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EFFECT OF ALCOHOLIC SEED EXTRACT OF *CAESALPINIA BONDUCELLA* ON MORPHOMETRY OF ENDOCRINE BETA CELL IN NORMAL AND ALLOXAN INDUCED DIABETIC MALE ALBINO RATS

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ABSTRACT

Present study was designed to evaluate the efficacy of seed extract of *Caesalpinia bonducella* a medicinal plant in healing and ameliorating pancreatic beta cell in alloxan induced diabetic male albino rats. The rats were divided into six groups - Normal-Control, Diabetic-Control, Normal+200mg ASECB, Normal+400mg ASECB, Diabetic+200mg ASECB and Diabetic+400mg ASECB. Rats made diabetic by intra peritoneal injection of alloxan. Oral administration of different doses of ASECB to normal and diabetic rats was conducted for a period of 14 days. Morphometry of beta cell and its nucleus was studied. Diabetic control group showed a significant ($P<0.05$) fall in cellular and nuclear diameter of beta cell, while the diabetic rats treated with different doses of ASECB showed a significant increase in cellular and nuclear diameter. In normal treated rats, the cellular and nuclear size of beta cell more or less similar to control rats indicating that the plant extract has no side effects. The higher dose of 400mg is more effective than 200mg. It is evident from the present histological study that ASECB can ameliorate and restore the cellular and nuclear diameter of beta cell to normalcy. Thus ASECB could serve as a good healing agent.

KEYWORDS: Diabetes, *Caesalpinia bonducella*, Beta cells, Alloxan, Cellular, Nuclear.

INTRODUCTION

Diabetes medically known as Diabetes Mellitus (DM) is a disorder of metabolism of carbohydrates, proteins and lipids caused due to defect in insulin secretion, insulin action or both.[1] DM is a common ailment afflicting many from various walks of life in different countries and is one of the most challenging health problems in the 21st century. DM occurs either when the pancreas does not produce enough insulin (T1DM) or when the body cannot effectively use the insulin it produces (T2DM). It is concerning to know that over thirty crores of people worldwide are suffering with DM and about same number of individuals are addressing to develop diabetes. DM is a chronic lifelong disease that can be controllable but not cured. It is a silent killer. If uncontrolled, it can lead to deadly complications. A world wide survey reported that DM is affecting nearly 10% of the population every year.[2] Every eight seconds somewhere in the world someone dies from DM. It is projected to become one of the world main disablers and killers within the next 25 years.[3] Recent studies on geographic and ethnic influences have shown that people of Indian origin are highly prone to DM. One in every five urban adult Indians now suffer from DM. Statistical projections about India suggest that number of diabetes will rise from 15 million in 1995 to 79.4 million by 2025, making it the country with the highest number of diabetic in the world.[4, 5] WHO has issued a warning that India will be the diabetic capital of the world.

In routine histological preparations all the cells of islets called insulocytes appear to be similar. However by special staining techniques like Aldehyde fuchsin technique 4 types of cells like Alpha cells, Beta cells, Delta cells and PP cells (Pancreatic polypeptide) can be differentiated. The structural organization of these 4 cell types has a species difference. In human and rat, the beta cells occupy the central position of islet, while alpha, delta and PP cells are located peripherally.[6,7,8] The arrangement differs in monkeys[9] and horses[10,11] where alpha cells are localized centrally and the other cells are arranged peripherally, where as in snakes, alpha and beta cells are located centrally and delta and PP cells are arranged peripherally.[12]

