic animals, received citrate buffer solution (5 ml/kg/day). Group III: (low dosage *costus* treated group) diabetic animals received *costus* extract (200 mg/kg/day). Group IV (high dosage *costus* treated group) diabetic animals received *costus* extract (400 mg/kg/day). Through the experiment which lasted for 4 weeks after induction of diabetes, fluid intake was estimated and finally, the rats were weighed, fasted overnight and then sacrificed under ether inhalation. Biochemical, histological, immunohistochemical and morphometrical studies were done.

**Biochemical study:-**
Blood samples from all animals were collected in heparinized tubes. Plasma and serum were separated from blood by centrifuging the samples at 5000 rpm for 10 min. The samples were examined to determine plasma glucose levels by glucose oxidase method and insulin level by using an enzyme-linked immunosorbent assay (ELISA).

**Histological study:-**
Immediately after dissection, pancreata will be taken out, cleaned and fixed in 10% buffered formalin solution. After fixation, tissues are sampled by routine histological procedures and stained with *Hematoxylin & Eosin (H&E)* for histopathological observation.

**Immunohistochemical study:-**
Immunolocalization technique for anti-insulin will be performed on 5–6 *μm* thickness sections and stained with the streptavidin– biotin–peroxidase staining method (Jackson and Blythe 2013).

**Ultrastructural study:-**
Ultrathin sections will be prepared from the pancreata as detailed by *Kalender et al.* (Christensen 1971) and examined with a Transmission electron microscope.

**Morphometrical study:-**
Morphometric studies were curried at different magnifications in the non-serial H&E stained pancreatic sections (Adeyemi, Komolafe et al. 2010), using the image analyzer computer system (Leica Qwin 500) to evaluate the:
- Number of islets/section: determined at 40x magnification
- Diameter of islets
- Number of β-cells/islet: determined by direct counting method at 1000× magnification

**Statistical Analysis:-**
The data that give numerical values is expressed as mean ± standard deviation for each group and subjected to one way analysis of variance (ANOVA) with multiple comparisons between groups. $P < 0.05$ was considered statistically significant.

**Ethical Considerations:-**
Under anesthesia is done during painful procedures to avoid distress and pain. By applicable international laws and regulations our standards of animal care and administration met those required.

## Results:-

**Body weight and fluid intake:-**
Table (1) show alterations in body weights and fluid intake in different groups. Diabetic group (II) shows significant loss of body weights and increased fluid intake as compared to the control group (I). There are no significant differences between values of the diabetic group (II) and the group treated with low dosage *costus* (III). The high dosage *costus* group (IV) shows significant increase in body weights and significant reduction in fluid intake comparing with the diabetic group (II).

**Biochemical Results:-**
As compared to the control group (I), the diabetic group (II) showed significant increase in blood glucose level and significant decrease in insulin level. On the other hand, the high dosage *costus* group (IV) showed significant decrease in blood glucose level and significant increase in insulin level compared with the diabetic group (II). While the values of the low dosage *costus* group (III) have no significant difference compared with the diabetic group Table (2).

**Llight and electron microscopy observations:-**
**Control group (group I):-**
The pancreatic sections of control rats stained with H&E showed closely packed lobules of pancreatic acini containing regular and well-defined islets of *Langerhans* which, were embedded within the exocrine portion. The islets appeared as pale staining areas; consist of clusters of polygonal cells that have pale acidophilic cytoplasm and central rounded vesicular nuclei. They are arranged in branching cords with blood capillaries in between. (Fig. 1a).

Immunohistochemical reaction revealed the pancreatic β-cells with strong positive reaction for anti-insulin antibodies as dark brown granules in the cytoplasm of β-cells which form the majority of cells population of the islets. (Fig. 2a).

Ultrastructurally, islets of *Langerhans* of control rats consist mainly of β-cells which appeared oval or polygonal in shape. Their cytoplasm contains many characteristic β granules which are formed of electron dense core surrounded by a wide lucent halo. Euchromatic nucleus, mitochondria, rough endoplasmic reticulum and Golgi apparatus were seen among them. (Fig. 3a)

**Diabetic, untreated group (group II):-**
Study of pancreatic sections stained with H&E of diabetic rats revealed pathological changes of pancreatic islets. The islets appeared ill-defined, shrunken and distorted with marked decrease of β –cells and loss of the normal islets cellular cord arrangement. Many β –cells showed marked cytoplasmic vacuolation and pyknotic nuclei. Some β –cells showed large darkly stained nuclei with deeply acidophilic cytoplasm.(Fig. 1b).

