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Effect of *Syzigium cumuni* seed powder on histoarchitecture & ultrastructure of reproductive organ (testis) of diabetic Swiss albino mice

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ABSTRACT

The aim of study was to investigate the ameliorative effect of *Syzigium cumuni* seed powder on histoarchitecture and ultrastructure of reproductive organ (testis) of diabetic male mice *Mus musculus* Hyperglycemic condition was induced in male mice by injecting Alloxan Monohydrate leading to deficiency in insulin secretion. Fixed dose of *Syzigium cumuni* seed powder were given along with food for three weeks. Histoarchitecture and SEM of testis of control, diabetic and *syzigium cumuni* fed mice tissue were observed at a regular interval. *Syzigium cumuni* seed powder restores the histoarchitecture of pancreatic β cells and stimulates the secretion of pancreatic insulin. Syzigium *cumuni* seed powder treatment increased the number of spermatogenic cell in mice of Group-III when compared to that of diabetic animals Group-II. The Testis configuration was restored and Leydig cells were rejuvenated in *Syzigium cumuni* seed powder treated diabetic group after 21 days. The spermatogenic cells are seen to be recovered after treatment in stipulated dose. Ultra-structure of Testes among control groups animals (Group-I) after 21-day exposure and recovery periods have been observed. Ultra-structure of reproductive organ (Testis) of Hyperglycemic group animals (Group-II) after 21-day exposure and recovery periods has been observed. Ultra-structure of reproductive organ (Testis) of treated group animal (Group-III) after 21 day exposure and recovery period has been observed.In the surface electron micrograph of control section stages of spermatogenesis were clearly seen as in control animals. Spermatozoa were in groups, attached to the inner portion of the lumen of the seminiferous tubules. In the present study, ultra-micrograph of Testis treated with alloxan monohydrate induced diabetes group of animals after 21 day showed substantive damages in normal ultra-histological architecture. The complete cessation of spermatogenesis was observed and seminiferous tubules were lack of spermatozoa. Spermatogenic cells were disrupted and were seen as a lump in the seminiferous tubules under the scanning electron microscope. Some spermatogenic cells were highly eosinophilic, with shrinking cytoplasm and some had deep-staining pycnotic nuclei denoting cell death. Cytoplasmic vacuolization, cellular infiltration of acute inflammatory cells was seen in diabetic mice (Group-II) and it is found to be pronounced with after 21 days. *Syzigium cumuni* seed powder treatment increased the number of spermatogenic cell in mice of Group- III when compared to that of diabetic animals Group II, it was found to be increased with the exposure periods. The Testis configuration was retained and Leydig cells were almost rejuvenated in *Syzigium cumuni* seed powder treated diabetic group of days 21. Some abnormal Leydig cells are also present in few slides of days 21. The spermatogenic cells are seen to be recovered & glucose level were found to return to their normal levels.

Keywords: Hyperglycemia, Histoarchitecture, Ultrastructure, Spermatogenesis, *Syzigium cumuni.*

INTRODUCTION

Diabetes mellitus, is a group of diseases that negatively affect the body's ability to produce or respond to the hormone insulin. This results in elevated levels of glucose in blood because body cannot metabolize carbohydrate properly. Over 380 million people are affected worldwide and the WHO has predicted in 1999 that diabetes will become seventh cause of death world wide by 2030.

Diabetes mellitus is India's fastest growing disease. About 72 million cases recorded in 2017, figure expected to nearly double by 2025. Thus, it is gaining the status of a potential epidemic in India with more than 62 million increased by 64% across India over the quarter century, according to Nov 2017 report by ICMAR, Institute for Health Metrics & Evaluation, both research institutes & the Public Health Foundation of India.

Inactivity & excessive consumption of high calorie foods, exacerbate diabetes risk factors. For this reason, diabetes is often classed as a Life Style Disease."

