UTTAR PRADESH JOURNAL OF ZOOLOGY

41(15): 22-27, 2020
ISSN: 0256-971X (P)

MORPHO METRICAL ASSESSMENT OF PANCREAS SHOWING PATERNAL DIABETES HERITABLE TO FIRST GENERATION MALE LITTERS AND THERAPEUTIC IMPACT OF BLACK PLUM (*Syzigium cumuni*)

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AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Syed Hafeezul Hassan, Liaquat National Medical College, Pakistan.
(2) Marwa M. Rashed, Mansoura University, Egypt.

Received: 14 August 2020
Accepted: 19 October 2020
Published: 27 October 2020

Original Research Article

ABSTRACT

Present study focused on morpho metrical assessment of Pancreatic parameter (pancreatic mass and pancreatic diameter) of first generation male litters obtained from diabetic male mated with normal female and therapeutic impact of black plum. Alloxan persuade diabetic male mice leads to decrease in sufficient insulin dissimulation in them. Fixed dose of Black plum (*Syzigium cumuni*) seed powder were fed along with food to First generation and morphometry of pancreas was done. Black plum (*Syzigium cumuni*) seed powder reinstate the histoarchitecture of pancreatic β cells and accelerate the secretion of pancreatic insulin in First generation male mice. Blood glucose levels were observed to recur to their normal levels. Significant reduction in the alterations or abnormalities (i.e. smaller and more irregular shaped islets) in First generation male mice were noticed. Hyperglycemia may affect the epigenetic modifications during spermatogenesis and these epigenetic alterations in pancreatic structures cannot be normalized by giving normal diet implying that these alterations were transgenerational and may inherited through male germ line and move onto more than one generations. In near future, transgenerational or epigenetic factors may be regarded as huge aspect in assessing risk of diabetes.

Keywords: Epigenetic; histoarchitecture; hyperglycemia; inherited; spermatogenesis; *Syzigium cumuni;* transgenerational; offsprings.

1. INTRODUCTION

Diabetes mellitus is the most common endocrine chronic, and progressive disorders characterized by elevated level of blood glucose. It occurs either when pancreas does not produce enough insulin hormone, which regulate blood sugar or when body cannot effectively use the insulin it produces.

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Diabetes mellitus, long considered a disease of minor significance to world health, is now taking its place as one of the major threats to human health in the 21st century. The history of diabetes is very old but modern civilization and its life style has made it an epidemic. The past two decades have seen an explosive increase in the number of people diagnosed with diabetes worldwide [1]. Conspicuous changes in the human environment and his behaviour and lifestyles accompanied by globalisation have resulted in escalating rate of obesity and diabetes. High content of starchy diets, fast food, and beverages and stressful life are the major contributors of the increased occurrence of diabetes [2].

There are two common type of diabetes – Type 1 which is an autoimmune disorder where the T lymphocytes are involved in the destruction of β cells of pancreas. It is quite common in children and young. Type 2 diabetes is highly prevalent and 90% of the globe population is affected by this disorder. It is due to obesity and development of peripheral resistance to insulin accompanied by a malfunctioning pancreas.

Dietary factors play a key role in the development of various human diseases including hypertension, cardiovascular, and other metabolic diseases, hyperlipidemia, thrombosis and diabetes. Medicinal plants continue to provide valuable restorative elements, in both modern medicine and in traditional system. The doubts about the efficacy and safety of the oral hypoglycemic elements have promoted a search for safer and more effective drugs in the treatment of diabetes (Reaven et al; 1983). In spite of the fact that insulin has become one of the most important restorative element known to medicine, researchers have been mainly effort to insulin substitutes from synthetic or plant sources for the treatment of diabetes. Many herbs have remained a substitute to conventional therapy especially in poor areas where insulin is not readily available (Sanchez et al; 1994).

Syzigium cumuni belongs to Myrtaceae family, commonly known as Jamun in India, are best known for their ability to regulate blood sugar level. It contains compound called jamboline and jambosine that minimize the rate at which sugar is relased into the blood stream, and also increase the production of insulin. It possesses powerful antioxidants like flavonoids and also phenolic compounds that help keep harmful free radicals at bay [3].

Aim of present experiment was to enlight the transgenerational effects of paternal diabetes through morphometric studies of pancreas on alloxan conduced diabetic male mice and their First generation obtained after mating with normal female mice.

1.1 Morphometrics

Morphometry refers to quantitative analysis, a concept that encompasses size and shape. Morphometric [4] analysis is commonly performed on organisms, are useful in analyzing developmental changes in forms, for estimating quantitative –genetic parameter of shape, detecting changes in shape.A major objective of morphometrics is to statistically test hypothesis about factors that effects shape.

Histomorphometric studies was done with the help of a Linear Scale –Occular Micrometer & an Area – Measuring Occular grid inserted into the eye piece. Occular micrometer & Occular grid were calibrated with a 1 mm stage micrometer. 24 histological stained sections (8 from each group) were used for morphometric analysis.

