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ALPHA CELLS A 'THERAPEUTIC TARGET': EFFECT OF CITRULLUS COLOCYNTHIS ON ALPHA CELL COUNT IN HEALTHY AND ALLOXAN INDUCED DIABETIC MALE ALBINO RATS

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ABSTRACT

Introduction The molecular mechanism of diabetes involves both the beta and alpha cell dysfunction; however, the molecular mechanisms explaining the role of alpha cells in diabetes remains enigma. **Objective:** The objective of this research was to study the response of alpha cells to "Citrullus colocynthis" in euglycaemia and experimental hyperglycemia. **Methods:** Forty eight Albino rats were randomly divided into six groups A1, A2, B1, B2, C1 and C2, each comprising of 8 rats. Diabetes was induced in A2, B2 and C2 with single iv injection of 50 mg/kg Alloxan. Rats having fasting blood glucose greater than 250 mg/dl were labeled as diabetic. Aqueous seed extract of Citrullus colocynthis was given to diabetic rats in groups B2 and C2 and their controls in groups B1 and C1 for 14 days. **Results:** Alpha cell count was significantly increased (3.706± 0.656; $p<0.001$) in response to alloxan and it was decreased (Insignificantly) in diabetic rats who received high dose of CCT. Alpha to beta cell ratio was increased in diabetic rats which was improved towards control values after treatment with Citrullus colocynthis. There was increase, though insignificant, in alpha cell count in control rats after CCT treatment. There was no significant effect on islet area and relative tissue body weight index. **Conclusion:** Citrullus colocynthis regulates alloxan induced alpha cell hyperplasia and alpha to beta cell ratios. Alpha cells respond differently in normal and diabetic rats. Altered alpha cell response offers therapeutic target to alleviate diabetes.

KEYWORDS: Alloxan, diabetes, alpha cells, Citrullus colocynthis.

INTRODUCTION

Diabetes mellitus was labeled as 'Bi hormonal disease' decades back by Unger and Orci.[1] Alpha cells play a crucial role in the development of Type 2 diabetes.[2] The mechanism of diabetes initiation and progression involves appropriate functioning of both the beta and alpha type cells of the endocrine pancreas with their secretory hormones and, more importantly, the cross talk between these morphological and biochemical elements.[1]

Diabetes is found to be associated with alpha cell hyperplasia[3] and the resultant increased functioning of alpha cells leading to hyperglucagonaemia.[4] The increased expression of tyrosine phosphatase is an evidence for hyperfunctioning of alpha cells in diabetes.[5] Glucose is known to directly inhibit the alpha cell secretion[6] but hyperglucagonemia of diabetes is not suppressible with glucose infusion.[7 8 9]

Hyperplasia and hyper functioning of alpha cells might be due to GLP-1 (Glucagon like peptide), which is also produced by the alpha cells because Inhibition of GLP-1 degradation by Dipeptidyle Peptidase blockers, like Sitagliptin, is known to alleviate diabetes induced alpha cell proliferation and beta cell death which suggests that antidiabetic therapies halting the reactionary process of alpha cell proliferation in diabetes can alleviate beta cell loss and hyperglycemia.[10]

In this context, effect of medicinal herbs having anti diabetic potential on alpha cells still need to be unraveled. *Citrullus colocynthis*, also named as 'tumba' or 'bitter apple', is known to have phytochemicals with medicinal virtues such as antioxidant[11], antidiabetic[12 13,] hypoglycemic, hypolipidemic[14] and insulinotropic[15]. However, its effect on alpha cells has not been studied yet which is reported herein.

METHODOLOGY

Grouping and intervention

Forty eight male rats of Wistar strain weighing 100-150 gm, were procured from the animal house of university of Health Sciences Lahore. Animal procedures were approved by the institutional review board and performed in accordance with the Helsinki's guide. Animals were randomly divided into six groups A1, A2, B1, B2, C1 and C2, each comprising of 8 rats, kept in separate steel cages and fed with rat chow and water ad libitum. After one week period of acclimatization, diabetes was induced in groups A2, B2 and C2, wherein, A2 served as disease control and groups B2 and C2 were given 1ml/kg and 2ml/kg of aqueous seed

extract of Citrullus colocynthis respectively. Group A1 served as normal control and animals were given normal saline equal in amount used as solvent to dissolve Alloxan. Groups B1 and C1 were given 1ml/kg and 2ml/kg of aqueous seed extract of Citrullus colocynthis respectively, once daily through oral gavage using feeding tube, for 14 days.