Immunohistochemistry study of diabetic group revealed marked reduction in immunoreactivity for anti-insulin antibodies inside the β-cells. (Fig. 2b)

The ultrastructure of pancreatic islets of diabetic rats revealed marked pathological changes in the β-cells in the form of apparent loss of their secretory granules leaving empty spaces. Their nuclei showed irregular counters and some of them appeared dark electron dense and others showed peripheral aggregation of heterochromatin. Mitochondria were vacuolated with loss of their cristae and matrix. Golgi apparatus was dilated and congested blood capillaries were also seen (Fig.3b).

**Low dosage costus treated group (group III):-**
Pancreatic sections stained with H&E of the low dosage *costus* treated group showed no marked amelioration of pathological changes; the islets still ill-defined and shrunken with loss of normal cellular arrangement. Many β –cells still showed marked cytoplasmic vacuolation and pyknotic nuclei. (Fig. 1c)

Immunohistochemistry slides revealed mild positive insulin reactivity of some cells but the majority of β-cells displayed negative immunoreactivity. (Fig.2c)

The ultrastructure study showed no marked improvement in β –cells. Most cells were shrunken with vacuolated cytoplasm and heterochromatic nuclei. β secretory granules appeared empty and few cells appeared with normal characteristic granules. (Fig.3c).

**High dosage costus treated group (group IV):-**
Pancreatic sections stained with H&E of the high dosage *costus* treated group showed apparent improvement in the structure of islets. The islets increased in size and partially restored their normal cellular arrangement. β–cells showed minimal cytoplasmic vacuolation and pyknotic nuclei. Majority of cells appeared having central vesicular nuclei and pale acidophilic cytoplasm. (Fig. 1d)

Immunohistochemistry study revealed increased positive insulin reactivity of β -cells in comparison to the diabetic group (group II) and low dosage costus treated group (group III). (Fig. 2d)

The ultrastructural study showed more or less normal β cells containing euchromatic nuclei with clumps of heterochromatin. The β granules were increased in number and restored their normal structure; having electron dense core surrounded by lucent halo space. Numerous rounded and elongated mitochondria were observed. Some cells were still affected showing empty spaces and heterochromatic nuclei with irregular counter. (Fig. 3d).

**Morphometric Results:-**
The morphometric study evaluated the number of islets/section, diameter of islets and number of β cells/islet. There was a significant reduction of all these parameters in the diabetic group (group II) in comparison to that of the control group (group I). These parameters increased in the low dosage costus treated group, but this increase was not statistically significant. The high dosage *costus* treated group showed significant increase in the studied parameters compared to the diabetic group. (Table 3).

**Table 1:-** body weight and fluid intake in different groups

| group | body weight (g) | fluid intake (ml) |
|---|---|---|
| group (I) | 247 ± 4.3 | 37 ± 3 |
| group (II) | 184 ± 2.1[a] | 203 ± 5[a] |
| group (III) | 197 ± 3.4 | 164 ± 2 |
| group (IV) | 237 ± 1.1[b] | 91 ± 7[b] |

Values are Mean±SD.
[a] significant at P < 0.001 as compared to control.
[b] significant at P < 0.001 as compared to diabetic.