2. MATERIALS AND METHODS

2.1 Plant Material

Syzigium cumuni seeds from trees grown in the campus of PG department of Botany,TMBU Bhagalpur were collected fresh and seeds were air dried in the sun, reduced to coarse powder with the help of mortar and pestle and kept in airtight jar till utilization.

2.2 Experimental Animals

Swiss albino mice *Mus musculus* of weight about (30±5) gram were used throughout the experiment. The animals were procured from CDRI Lucknow. Mice were maintained at the Animal House of University Department of Zoology, T.M. Bhagalpur University under standard environmental conditions. Standard environmental conditions such as temperatue (26±2)°C, relative humidity (45-55%), and 12 hrs dark/light cycle were maintained in quarantine. They were fed with standard diet. Food and water were given *ad libitum*. Rice husk was used as bedding material and replaced daily. Animal handling was performed as per good laboratory practice (Work Manual, CDRI). The mice of 12 weeks of age were acclimatized in the laboratory condition for one week before the experiment [5].

2.3 Induction of diabetes

Alloxan (2,4,5,6-tetraoxypyrimidine, 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative and was originally isolated in 1838 by Brugnatelli and got its name in 1838 by Friedrich

Wohler and Justus von Liebig. Alloxan is a toxic glucose analogue, which selectively destroys insulin producing cell in pancreas when administered to rodents and many other animal species. This causes an insulin dependent diabetes mellitus, also known as Alloxan Diabetes, in these animals, with characteristics similar to type 1 diabetes in humans [6].

Alloxan is a urea derivative which causes selective necrosis of the β- cells of pancreatic islets. It has been widely used to induce experimental diabetes in animals species such as rabbits, rats, mice and dogs by varying the dose of alloxan [7].

The drug Alloxan-monohydrate was purchased from Loba Chemicals, Mumbai. All other chemicals used in the entire experiments were of analytical grade and were acquired from commercial sources.

The experimental animals were kept on fast before induction of diabetes. Diabetes was induced intraperitonally by administrating alloxan-monohydrate [8].

Total dose of Alloxan monohydrate (450 mg/kg/bw) was administered in three injections at intervals of 48 h (150 mg/kg/bw) each time.

2.4 Experimental Design

The experimental mice were divided into three groups of 10 animals in each.

Group-F-0 (Control) Paternal diabetic mice

Group-F-1(Generation obtained from F-O i.e.when diabetic male mated with normal female mice)

Group-F-T(F-1 mice fed with *Syzigium cumuni* seed powder).

The total experimental protocol was maintained for about day six – nine months, after obtaining F-1 generation. Blood glucose level of mice observed on 7^{th}, 14^{th} and 21^{st} days for all *Syzigium cumuni* fed F-1 male mice.

2.5 Pancreatic Mass

Pancreas were dissected out, washed in saline (.09%NaCl), dried with the help of blotting paper and wet weight of gland was measured using digital scale balance.

2.6 Islet Density

It is the number of islets per microscopic field.Four random fields were selected per section. Islet size was estimated by measuring diameter of islet under microscope [9].

Calculation

Volume Density (VD)= P (Islet)/ P(Reference)

Where

P(Islet)&P(Reference) were no. of test points falling on islet's profile & on the reference space respectively.

2.7 Determination of Islet Diameter

Morphometery used to calculate islet diameter using a graticule from a calibrated linear scale major axes (a), minor axes (b) and axes of islets at right angle to major axes (a) were measured and mean islet diameter was calculated.

Calculation

- Diameter of islet(D) = $\sqrt{ab}$

Where,

D = diameter of islet
a = major axis
b = minor axis

- No. of islets per unit area(NA)=N/AT

Where,

N = No. of sectioned profile of islets.
AT = Area of section.

Islet area was measured in each pancreatic section at 400 X magnification using ocular grid.

2.8 Statistical Analysis

The data obtained in different quantitative estimation were processed in MS-Excel for calculating standard error and Analysis of Variance.

2.9 Analysis of Variance

With the help of processed data, the value of D.F. (Degree of Freedom), S.S (sum square). MSS (Mean sum square) and variance ratio were determined and put in ANOVA table.

3. RESULTS

Morphometric study grabes attention on significant differences in the diameter of the pancreatic islets in

diabetic individuals (Group II) to those of *S. cumuni* fed individuals, (Group F1-T) when compared with control ones (Group F0). Transgenerational changes in pancreatic morphometry (percent volume density and diameter) and pancreatic mass at birth and at weaning stage are predicted in Table 1 and Table 2.

The pancreatic percent volume density at birth was recorded (20.4±0.79)% in F-0 group of mice and this value was decreased to (19.1±0.74)% in group-F-1, while in F1-T group it was recovered to (20.1±0.74)%.

The pancreatic percent volume density at weaning was recorded (20.8±0.67)% in F-0 group of mice and this value was decreased to (19.3±0.62)% in group-F-1, while in F1-T group it was recovered to (20.1±0.82)%.

The pancreatic diameter at birth was recorded (78.3±1.12) µm in F-0 group of mice and this value was decreased to (74.6±0.69) µm in group-F-1, while in F1-T group it was recovered to (77.2±0.61)µm.