Aim of present study deals with the investigation on the effect of diabetes on histological and ultra-structural change occurring in reproductive organ (testis) of male mice, Mus *musculus* and their possible recovery using *Syzigium cumuni* seed powder along with the food supplementation for 21 days. Over the centuries, Indian herbal drugs have served as a major source of medicines for the prevention and treatment of diseases including diabetes mellitus. It is estimated that more than 800 species of plant exhibit hypoglycemic properties, including many common plants such as bitter gourd, guduchi, *Carica papya*, *Syzigium cumuni* etc. (*Zarrow et al*., 1964, Ahmad *et al*., 2008).

Indian black berry belongs to family myrtaceae. Black berry was originated in India which has now been spread to in many countries of South Western Asia & Eastern Africa.

MATERIALS AND METHODS

Plant material

Syzigium cumuni seeds were collected fresh from plants grown in University Department of Botany campus, Bhagalpur, Bihar, India. Taxonomic identification was authenticated by the Department of Botany, T.M Bhagalpur University. The seeds were air dried, reduced to coarse powder with the help of mortar and pestle and kept in airtight container until the time of use.

Experimental animal

Swiss albino mice *Mus musculus* weighing about 30±5 gram were obtained from CDRI Lucknow. Mice were maintained at the Animal house of University Department of Zoology, T.M. Bhagalpur University under standard conditions and fed with standard diet. Food and water were given *ad libitum*. Rice husk was used as bedding material and changed 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 (Zarrow *et al*., 1964 and Ahmad *et al*., 2008).

Drugs and Chemicals

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

Alloxan is most prominent chemical compound used in diabetogenic research. In research it is used for induction of Type 1 diabetes. 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 such as rabbits, rats, mice and dogs by varying the dose of alloxan.

The experimental animals were kept on fast before induction of diabetes. Diabetes was induced intraperitoneally by administrating alloxan-monohydrate. Total dose of Alloxan monohydrate (450mg/kg/bw) was administered in three injections at intervals of 48 h (150mg/kg/bw) each time.

Experimental Design

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

Group-I (Control)

Group-II (Diabetic control)

Group-III (Diabetic fed with *Sygizium cumini* seed powder).

The total experimental protocol was maintained for 21 days (3 weeks) after induction of diabetes as per

method suggested by (10). Experiments were performed on the frequency of 7, 14 and 21 days for all the test animals.

Histological process

At the end of the experiment animals were sacrificed and their organs were removed and fixed in Bouins solution, and after overnight fixing, organ samples were then washed through a graded ethanol series, then dehydrated by passing the tissue through increasing percentage of alcohol, then cleared in xylene and embedded in paraffin and sectioning was done and stained with Haematoxylin Eosin and then mounted with DPX for histological assessment under light microscope (Pears,1985). Selected sections of testis of control and experimental groups are examined under low and high magnification respectively. (Fawcet and Bloom, 1972 and Pears, 1985).

Micro anatomical process for SEM

Control and treated Swiss Albino Mice (*Mus musculus*) were sacrificed under chloroform anesthesia and the testes were removed immediately after dissection. After those testes were transversely cut and exposed the tissues were rinsed in Phosphate buffer 5-10 minute along with tween solution for removal of mucous from the tissue. After rinsing in buffer, the tissues were fixed in 2.5% gluteraldehyde for 24 hr at 4^0C. After fixation, the tissues were removed, rinsed in buffer and post-fixed in 1% OsO_4 for 2 hr and again rinsed in 0.1 M Phosphate Buffer and dehydrated in graded acetones, followed by amyl acetate. Then tissues were dried by critical point method with liquid carbon monoxide. The tissues were cemented to metal Stub and coated with gold to a thickness of approximately 20mm and were examined under SEM.

RESULT

Histology of reproductive organ (Testis) of different test groups animal (Group-I, Group-II and Group-III) after 21 days exposure and recovery periods have been observed. In the present study, HE stained 5µ section of Testis of alloxan monohydrate induced diabetes group (Group-II) animals after 21day (Fig.1b) showed severe damages in normal histological architecture.

Cytoplasm vacuolization, cellular infiltration of acute inflammatory cells was seen in diabetic mice (Group-II) and it was found to be pronounced with increased exposure period of 21 days (Fig.1b).