**Table 2:-** Levels of blood glucose and insulin in different groups.

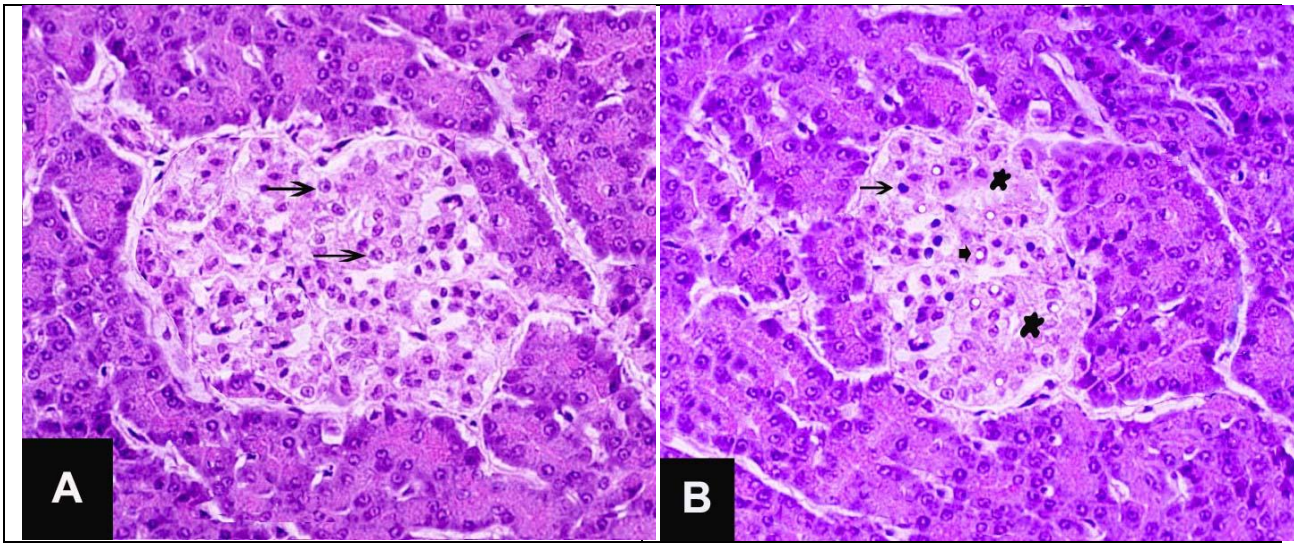
| Group | Blood glucose (mg/dl) | Insulin level (µU/ml) |
|---|---|---|
| group (I) | 100.47 ± 3.22 | 51.24 ± 2.51 |
| group (II) | 367.25 ± 6.50[a] | 11.62± 3.07[a] |
| group (III) | 248.08 ± 2.84 | 15.23± 3.98 |
| group (IV) | 207.06 ± 7.85[b] | 33.76± 4.03[b] |

Values are Mean±SD.
[a] significant at P < 0.001 as compared to control.
[b] significant at P < 0.001 as compared to diabetic.

**Table 3:-** Morphometric studies of different groups

| Parameter | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| Number of islets/pancreas (N/10 $mm^2$) | 19.09 ± 0.69 | 5.11 ± 0.41[a] | 7.34 ± 0.30 | 12.39 ± 0.72[b] |
| Diameter of islets (µm) | 125.99 ± 11.87 | 63.97 ± 6.42[a] | 82.41 ± 4.01 | 107.12 ± 8.21[b] |
| Number of β cells/islet (N/1000 µm2) | 9.95 ± 0.70 | 2.55 ± 0.26[a] | 3.49 ± 0.77 | 7.45 ± 0.86[b] |

Values are Mean±SD.
[a] significant at P < 0.001 as compared to control.
[b] significant at P < 0.001 as compared to diabetic.



