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Comparative Assessment of Pancreatic Morphometry Among Hyperglycemic (F₀), F₁ Generation and *Syzigium cumuni* Treated Offsprings (F₁-T) of Swiss Albino Mice

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Abstract: Now-a-days with the changes in population lifestyle, prediabetes is constantly on the surge. Management of prediabetes currently depends fully on lifestyle modifications like weight loss, exercise and diet control. Better drugs with acceptable safety profile are needed for better control of prediabetes. The present research assessment sought to compare the effectiveness of *Syzigium cumuni* (Black plum) seed powder on the pancreas through pancreatic morphometry on the generations obtained when hyperglycemic (Alloxan monohydrate persuaded) male mice mated with normal female.

Morphometry refers to quantitative analysis of shape and size. Histomorphometric studies were performed with the help of Linear Scale Ocular Micrometer and Area-Measuring Ocular grid inserted into the eye piece, both calibrated with a 1 mm stage micrometer. Histological slides of pancreas were used for morphometric analysis. Hyperglycemic conditions in the male mice were persuaded by injecting alloxan monohydrate, which prompt insulin deficiency in them and their obtained generation also. These mice were kept in Groups F₀ and F₁, respectively. Now these F₁ groups were given fixed dose of *Syzigium cumuni* (Black plum) seed powder along with food and were kept in Group F₁-T. Comparative morphometric assessment of pancreas were performed among F₀ (Diabetic Male), F₁ (generation of F₀), F₁-T (F₁ generation fed with *Syzigium cumuni* seed powder along with food) at the time of birth and at weaning time.

Drastic decrease is noticed in pancreatic diameter at the time of birth and at weaning time in case of F₁ when compared with F₀. Now when these F₁ were given *Syzigium cumuni* seed powder along with the food, parameters were observed to be improved. Hyperglycemia may affect the epigenetic modifications during spermatogenesis and inherit through male germ line and these changes may passed onto more than one generations. *Syzigium cumuni* (Black plum) seed powder sparks the secretion of pancreatic insulin, and it also brings back the histoarchitecture of β -cells of pancreas in F₁-T generation.

Presence of various identified phytochemicals in *Syzigium cumuni* (Black plum) seed powder with their described mechanisms of action shows that some compounds target multiple metabolic routes, thereby, becoming a potential pharmacological tool.

Keywords: Prediabetes, Histoarchitecture, Hyperglycemia, Pharmacological, Phytochemicals, Spermatogenesis, Transgenerational, Alloxan, *Syzigium cumuni*

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Introduction

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 surge in the number of people diagnosed with diabetes worldwide. 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 (Kerrison, 2017).

Diabetes mellitus is a group of metabolic disorders resulting in hyperglycaemia. Genetic and environmental factors contribute to the insufficient secretion of insulin, decreased response to insulin either endogenous or exogenous, raised glucose production, with or without abnormalities in fat and protein metabolism. The resultant hyperglycaemia might lead to acute symptoms and metabolic abnormalities (Powers, 2018).

It is a very complex metabolic disorder associated with developing insulin resistance, impaired insulin signaling and β -cell dysfunction, abnormal glucose levels and lipid metabolism, sub-clinical inflammation and increased oxidative stress. These metabolic abnormalities lead to long-term pathogenic conditions including micro- and macro-vascular complications, neuropathy, retinopathy, nephropathy, and a consequent decrease in quality of life and a hike in the rate of mortality (Kerrison, 2017). The main leading factor in progression of diabetes is diet. Both experimental and epidemiological evidences have proved that consumption of vegetables rich in phenolic compounds and possess high antioxidant capacity may have inverse relationship with the incidence and prevalence of diabetes (Liu *et al.*, 2020). Dietary control remains one of the most dominant and desirable avenues for the prevention and management of chronic degenerative diseases such as diabetes and cardiovascular diseases. Although, throughout the

world various conventional herbal plants have been used as traditional medicine to treat diabetes, a lot remains to be done scientifically to confirm the efficacy of these herbal plants (Yeh, 2003).

WHO (World Health Organisation) projects that diabetes will be the 7th leading cause of death in 2030. India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the “Diabetes capital of the world”. Although the prevalence of type 1 and type 2 diabetes mellitus (Powers, 2015) is increasing worldwide, the prevalence of type 2 diabetes mellitus is rising much more rapidly, presumably because of increasing obesity, reduced activity levels as countries become more industrialized and also due to ageing of the population. Over the time, diabetes can increase the risk of health-related problems including nerve damage, kidney damage, blindness, amputation of lower limbs and cardio-vascular diseases. Although diabetes cannot be cured, the disease can be managed by non-pharmacological and pharmacological strategies, where improvements in glycemic control are important factors in delaying the onset and progression of diabetes-related complications (Kerrison, 2017).