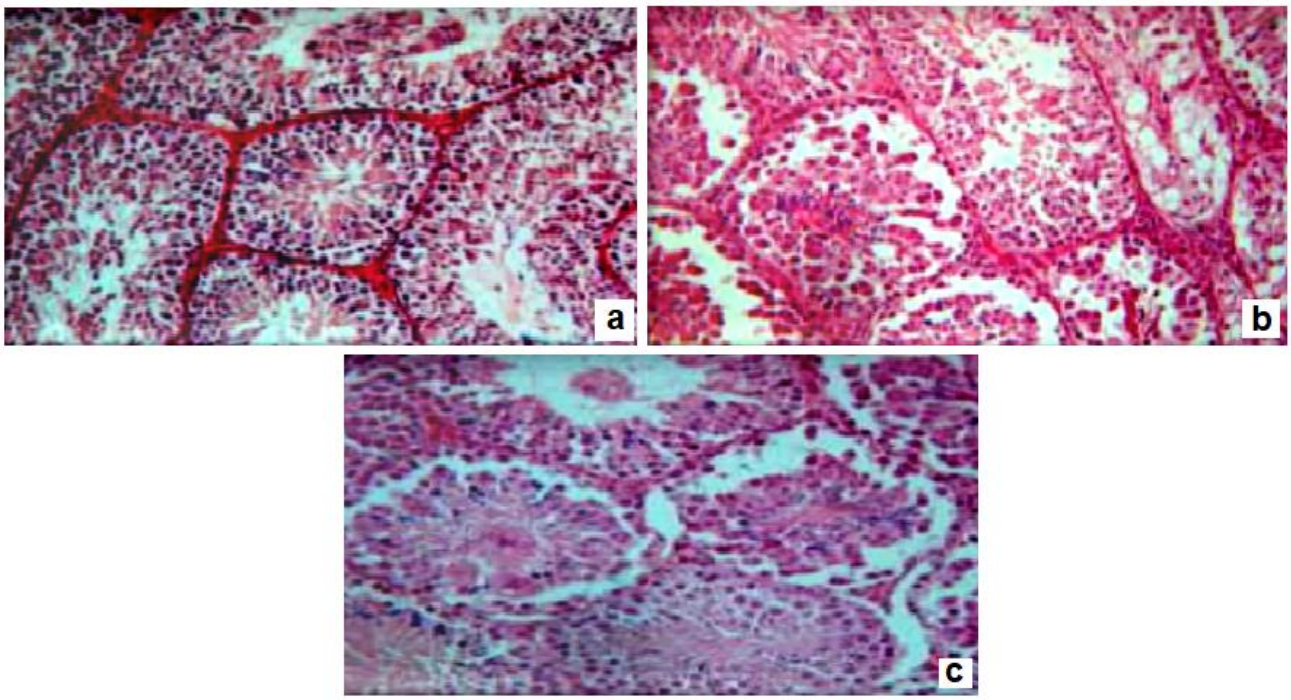

Figure 1a: Testicular autopsy of control mice
b. Testicular autopsy of Diabetic mice
c : Testicular autopsy of treated mice with *Syzigium cumuni* seed powder