Induction of diabetes

After acclimatization for a week, rats in groups A2, B2 and C2 were kept fasted for 12 hours. Diabetes was induced with single injection of 50 mg Alloxan per kilogram of body weight of rat, dissolved in 0.5ml ice cold saline just before injection. Injection was given intravenously in the proximal part of the dorsal tail vein of rats. It was given as a quick bolus to avoid decay of alloxan due to its shorter half-life. Fasting blood sugar levels were checked using Accu Check (Germany). Blood sample was taken from rat tail by pricking the tip of rat tail, after warming and tapping it to improve circulation. Rats having fasting blood sugar (FBS) greater than 250 mg/dl, 72 hours after having rendering them diabetic were labeled as diabetic.

Preparation of extract

Citrullus colocynthis plants were collected form a village of Sindh, Pakistan. The identification of the plants was confirmed by botanist in a local University. Plants were shade dried and seeds were separated and grinded to powdered form using a grinding mill. Seed powder weighing 200 gm was dissolved in 400 ml distilled water.[16] The solution was filtered using a four times folded gauze and filtrate was used for the experiment.

Histological procedures

At the end of experiment, rats were euthanized using chloroform and dissected to remove pancreata. Each pancreas was freed from all its attachments with stomach and intestine, weighed and fixed in bouin's fixative for 48 hours. Bouins's fixative was prepared according to method given in Bancroft.[17]Tissues were washed with 70% ethanol to remove picric acid. Pancreatic tissues were cut into 5 µm thick sections which were stained with H&E and Modified Aldehyde Fuchsin stain.[17]

Morphological analysis was performed for the mean islet area, alpha cell count per unit islet area, alpha to beta cell ratio. Eight islets were randomly selected per section and total 64 islets were studied per group. Number of alpha cells per 1000 mm^2 was recorded by using point counting method of Weibel.[18] The correlations of alpha cells with body weight,

pancreatic tissue weight, beta cells and with fasting blood sugar levels were also calculated. Relative tissue body weight index (RTWI) was calculated according to formula:[18]

$$RTWI = \frac{\text{Tissue weight}}{\text{Body weight}} \times 100$$

Statistical analysis

Data was analyzed using SPSS V20. Quantitative parameters were analyzed with one way analysis of variance (ANOVA) followed by Post Hoc Tukey test. Pearson correlation was applied to see the correlations.

RESULTS

The relative tissue body weight index was decreased in alloxan treated groups and no significant different was found after treatment with CCT (Fig. 1). Mean islet area was decreased in alloxan treated rats; however, the difference was not statistically significant when compared among the groups (Fig. 2). The number of alpha cells (No/1000mm^2) was 1.666±0.189 in group A1, 2.326±0.550 in group B1 and 2.197±0.616 in group C1. Alpha cell count increased in diabetic groups A2 (3.706±0.656). Low dose of CCT did not decrease the alpha cell count in B2 (3.897±1.159); whereas, mild decrease was observed in group C2 (3.066±1.360) which received high dose of CCT (Fig. 3); however, group C2 didn't differ significantly from A2 and B2. The difference was statistically significant ($p<0.001$) when groups A2 and B2 were compared with control groups A1, B1 and C1 each; however, group C2 only differed significantly from group A1 (p-value=0.002).

Mean value of alpha to beta cell ratio was significantly (p value<0000) increased in diabetic rats (Fig. 4 & 5). In CCT treated diabetic groups, ratio was reduced in a dose dependent manner (Fig. 3). There was significant negative correlation between alpha cells and beta cells (**r= -0.631**; p-value=0.000) and positive correlation between alpha cell count and fasting blood sugar levels (**r= 0.634**; p-value=0.000) at the end of the experiment. However, alpha cell count showed weak negative correlation with animal weight **(-0.490**; p-value=0.000) and strong negative correlation with pancreatic tissue weight **(-.744**; p-value=0.000).