The pancreatic diameter at weaning was recorded 80.4±1.48 µm in F-0 group of mice and this value was reduced to 77.4±0.94 µm in group-F-1,while in F1-T group it was recovered to (79.6±1.10) µm

Statistical analysis showed that the source of variance (Analysis of Variance; ANOVA for single factor) as between groups (between treatment) and with in groups (control) were found to be significant at 95% and 99% of confidence ($p<0.05$, $p<0.01$).

4. DISCUSSION

In this study, it has been observed that paternal diabetes i.e. when a diabetic male mice mated with a normal female mice, affects the metabolic parameters in F-1 generation. One of the parameters investigated in this study was morphometrical assessment of pancreas of male mice. However, First generation offsprings were normoglycemic under control conditions but secrete low levels of insulin compared to their parents [10]. The maintenance of glucose levels in F-1 male generation may reflect an increased sensitivity to insulin and a subsequent increase in the glucose uptake by peripheral tissue [11].

Moreover, F-1 groups had significantly smaller and more irregularly shaped islets as compared to F-0 groups. Overall, the F-1 neonatal islets showed a less organized histoarchitecture compatible with a delay in islet maturation. Furthermore, at birth, the islets are normally arranged like pearls on a string [12], but this structure was not observed in the F-1 offspring. The disorder of endocrine cell types within the islet detected both at birth and weaning suggests that islet cell distribution is not altered with age.

Our observations of the F1 generation are consistent with single-generation animal studies [13].

Table 1. Transgenerational changes in pancreatic mass (G) in male litter offspring and therapeutic impact of black plum seed powder

AGE	F_0	F_1	F_1T
AT BIRTH	0.014±0.0003	0.012±0.0005*	0.013±0.0006**
DAY- 7	0.061±0.0008	0.057±0.0010*	0.059±0.0012**
DAY- 14	0.088±0.0007	0.085±0.00010*	0.087±0.0011**
DAY- 21	0.131±0.0017	0.117±0.0015*	0.121±0.0018**
AT WEANING	0.193±0.0019	0.158±0.0014*	0.161±0.0018**

*N=10 Values are given as mean±SE for groups of ten mice. Values are statistically *significant (p<0.05); ** highly significant (p<0.01)*

Table 2. Transgenerational changes in pancreatic morphometry in male litter offspring and their possible recovery by *Syzigium cumuni* seed powder

Islet of Langerhan's	F_0	F_1	F_1T
AT BIRTH			
Volume Density %	20.4±0.79	19.1±0.74*	20.1±0.74**
Diameter (µm)	78.3±1.12	74.6±0.69*	77.2±0.61**
AT WEANING			
Volume Density %	20.8±0.67	19.3±0.62*	20.3±0.82**
Diameter (µm)	80.4±1.48	77.4±0.94*	79.6±1.10**

*N=10 Values are given as mean± SE for groups of ten mice. Values are statistically *significant (p<0.05); ** highly significant (p<0.01)*

Diabetes may influence the epigenetic modification during spermatogenesis and that these epigenetic dysregulation or alterations in pancreatic structure cannot be normalized by providing normal diet, implying that these changes are transgenerational and may be inherited through the male germ line and passed onto more than one generation, which in turn may increase the risk of diabetes in offspring [14].

These findings also show that, in the near future, epigenetic factors, which are inheritable, may be regarded as an important genetic factors in determining risk of having diabetes.

Syzigium stimulates the secretion of pancreatic insulin and restore the architecture of the pancreatic β-cell in diabetic experimental animal cells [15,16]. These anti-diabetic or antihyperglycemic properties of *S. cumini* seed are may be due to a single component or a combination of phytochemicals, such as triterpenoids, anthocyanins, oleic acid, essential oils, glycosides, saponins, and several members of the flavonoids (e.g., rutin, quercetin, myricetin [17,15] which directly or indirectly effect on insulin resistance and β-cell function.

5. CONCLUSIONS

The result of the present study indicates that *S. cumuni* seed powder has a protective effect on alloxan monohydrate induced diabetes.

From the above study, it can be inferred that *Syzigium cumuni* seed powder is beneficial in the preventing as well as in treatment of metabolic aberrations caused by diabetes. The optimum dosage for both conditions need to be established via clinical studies involving human subjects. Prior to this there is a need to ascertain its safety on prolonged consumption on vital organs of the mice, determine the acute, subchronic toxicity level as well as LD_{50}. These data are utmost important for projecting the commercial and global use of *Syzigium cumuni* seed powder as a diabetic prevention and curative natural product.

ACKNOWLEDGEMENT

I am very thankful to my research supervisor Dr. Manish Chandra Varma, Ph.D., F.Z.S.I., F.S.L.Sc, F.I.E.S, M.N.A. Sc., Retd. Professor and & Head, University Dept. of Zoology, T.M. Bhagalpur University, for his constant guidance, encouragement and keen interest throughout the period of the work. I am also obliged to PG Department of Botany, TMBU for providing me fresh *Syzigium cumuni* seed.

CONFLICT OF INTEREST

I do not have any conflict of interest for this experiment.

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