Surface electron microscopy of testis

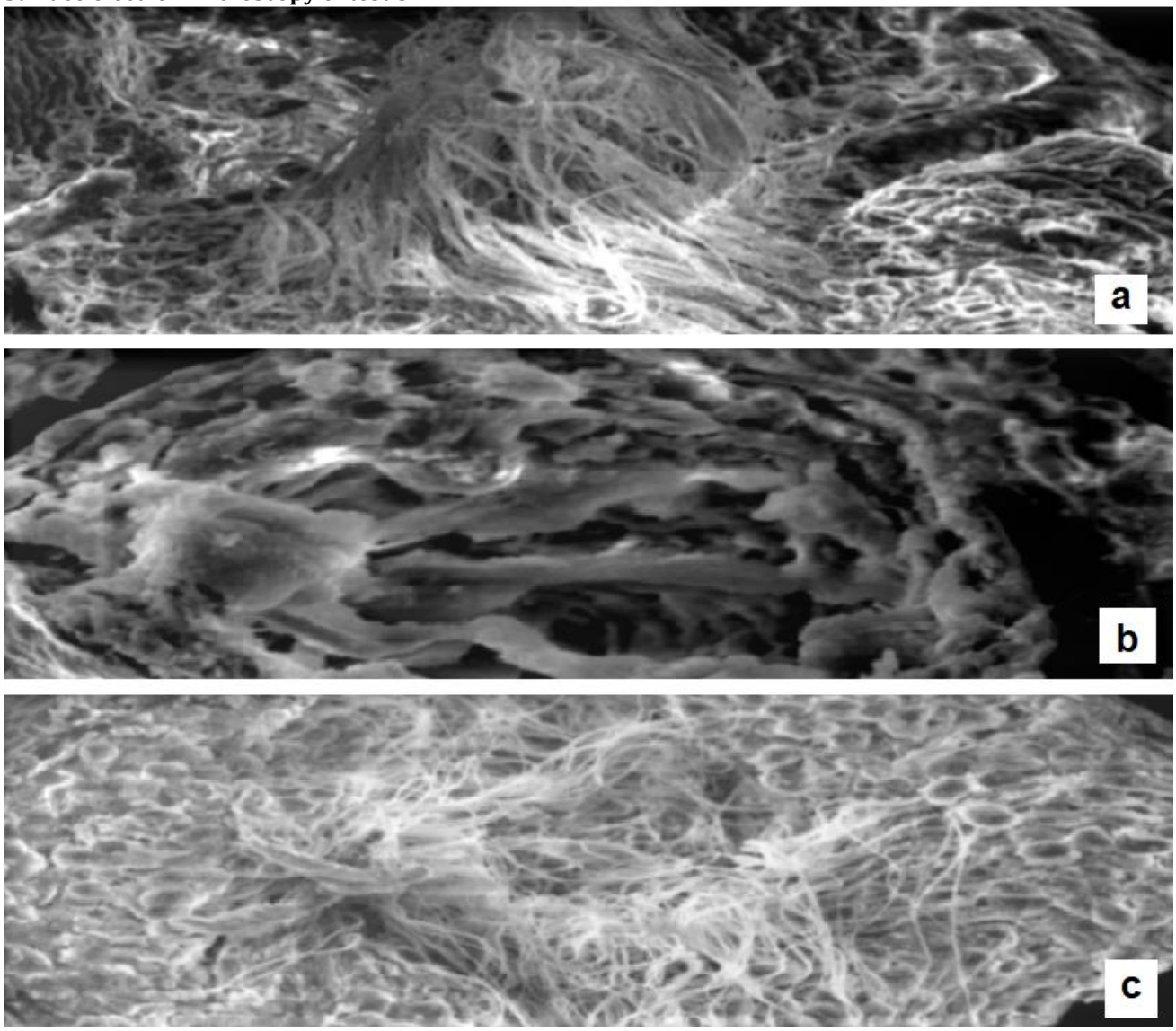

Figure 2 **a:** SEM micrograph of testis of control mice
b: SEM micrograph of Diabetic mice testis
c: SEM micrograph of *Syzigium cumuni* seed powder treated mice testis

Syzigium cumuni seed powder treatment increased the number of spermatogenic cell in mice of Group-III (Fig.1c) when compared to that of diabetic animals Group-II(Fig.1b). The Testis configuration was restored and Leydig cells were rejuvenated in *Syzigium cumuni* seed powder treated diabetic group after 21 days. The spermatogenic cells are seen to be recovered after treatment in stipulated dose.

Ultra-structure of Testes among control groups animals (Group-I) (Fig.2a) after 21-day exposure and recovery periods have been observed.

Ultra-structure of reproductive organ (Testis) of Hyperglycemic group animals (Group-II) (Fig.2b) after 21-day exposure and recovery periods has been observed. Ultra-structure of reproductive organ (Testis) of treated group animal (Group-III) (Fig. 2c) after 21-day exposure and recovery period has been observed.

In the surface electron micrograph of control section stages of spermatogenesis were clearly seen as in control animals. Spermatozoa were in groups, attached to the inner portion of the lumen of the seminiferous tubules.