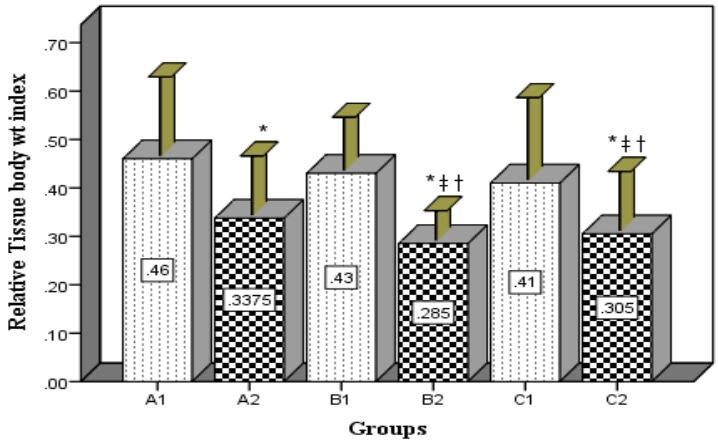

Fig 1: Bar chart showing comparison of RTWI. It was significantly decreased in diabetic control group (A2).*significantly different when compared with A1; †=significantly different when compared with B1; ‡=significantly different when compared with C

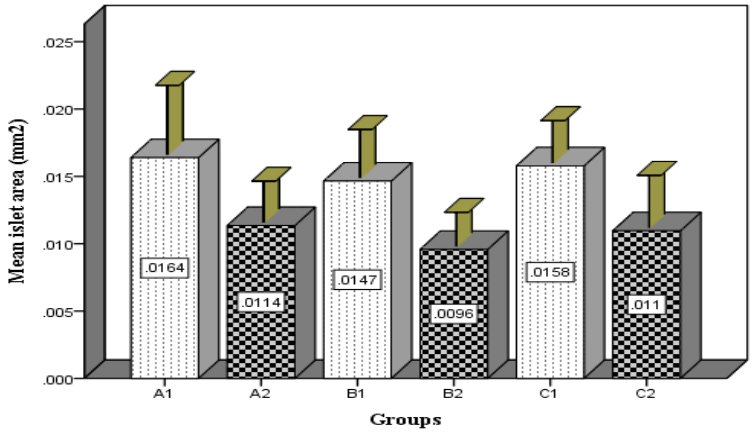

Fig. 2: Bar chart showing mean islet area (mm2) among the groups. The difference among the groups was not statistically significant.

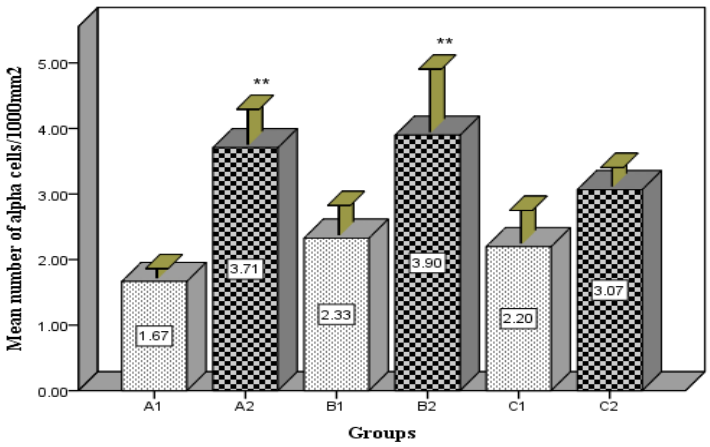

Fig. 3: Bar chart showing comparison of mean values of alpha cell count per 1000 mm2 of islet area. Alpha cell count is increased in diabetic groups A2, B2 and C2. ** Significantly different when compared with control groups A1, B1 and C1. C2 was different from only A1.