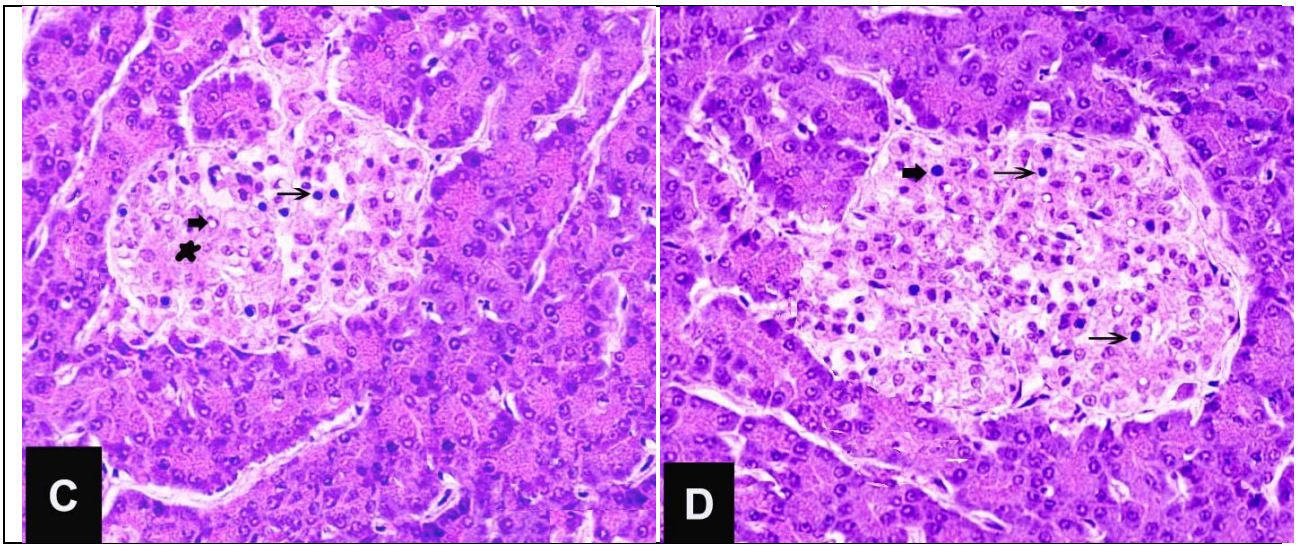
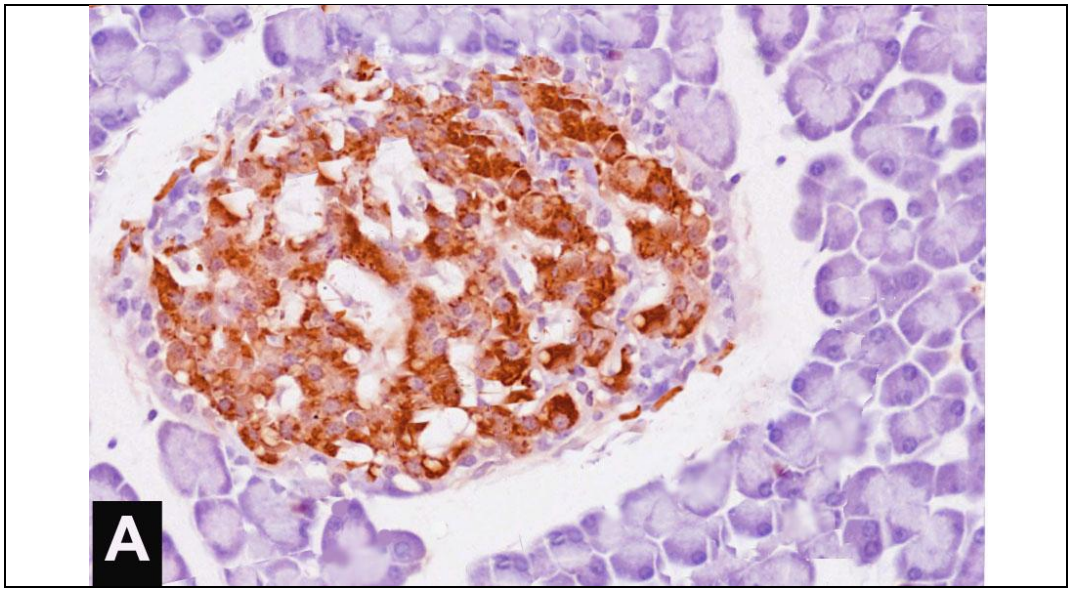