In the present study, ultra-micrograph of Testis treated with alloxan monohydrate induced diabetes group of animals after 21 day showed substantive damages in normal ultra-histological architecture. The complete cessation of spermatogenesis was observed and seminiferous tubules were lack of spermatozoa. Spermatogenic cells were disrupted and were seen as a lump in the seminiferous tubules under the scanning electron microscope. Some spermatogenic cells were highly eosinophilic, with shrinking cytoplasm and some had deep-staining pycnotic nuclei denoting cell death. Cytoplasmic vacuolization, cellular infiltration of acute inflammatory cells was seen in diabetic mice (Group-II) and it is found to be pronounced with after 21 days (Fig. 2b). *Syzigium cumuni* seed powder treatment increased the number of spermatogenic cell in mice of Group- III when compared to that of diabetic animals Group II, it was found to be increased with the exposure periods. The Testis configuration was retained and Leydig cells were almost rejuvenated in *Syzigium cumuni* seed powder treated diabetic group of days 21. Some abnormal Leydig cells are also present in few slides of days 21. The spermatogenic cells are seen to be recovering in days- 21 treatment with *Syzigium cumuni* seed powder in fixed dose.

DISCUSSION

The present study showed that significant alterations in the histological and ultra-structural patterns in the testis. Similar changes accompanied by the accumulation of immature cells within the tubular lumen were also observed under the influence of Alloxan monohydrate induced hyperglycemic mice. More conspicuous degenerative changes in testicular tissues and an increase in sperm head abnormalities were observed in H& E stained section of Alloxan monohydrate induced hyperglycemic mice. (O'Neill *et al*., 2010).

The release of immature germ cells within the tubular lumen in alloxan monohydrate induced mice reported degenerative changes in the internal milieu of testes (Fig.1a). The cytological changes observed in the acrosomal cap may hamper the potentiality of these cells to mature into functional sperm. The restoration of morphological features of the seminiferous tubules was observed when the mice fed with *Syzigium cumuni* seed powder at fixed dose (200mg $kg^{-1}bw^{-1}$) for three weeks in hyperglycemic mice recorded visible changes in the histoarchitecture of hyperglycemic mice towards normal.

The hyperglycemia affects the spermatogenic cycle finally the morphology of spermatozoa was changed and retained in the epididymis. Diabetes effects include apparent deleterious consequences, such as lower sperm motility and count and an increase in detached sperm head that could help in the fertilization. Similar effects on testis were also reported when exposed to other toxic chemicals and ameliorated by some other herbs (methyl chloride-Working *et al*.,1985); endosulfan, phosphamidon and mannose – (Khan and Sinha 1996).

However, these effects were again retained after a short recovery period, suggesting that the changes caused by toxicants are mostly reversible (Ghosh and Surawanshi, 2001). More puzzling and of potential interest is the finding that the percentage of normal healthy sperm is increased following exposure and that this effect does not seem to be readily reversible. It may suggest that diabetes interferes with sperm capacitating, therefore rendering the cells more resistant to undergo the acrosome reaction (Arikawe *et al*., 2006). If this is indeed the case, the present results suggest that this effect may be long lasting and may potentially affect fertility at a longer time despite otherwise normal sperm parameters. Studies in mice models suggest mechanisms including oxidative stress, DNA damage to sperm, altered hormonal profiles, and abnormal progression through spermatogenesis (Desjardins, 1978)

CONCLUSION

The present work indicates improvement recorded in histoarchitecture and SEM of Testis. Their morphoanatomical alteration caused by alloxan monohydrate induced hyperglycemia was successfully ameliorated. The testicular abnormalities of sperm were reduced by oral administration of *Syzigium cumuni* seed powder at fixed dose (200mg $kg^{-1}bw^{-1}$) for three weeks in different groups of mice (Group-III) used in this study suggesting its protective potential against histopathological alteration. It can be suggested that this *Syzigium cumuni* seed powder at fixed dose (200mg $kg^{-1}bw^{-1}$) could be useful in reducing the defects associated with Hyperglycemia.

Conflict of Interest

The author declares that there is no conflict of interest.

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