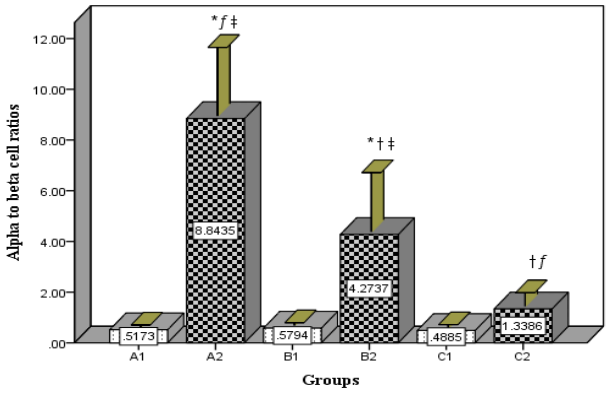

Fig. 4: Bar chart showing comparison of alpha to beta cell ratios. It was significantly increased in diabetic control group (A2).*significantly different when compared with A1, B1 and C1; *ƒ*=significantly different when compared wit

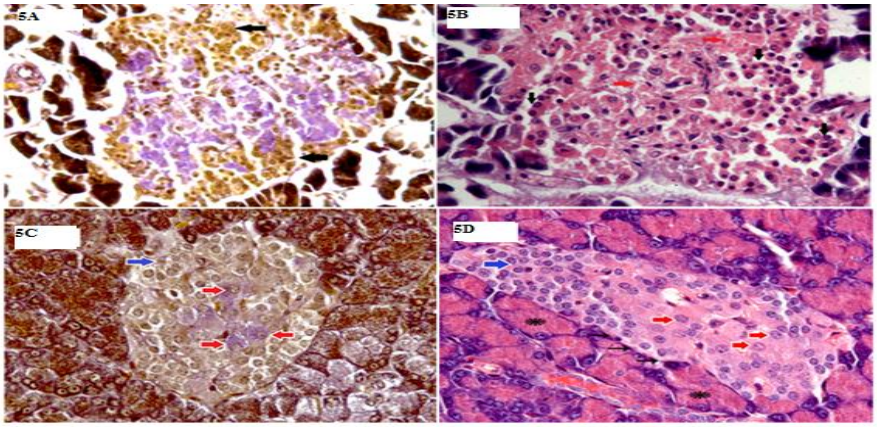

Fig. 5: Photomicrograph showing pancreatic islets from ALX treated group A2 (Fig. 5A & B) and ALX+CCT treated group C2 (Fig. 5C & D). Fig 5A shows islet cells stained with Modified Aldehyde Fuchsin stain illustrating alpha cells as brown cells arranged at periphery and beta cells as purple cells in the core. Beta cells show absence of nuclei as a sign of necrosis. Fig 5C/D shows re appearance of some nucleated insulin positive cells in the core (Red arrow) which are still less in number than the peripherally arranged alpha cells (Blue arrow)

DISCUSSION

This study was conducted to observe the effect of antidiabetic herb "Citrullus colocynthis" on alpha cell count. Upon treatment with alloxan to prepare the rodent model of diabetes, pancreatic beta cells were specifically damaged, whereas alpha cells escaped alloxan induced necrosis and showed histological preservation (Fig. 5). Our finding is in agreement with previous work.[19] The alpha cells might have spared alloxan induced injury because of the

presence of stronger anti-oxidant defense system as compared to beta cells. The mechanism of action of alloxan is production of reactive oxygen species (ROS) and the resultant oxidative stress leads to beta cell loss.[20] Likewise, alpha cells are spared in diabetic islet injury in humans which may be because the alpha cells are known to be resistant to glucotoxicity and lypotoxicity of Type 2 diabetes due to stronger antioxidant defense system[21] with increased expression of catalase[22] as compared to beta cells; whereas, beta cells have very low levels of these enzymes.[23] Moreover, alpha cells are less susceptible to hypoxia[24] and express more pro-survival proteins like Bcl-xl as compared to beta cells.[25] There was no significant change in RTWI after CCT treatment which may be due to shorter duration of the study. Extended work in this area may revert Alloxan induced change in RTWI. In the current investigation, significant increase in number of alpha cells (Fig. 3) was observed in alloxan treated groups. Our finding is consistent with the previous studies which have documented beta cell loss and alpha cell hyperplasia in animal model of diabetes.[19 10] Alpha cell expansion has previously been reported to be a reaction to beta cell injury.[10] The higher proportion of alpha cells to beta cells has also been found in autopsied samples of pancreata of humans having type 2 diabetes. It is postulated that increase in alpha cells appears to be relative is due to decrease in beta cells, resulting in loss of inhibitory effect of beta cells on alpha cells.[26]