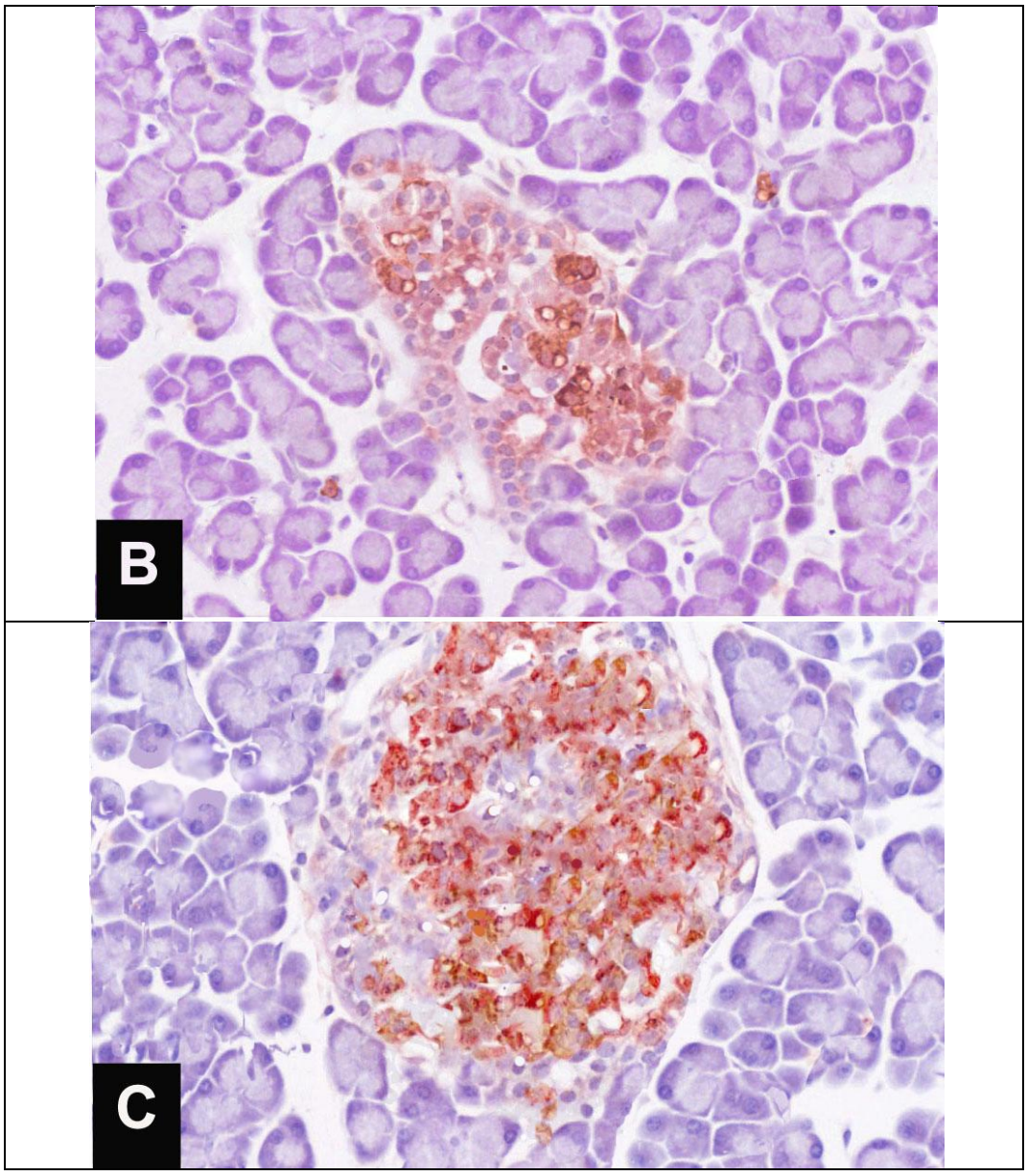