Syzigium cumuni:

Syzigium cumuni (black plum), growing extensively in different agro-climatic countries possess several medicinal properties (Ayyanar *et al.*, 2013). The extracts of various parts of *S. cumuni* contains phytochemicals including tannins, anthocyanins, terpenes, flavanols and aliphatic-acids (Sharma *et al.*, 2011; Srivastava *et al.*, 2020, 2021). All parts of *S. cumuni* are rich in polyphenols (Sharma *et al.*, 2008). Both fruit and flowers of *S. cumuni* are enriched in anthocyanins as cyanidin, delphinidin, peonidin, pelargonidin, petunidin, and malvidin (Ramaya *et al.*, 2012). The seeds of *S. cumuni* contain rucetin and quercetin while leaves have been reported to contain kaempferol, myricetin, quercetin and their glycosides (Ayyanar, 2013). The plant has been

reported to contain ellagic acid, triterpenoids, acetyl oleanolic acid, quercetin, isoquercetin, myricetin and kaempferol. *S. cumuni* possesses antihypoglycemic, antimicrobial, hypolipidemic, anti-allergic, anti-inflammatory, cardio-protective, hepato-protective and antineoplastic properties (Baliga, 2013).

Syzigium cumuni belongs to the family Myrtaceae, is a large evergreen tree up to 30 m height and a girth of 3.6 m with a bole upto 15 m found throughout India upto an altitude of 1,800 m (Ross, 2003). Most of the plant parts of *S. cumuni* are used in traditional system of medicine in India. The original home of *Syzigium cumuni* is India or the East Indies. It is also found in Thailand, Philippines, Madagascar and some other countries. The plant has been successfully introduced into many other tropical countries such as the West Indies, East and West Africa and some subtropical regions including Florida, California, Algeria and Israel (Ross, 2003).

The present study was conducted-- (i) to investigate the antidiabetic efficacy of *Syzigium cumuni* seed powder in alloxan monohydrate persuaded hyperglycemic Swiss albino mice; and (ii) to analyze the effectiveness of *Syzigium cumuni* seed powder in pancreatic morphometry of hyperglycemic (F₀), F₁ generations and *Syzigium cumuni* treated offsprings (F₁-T) of Swiss albino mice.

Materials and Methods

Plant Material:

Fresh *Syzigium cumuni* seeds were collected from trees grown in the campus of University Department of Botany, Tilka Manjhi Bhagalpur University, Bhagalpur, India and air dried in the sun, reduced to coarse powder with the help of mortar and pestle and kept in airtight jar till utilization.

Experimental animal:

Thirty Swiss albino adult male mice (12 weeks old; bw 25-30 g) were used for investigation. The

animals were procured from CDRI Lucknow, India. Mice were maintained at the Animal House of University Department of Zoology, Tilka Manjhi Bhagalpur University, Bhagalpur. The animals were housed under standard laboratory conditions such as temperature (26±2 °C), relative humidity (45-55%), and 12 h dark/light cycle and provided with pelleted diet and water ad libitum.

Experimental Design, Drug and Dosage:

After recording the initial body weights, all animals were divided into three groups of ten each and treated as follows:

Group F₀: Mice made diabetic by intraperitoneal injection of alloxan monohydrate.

Group F₁: Generation obtained from F₀ by mating F₀ males with normal females.

Group F₁-T: Feeding *Syzigium cumuni* seed powder along with food (200 mg/kg bw/day) for 28 days followed by sacrificing the animals after cessation of the treatment.

Induction of Hyperglycemia:

The experimental animals were kept on fast before induction of diabetes (Harrowfield *et al.*, 1973). Hyperglycemia was persuaded intraperitoneally by administering alloxan monohydrate, which was procured from Loba Chemicals, Mumbai. All other chemicals used in the present study were of analytical grade and were procured from commercial suppliers. 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.

Alloxan is a urea derivative which causes selective necrosis of the β -cells of pancreatic islets. It has been widely used to induce hyperglycemia in experimental animal species such as rabbits, rats, mice and dogs (Yashimoto *et al.*, 1993).

Animal Sacrifice and Organ Collection:

After recording the final body weights the animals were sacrificed by cervical dislocation. Among ten animals from each group, five animals were used

for the histological assessment. The pancreas (Powers, 2018) were dissected out, blotted free of blood and processed for the following studies:

Morphometric Study of Pancreatic Islets:

Morphometry refers to quantitative analysis, a concept that encompasses size and shape. Morphometric (Weibel, 1963) analysis is commonly performed on organisms which is very useful in analyzing developmental changes in forms, for estimating quantitative-genetic parameter of shape and detecting changes in shape. A major objective of morphometrics is to statistically test hypothesis about factors that affects shape.

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

Pancreatic Mass:

Pancreas were dissected out, washed in saline (0.9 % NaCl), dried with the help of blotting paper and wet weight of gland was measured using digital scale balance.

Islet Density:

Islet density 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 (Hoftiezer, 1973). Density was calculated as follow:

$$\text{Volume Density (VD)} = P(\text{Islet})/P(\text{Reference})$$

Where, P (Islet) is number of test points falling on islet's profile, and P (Reference) is reference space.

Determination of Islet Diameter:

Morphometry is 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.