Strong positive correlation found in our study between alpha cell count and fasting blood sugar levels implies the possible role of hyperglycemia in alpha cell expansion. However, alpha cell expansion has also been found even before significant hyperglycemia[10] which suspects the role of hyperglycemia in alpha cell proliferation. Strong negative correlation between alpha cell count and pancreatic weight shows that degree of damage to pancreatic tissue affects the alpha cell response.

Alpha cell dysfunction associated with diabetes is considered to be the defect of glucose to suppress glucagon secretion affecting the signaling rather than alpha cell mass.[26] On the other hand, it is suggested that all that matters is the lack of insulin and lack of its inhibitory effect on alpha cells unleashing the alpha cell secretion of glucagon.[10] It is also believed that the abnormal alpha cell response might be due to reduced entry of glucose through the thickened intra islet capillaries eliciting hypoglycemia and resultant alpha cell stimulation and glucagonaemia; or decrease entry of glucose into the alpha cells due to lack of insulin[28] or malfunctioning of beta cells rather than alpha cell expansion through proliferation.[8]

In the present study, we speculate that alpha cell expansion might have occurred due to stimulating molecular signals by the damaged beta cells. Literature supports that after beta cell loss, alpha cells increase in number and can get transformed into insulin secreting cells.[29] With high dose of *Citrullus colocynthis* extract, alpha to beta cell ratio reduced and was comparable with normal control which seemed to be due to increase in number of beta cells and decrease in alpha cells seems to play minor role because alpha cell count remained high even when treated with CCT after having rendering rats diabetic.

Moreover, the role of *Citrullus colocynthis* in reappearance of beta cells might be due to its antioxidant effect and secretion of some analogue of glucagon like peptide (GLP). Literature supports that in conditions of beta cell stress, alpha cells respond by proliferating and producing GLP-1, a growth hormone for beta cells.[29 30] Takeda et al.[10] observed alleviation of both the beta cell apoptosis and alpha cell proliferation with Sitagliptin, which makes GLP-1 available by inhibiting its degradation.

It could be due to cytokine interleukin-6 (IL6) lowering effect of Citrullus colocynthis. It is known to reduce IL6 levels which increases in diabetes and obesity.[31] It is said that IL 6 is required for alpha cell expansion.[32] Other explanation for mild decrease in alpha to beta cell ratio might be the presence of fatty acids like palmitate (8 %) and oleate (76 %) of seed oil content) in Citrullus seed[33] and these are known to decrease alpha cell count.[34] However, it looks like that these can play role in obesity induced diabetes.

CONCLUSION

We herein, provide evidence that *Citrullus colocynthis* treatment inn alloxan induced diabetes makes alpha to beta cell ratios comparable with control. It seems to be due to the increase in beta cells count mainly which in turn might regulate the alpha cell proliferation. *Citrullus colocynthis* increases alpha cell count, though insignificantly, in normal rats due to its hypoglycemic effect. The alpha cell response to anti-diabetic herb is different in the presence of euglycaemic and hyperglycemic conditions. So, therapeutic targets to alpha cell regulation need to be considered to halt the progression in diabetes.

LIMITATIONS & FUTURE IMPLICATIONS

Biochemical analysis for serum levels of insulin, glucagon or GLP-1 was not included which could have been an important evidence for the functional status of alpha cells and role of glucagon in diabetes. Further research is needed to explore the molecular and biochemical

mechanisms involved in alpha-beta cell signaling in response to metabolic stress and role of *Citrullus colocynthis* as GLP-1 analogue. This study suggests that therapeutic strategies halting the diabetes induced alpha cell proliferation should be considered in diabetes treatment.

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