**Fig. 1:-** Photomicrographs of pancreatic sections of the different groups, stained with Hematoxylin and Eosin; (A) Normal control rats: showing regular and well-defined islets of Langerhans (pale stained areas) surrounded by pancreatic acini. β –cells appear with rounded vesicular nuclei and pale acidophilic cytoplasm (arrows). They are arranged in branching cords with blood capillaries in between. (B) Diabetic group: showing ill-defined, shrunken islets with necrotic areas (stars). Many β –cells showing marked cytoplasmic vacuolation (arrow head ) and pyknotic nuclei (arrow). (C) Low dosag costus treated group: showing no marked amelioration of pathological changes than that of diabetic group; the islets still ill defined and shrunken with some necrotic areas (star). Many β –cells still showing marked cytoplasmic vacuolation (arrow head) and pyknotic nuclei (arrow). (D) High dosage costus treated group: showing apparent improvement in the structure of islets which increased in size and partially restored their normal cellular arrangement. β–cells showed minimal cytoplasmic vacuolation (arrow head) and pyknotic nuclei (arrows). Majority of cells appeared having central vesicular nuclei and pale acidophilic cytoplasm. H&E X 400.

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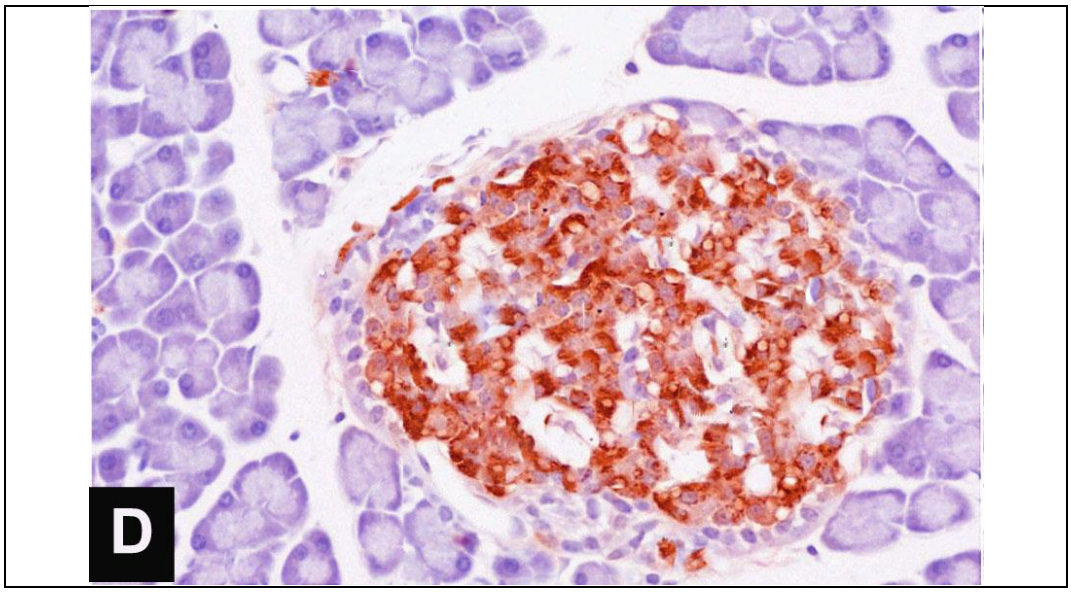
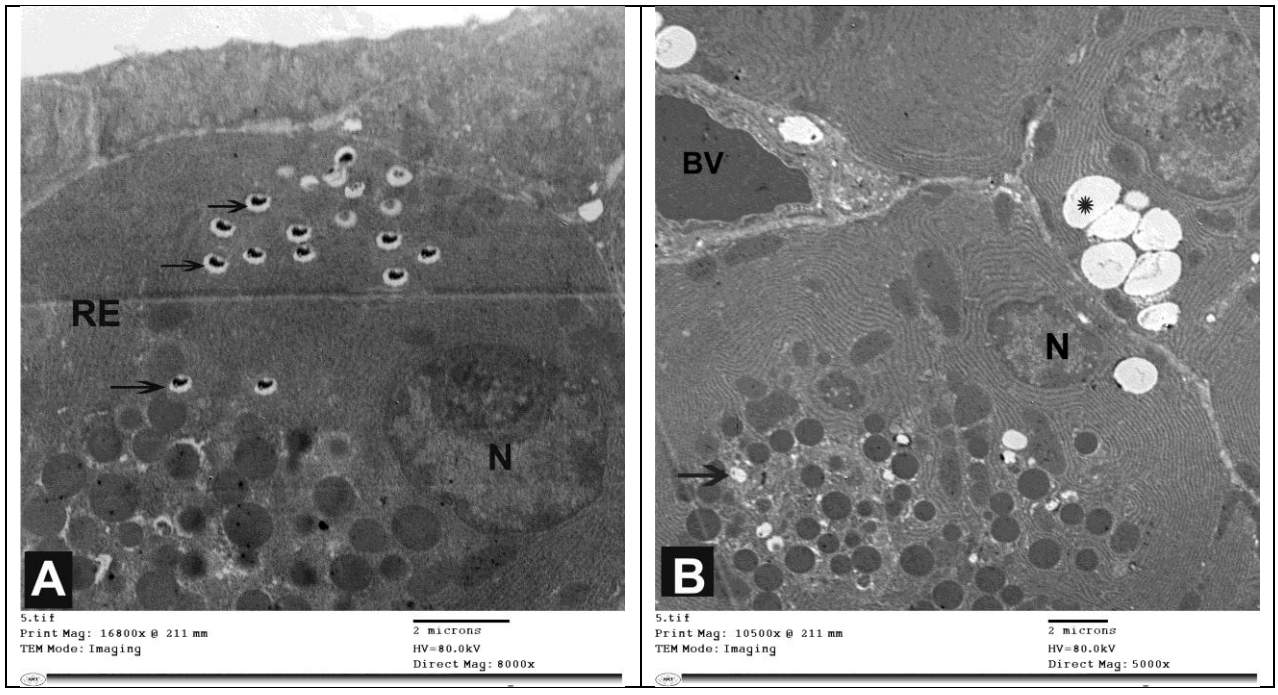