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

Where, a = major axis, and b = minor axis.

$$\text{No. of islets per unit area (NA)} = N/AT$$

Where, N = No. of sectioned profile of islets, and AT = Area of section.

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

Statistical analysis:

All the data were analyzed statistically by one way ANOVA. Values were considered significant at $P < 0.05$.

Results

Morphometric study grabs attention on significant differences in the diameter of the pancreatic islets indiabetic individuals (Group II) to those of *S. cumuni* fed individuals, (Group F_{1-T}) when compared with control ones (Group F₀). Changes in pancreatic morphometry (per cent volume density and diameter) and pancreatic mass at birth and at weaning stage are recorded in Tables 1 and 2.

The pancreatic per cent volume density at birth was recorded as 20.4 ± 0.79 % in F₀ group of mice and this value decreased to 19.1 ± 0.74 % in group-F₁, while in F_{1-T} group it recovered to 20.1 ± 0.74 %.

The pancreatic volume density per cent at weaning was recorded as 20.8 ± 0.67 % in F₀ group of mice and this value decreased to 19.3 ± 0.62 % in group-F₁, while in F_{1-T} group it recovered to 20.1 ± 0.82 %.

The pancreatic diameter at birth was recorded as 78.3 ± 1.12 µm in F₀ group of mice and this value decreased to 74.6 ± 0.69 µm in group-F₁, while in F_{1-T} group it recovered to 77.2 ± 0.61 µm.

The pancreatic diameter at weaning was recorded as 80.4 ± 1.48 µm in F₀ group of mice and this value reduced to 77.4 ± 0.94 µm in group-F₁, while in F_{1-T} group it recovered to 79.6 ± 1.10 µm.

Table 1: Comparative assessment of pancreatic mass (G) in F₀, F₁ and F_{1-T}

AGE	F ₀	F ₁	F _{1-T}
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 expressed as mean ± SEM; * and ** indicate significance P<0.05 and P<0.001, respectively

Table 2: Comparative assessment of pancreatic morphometry in F₀, F₁ and F_{1-T}

Islet of Langerhang's	F ₀	F ₁	F _{1-T}
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 expressed as mean ± SEM

Statistical analysis showed that Analysis of Variance (ANOVA for single factor) between groups (between treatments) is significant (P<0.05).

Discussion

In this study, it has been noticed that paternal diabetes i.e. when a diabetic male mice mated with a normal female mice, affects the metabolic parameters in F₁ generation. One of the parameters investigated in this experiment 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 (Adeela *et al.*, 2012). The maintenance of glucose levels in F₁ male generation may reflect an

increased sensitivity to insulin and a subsequent increase in the glucose uptake by peripheral tissue (Waterland, 2003). Moreover, F₁ groups had significantly smaller and more irregularly shaped islets as compared to F₀ groups. Overall, the F₁ 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 (Jensen, 2004), but this structure was not observed in the F₁ offspring. The disorder of endocrine cell types within the islet detected both at birth and weaning hints that islet cell distribution is not altered with age.

Observations of the F₁ generation are consistent with single-generation animal studies (Erlandsen *et al.*, 1976). 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 (Pinney *et al.*, 2012). These findings also show that, in the near future, epigenetic factors, which are inheritable, may be regarded as an important genetic factors in accessing risk of having hyperglycemia. *Syzigium cumuni* stimulates the secretion of pancreatic insulin and meliorate the architecture of the pancreatic β -cell in hyperglycemic experimental animal cells (Sharma *et al.*, 2008, 2011). These anti-diabetic or antihyperglycemic characteristics of *S. cumuni* seed 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 (Ramya *et al.*, 2012; Sharma *et al.*, 2011) which directly or indirectly affect on insulin resistance and β -cell function.

Conclusion

The outcomes of present research assessment demonstrates significant efficacy of *Syzigium cumuni* i.e. jamun seed powder on pancreatic morphometry of alloxan monohydrate persuaded hyperglycemic Swiss albino mice. The effect of seed powder provokes supplementation in improving glycemic control and dyslipidemia in hyperglycemia. Thus, it may emerge as potential multitargeted drug and play a vital role in reducing morbidity and mortality associated with hyperglycemia.

The present outcomes concluded that *Syzigium cumuni* seed powder reduces blood glucose levels in hyperglycemic mice, and also have rejuvenatory effect on pancreatic islets. The results of the assessment suggest that the *S.*

cumuni seed powder can be used as a supplementary or alternative herbal remedy for the treatment of hyperglycemia.

The remarkable antihyperglycemic activity as observed during present research work deserves further investigations involving constituents of the *S. cumuni* seed powder that can be a good platform for the development of a new horizon for antihyperglycemic drugs. The results of the present study suggest that *S. cumuni* seed powder can be used as a supplementary or alternative herbal remedy for the treatment of hyperglycemia and its related complications. These results confirmed the use of *S. cumuni* seed in traditional system of medicine to treat diabetes. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of antihyperglycemic effect of *Syzigium cumuni* seed.

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