Different types of oral hypoglycemic agents such as biguanides, sulphonylurea are available along with insulin for the treatment of DM[13] but side effects are associated with their uses.[14,15] Though there are various approaches to reduce the ill effects of DM and its secondary complications, herbal formulations are preferred throughout the world due to lesser side effects,

natural origin and low cost. In fact many of the currently available drugs were derived either directly or indirectly from the plants. In many places throughout the world, DM is kept under control by the use of medicinal plant treatment. The WHO approves the use of plant drugs for different disease including DM.[16] There are hundreds plants worldwide that are used in the treatment of DM. Many plants with hypoglycemic property have been listed in many reviews.[17, 18, 19] India is the largest producer of medicinal herbs and is called Botanical garden of the world.[20] A large number of plant extract and their isolated components have been screened by various scientists throughout the world as antidiabetic during the last few decades. But the therapeutic efficacy, mechanism of action and safety of most of the herbals used has not been worked out. Only a small number of plants have received scientific and medical evaluation to assess their efficacy. Hence, the search for highly effective, non-toxic plant based antidiabetic drug is still continued. *Caesalpinia bonducella* is a medicinal plant belonging to the family Fabaceae. To our knowledge, though hypoglycemic property of *C. bonducella* was reported by other workers and from our laboratory, yet its effect on morphometry of beta cells in alloxan induced diabetic rats are not available. Hence the present study was carried out to investigate the effect of alcoholic seed extract of *Caesalpinia bonducella* (ASECB) on the cellular and nuclear diameter of beta cells in normal and alloxan induced diabetic male albino rats.

MATERIALS AND METHODS

Experimental animals used

Adult male albino rats of Wistar strain developed from Norwegian rat (*Ratus norvegicus*, Family: Muridae, Order: Rodentia) aged about 80-90 days, weighing between 200-240 gms bred in animal house of Department of Post Graduate Studies and Research in Zoology, Karnatak University, Dharwad, India were selected as an experimental model in the present investigation. The rats were fed with rat pellet feed supplied by M/s Krish Scientist's Shoppe, agents for scientist's choice laboratory animal feed, Bangalore, India and water *ad libitum* throughout the experimental period. The experiments were designed as per guidelines of Institutional Animal Ethics Committee (IAEC), vide Registration No. 639/ 02/a/CPCSEA.

Induction of diabetes

Alloxan monohydrate ($C_4H_2N_2O_4H_2O$) was used as diabetes inducer in rats. The purpose of choosing alloxan monohydrate as diabetes-inducing agent was that it causes a massive reduction of the beta cells of the islets of langerhans and induces hyperglycemia.[21] DM was induced in normal healthy male albino rats by single intraperitonial freshly prepared injection of alloxan (150 mg/kg body weight)[22] dissolved in normal saline (2 ml/kg bw). The animals were allowed to fast for 12 hours prior to alloxan injection. After 3 days of alloxan injection, the glucose level was measured. Rats showing fasting glucose levels >250 mg/ dL were considered as diabetic and selected for the investigation.

Procurement of seeds of *Caesalpinia bonducella* and preparation of the extract

C. bonducella seeds were collected from the local market in Dharwad district, Karnataka, India. The seed extract was prepared by following the method of Shukla et al.,[23] The seeds were shade dried, coarsely powdered, sieved and 50gm powder was extracted with 500ml of 95% ethanol by using a Soxhlet apparatus for 16 hours. The crude extract obtained was filtered through Whatman paper and the filtrate was evaporated to dryness on rotary flash evaporator under reduced pressure to obtain a greenish black jelly residue. The seed extract so obtained was then stored in airtight glass containers and refrigerated till further use.

Experimental design

A total of 36 rats (18 normal, 18 diabetic) were used. The rats were randomly divided into 6 groups. All the experimental rats were placed on normal diet and observed daily for general health and behavior in the cages throughout the experimental period. Alloxan was administered and induced diabetes in Group-II, V and VI. Group-I served as normal-control, Group-II served as diabetic-control and both the groups did not receive any treatment. The experimental animals of Group-III, IV, V and VI were administered with ASECB. Six rats were used for each group (n=6).