**Fig 2:-** Photomicrographs of pancreatic sections of the different groups, stained with anti-insulin immunostain; (A) Control group: showing pancreatic β-cells with strong positive reaction for anti-insulin antibodies as dark brown granules in the cytoplasm of β-cells. (B) Diabetic group: showing marked reduction in immunoreactivity for anti-insulin antibodies inside the β-cells with necrotic areas. (C) Low dosage costus treated group: showing mild positive insulin reactivity of some cells. (D) high dosage costus treated group: showing increased positive insulin reactivity of β –cells compared to the diabetic group.



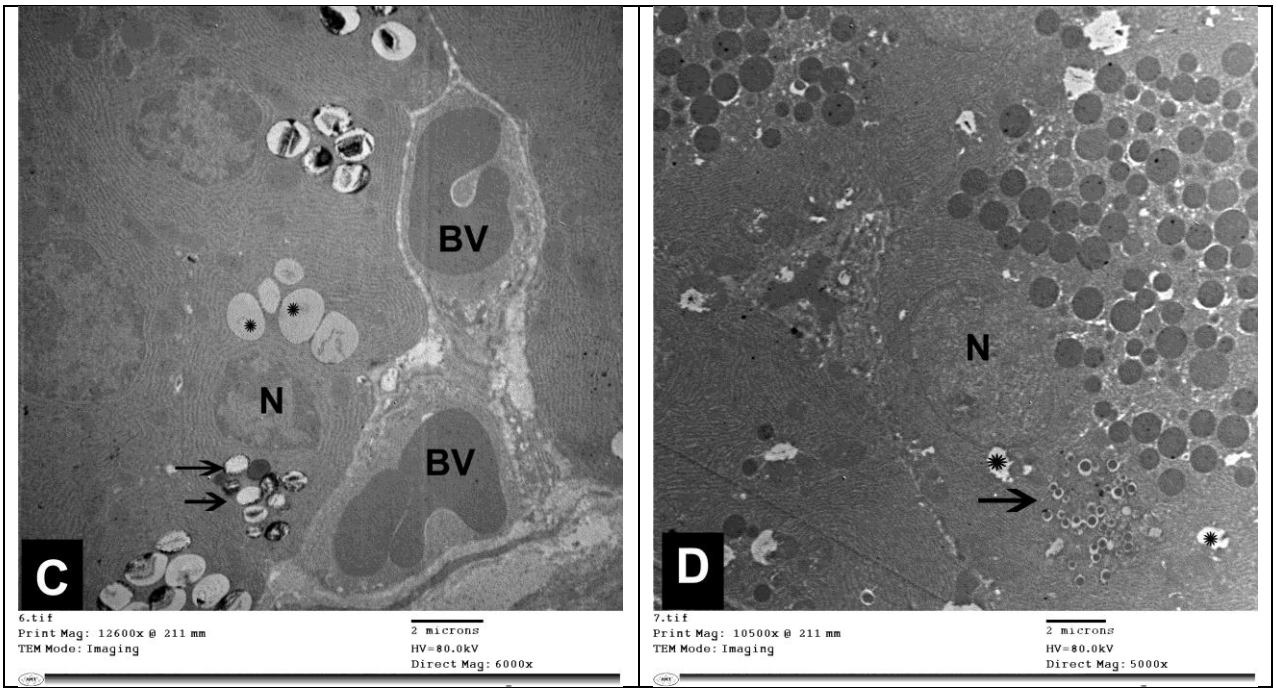



**Fig. 3:-** Electron micrographs of different studied groups. (A) Control group: showing islets of Langerhans consist mainly of β-cells which appeared oval or polygonal in shape. Their cytoplasm contains many characteristic β granules (arrows), euchromatic nucleus (N), and rough endoplasmic reticulum (RE). (B) Diabetic group: showing marked pathological changes in the β-cells in the form of apparent loss of their secretory granules leaving empty spaces (arrow). There are marked cytoplasmic vacuolation (*) and small nuclei (N). Congested blood capillaries were seen (BV). (C) Low dosage costus treated group: showing no marked improvement in β –cells. Most cells were shrunken with vacuolated cytoplasm (*) and heterochromatic nuclei (N). Many β secretory granules appeared empty (arrows). (D) High dosage costus treated group: showing β cells containing euchromatic oval nuclei (N). The β granules were increased in number and restored their normal structure (arrow). Numerous rounded and elongated mitochondria were observed. But there were some cytoplasmic vacuolation (*).