Histopathological and Morphometric studies

GROUPS	TREATMENTS	DOSES
Group-I	Normal-Control	Administered 1ml distilled water /rat/day orally for 14 days
Group-II	Diabetic-Control	Administered 1ml distilled water/rat/day orally for 14 days
Group-III	Normal-Treated	Administered 200 mg ASECB/kg bw orally/rat/1ml for 14 days
Group-IV	Normal-Treated	Administered 400 mg ASECB/kg bw orally/rat/1ml for 14 days
Group-V	Diabetic-Treated	Administered 200 mg ASECB/kg bw orally/rat/1ml for 14 days
Group-VI	Diabetic-Treated	Administered 400 mg ASECB/kg bw orally/rat/1ml for 14 days

At the end of the experiment animals were kept overnight fast but the animals had free access of water and sacrificed after a short exposure to sodium pentobarbital. Pancreas was surgically removed, blotted to remove blood traces and stored in 10% formalin. For histopathological studies, the procedure for tissue preparation such as fixation, buffering, dehydration, embedding etc., were carefully standardized to minimize

experimental variations. The fixative, buffer and embedding medium were freshly prepared. The fixed pancreatic tissue were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Ribbons of ultrathin sections of 4-5 microns thickness with pale gold to silver interference colors were prepared and first stained with basic dyes Haematoxilin and Eosin (H&E) according to Conn procedure[24] and later sections were specifically stained with aldehyde fuschin for beta cells as described by Gomeri.[25] For the morphometric study, five blocks were chosen by lottery from each of six animals and thus a total of thirty blocks from each group was cut. From each block about fifty islets were selected from hundred randomly selected cross section of pancreas. Evaluation of morphometry of beta cell was carried out using a micro occulometer (Erma, Japan), previously standardized with the help of a microscopic scale. The beta cell and its nucleus size measurement were done at their longest axis at 400 X.

Statistical evaluation

All values were expressed as mean + S.E. Statistical evaluation was carried out using one-way analysis of variance (ANOVA).[26] P values of < 0.05 were taken as significant.

RESULTS

Cellular diameter of beta cell

Table-1 shows the mean values (in microns) and ANOVA results of cellular and nuclear diameter of beta cells. The mean diameter of beta cell in Group-I was 8.76±0.17. The diameter of beta cell in alloxan induced diabetic rats (Group-II) declined to 4.70±0.20. This sharp fall was statistically significant (P<0.05) compared to control rats. The beta cell diameter in rats of Group-III and IV which are normal but administered with 200mg and 400 mg ASECB respectively was 8.54±0.22 and 8.50±0.47, which was almost similar to that of normal control rats indicating that the plant extract has no side effects. There was increase of beta cell size in diabetic induced rats of Group V and VI, which were administered with 200 mg and 400 mg ASECB respectively. The beta cell size recorded in Group V and VI were 6.36±0.30 and 8.66±0.41 respectively, which was statistically significant (P< 0.05) compared to diabetic- control rats (Group-II). The increase in beta cell size observed in experimental animals of Group-V and Group-VI was mainly due to ASECB. The elevation was almost nearer to the levels of normal-control rats.

Nuclear diameter of beta cell

The mean nuclear diameter of the beta cell in Group-I, II, III, IV, V and VI was 4.86±0.22, 2.72±0.29, 4.98±0.07, 4.88±0.11, 4.10±0.25 and 4.76±0.24 respectively. The nuclear size of the diabetic control group (Group-II) significantly decreased (P<0.05) when compared to normal-control group. The diabetic induced rats after administration of ASECB (Group-V & Group-VI) showed significant increase (P<0.05) in nuclear size when compared to diabetic control rats (Group-II). The increase in nuclear size in noted in experimental animals of Group & VI was mainly due to administration ASECB. The increase in nuclear size in Group-V & VI was however not significant when compared to Group-I (Normal-control).

Table 1: Effect of treatment with ASECB on Cellular and Nuclear diameter of Beta cell in normal, diabetic and diabetic treated albino rats.

Groups	Treatment	Cellular diameter of Beta cells (µm)	Nuclear diameter of Beta cells (µm)
I	Normal-Control	8.76±0.17^{a}	4.86±0.22^{a}
II	Diabetic-Control	4.70±0.20^{b}	2.72±0.29^{b}
III	Normal+200mg ASECB	8.54±0.22^{a}	4.98±0.07^{a}
IV	Normal+400mg ASECB	8.50±0.47^{a}	4.88±0.11^{a}
V	Diabetic+200mg ASECB	6.36±0.30^{c}	4.10±0.25^{a}
VI	Diabetic+400mg ASECB	8.66±0.41^{a}	4.76±0.24^{a}

Note: *Groups with similar superscript letters (a, b, c) in the given column indicates not significant. While groups with dissimilar superscript letter indicate significantly different from each other.*

DISCUSSION

The pancreas is a mixed gland that produces digestive enzymes and hormones which regulate glucose, lipid and protein metabolism.[27] DM arises when beta cells are unable to meet insulin demands in the body, due to failure and destruction of the beta cell mass or increased insulin needs due to insulin resistance. Insulin is a protein that is essential for proper regulation of glucose and for maintenance of proper blood glucose levels.[28] Beta cell represents about 65% (by number) and about 85% (by volume) of the total islet mass.[29] The pancreatic beta cells of the islets of Langerhans are the only cells that produce insulin in humans as well as in almost all animals.[30] It is widely recognized that beta cell dysfunction, specifically the inability of beta cells to properly secrete insulin response to high blood glucose level, is among the earliest clinical features during progression to T2D.[31] It has been well documented that oxidative stress plays a important role in beta cell dysfunction and apoptosis.[32] Because of poor antioxidant capacity, beta cells are vulnerable to the oxidative stress induced by both T1D insulitis and T2D glucotoxicity.[33] Studies in the last several decades have shown that plant based therapies have a potential to control and treat diabetes and its complications.[34, 35] The results found in the current morphometric study show

that the cellular and nuclear diameter of beta cells of diabetic control rats (Group-II) were significantly ($P<0.05$) decreased than the corresponding values of the control group. The decrease in cellular diameter correspondingly decreased the nuclear size. This decrease in cellular and nuclear size and decrease in cell population might be attributed to the reduced secretion of insulin.

There was a significant increase in the overall diameter of beta cell on the supplementation of plant extract to diabetic animals. The nuclear size of the beta cell also proportionately increased in diabetic treated group. The beta cells were restored to normalcy. ASECB has possessed many of the active phytocomponents with antioxidant property and this property of the plant might have prevented the further deterioration and destruction of beta cell due to oxidative stress but in turn played a vital role in stimulating the shrinked beta cells to rejuvenate. This has resulted in secretion of insulin in sufficient quantity and thus hypoglycemia was achieved in diabetic treated rats (Group-V & VI). The present data are in consistent with those of our previous histological studies on endocrine pancreas reported earlier from our laboratory[36, 37] reporting increase in islet size, beta cell population and normalization of disturbed lipid profile in diabetic group treated with ASECB. Recovery of beta cells to normalcy was dose dependant of the plant extract. The restoration of beta cells and its nucleus was evident at higher dose level. The noticeable restoration of beta cells in diabetic treated rats corroborates the normalcy of blood glucose level in alloxan induced diabetic rats. In the current study the results of Group-III and IV (Normal-treated) were similar to the control one indicating safety and non toxicity of ASECB administration. These observations were in consistent with results of earlier researchers,[38,39] who observed that, there is no any observed toxicity of the plant extract in rats.

CONCLUSION

Based on the current work it can be concluded that the ASECB, though it does not completely recover the damaged beta cells, but it can ameliorate and restore the microscopical changes of beta cells to a large extent, which is secondary to control blood glucose level. Thus ASECB could serve as a good healing agent. Although promising results have been obtained, the exact mechanism responsible for the healing/regenerative effect of main principle in the ASECB on endocrine beta cell and its isolation is yet to be elucidated.

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