## Discussion:-

Type I diabetes is one of the most common chronic childhood conditions. Its incidence continues to increase worldwide and it has serious short-term and long-term implications (Daneman 2006). This disease is an autoimmune condition results from partial or complete destruction of β cells. Chronic hyperglycemia in diabetes causes increased oxidative stress which is postulated as one of the mechanisms that produce the deleterious effects of glucotoxicity (Baynes and Thorpe 1996). From ancient times, diabetes has been treated with natural antioxidants of herbal medicines with the emphasis on maintaining normoglycemia and protection from damaging effects of this disease. In this regard, we studied the effect of costus rhizome extract on the pancreatic β cells in alloxan-induced diabetic rats.

Alloxan-induced diabetes is one of the widely used models to induce Type I diabetes mellitus in the experimental animals (Viana, Medeiros et al. 2004, Etuk 2010). In the present study, treatment with alloxan led to histopathological changes in the pancreatic islets. There were marked decrease of β –cells and loss of the normal islets arrangement. Many β –cells showed marked cytoplasmic vacuolation and pyknotic nuclei. There were marked reduction in insulin secretion and significant increase in blood glucose level.

Alloxan has been found to be selectively toxic to pancreatic beta cells as it preferentially accumulates in the beta cells as glucose analogues (Jelodar, Maleki et al. 2005, Hamden, Boujbiha et al. 2009). Furthermore, the cytotoxic action of alloxan is mediated mainly by the generation of reactive oxygen species (ROS) (Dahech, Belghith et al. 2011). ROS produce fragmentation and damage of DNA of β –cells exposed to alloxan (Ebelt, Peschke et al. 2000).

The present study revealed that the treatment with costus rhizome extract significantly ameliorates the histopatholgical changes of the pancreatic islets. The islets increased in size and partially restored their normal arrangement. β–cells showed marked improvement in their ultrastructure. There were significant increase in insulin secretion and marked decrease in the serum glucose level. Dose dependent decrease in the serum glucose level was observed during the present study. The evident effect of *costus* extract was at dose of 400 mg/kg/day.

In the present study the hexane crude extract of *C. specious* rhizome was used. Efficacy of hexane extract in decreasing the serum glucose level and normalizing other biochemical parameters in diabetic rats, was confirmed previously (Daisy, Eliza et al. 2008, Eliza, Daisy et al. 2009). Aqueous and methanolic extracts of *C. speciosus* were, also, highly effective in bringing down the blood glucose level (Rajesh, Harish et al. 2009). In addition, it was reported that the ethanolic extract of *C. speciosus* could be used to improve the glucose and lipid metabolism as to reduce the imbalance between the generation of ROS and the scavenging enzyme activity in diabetic conditions (Bavarva and Narasimhacharya 2008, Revathy, Abdullah et al. 2014).

Action of *C. speciosus* to induce reduction in the level of glucose was suggested to be due to insulin like effects of *C. speciosus* or its ingredients which lead either to increase in glucose uptake mechanism possibly by inhibiting the process of gluconeogenesis (Ali, Khan et al. 1993). Moreover, the *C. speciosus* might also enhance the regeneration process of β–cells in pancreas (Bansal, Ahmad et al. 1981, Shanmugasundaram, Gopinath et al. 1990). Further, *C. speciosus* might inhibit the expression of nitric oxide synthase leading to increase insulin secretion (Gunawardana, Head et al. 2008).

In conclusion, based on the present findings, it is tempting to suggest that *C. speciosus* rhizome extract may possess a strong potential for development as natural protective agent that may be exceedingly effective at protection of pancreatic islets against toxic agents in a dose dependant manner. It can be used as a possible food additive or as a functional food recommended in human nutrition.

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