



Effect of *Moringa oleifera* leaf powder on sperm count, histology of testis and epididymis of hyperglycaemic mice *Mus musculus*

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Abstract: The aim of the study is to evaluate the effect of leaf powder of *Moringa oleifera* Lam. on male reproductive system of Swiss albino mice *Mus musculus*. The sperm count, its mobility and mortality, histology of testis and epididymis of normal and hyperglycaemic male Swiss albino mice have been investigated and attempt has been taken to evaluate the efficacy of *Moringa* leaf powder in repair mechanism in case of hyperglycaemia. Three sets of animal, i.e. Control (Group I), Hyperglycaemic (Group II) and Hyperglycaemic fed with *Moringa* powder (Group III) were taken for the experiment. Both normal and hyperglycaemic mice were fed with 200mg/ kg body weight of *Moringa* leaf powder. The sperm count (million /mm³) recorded decrease in hyperglycaemic mice (Group II) as compared to control (Group I) but improved in treated mice (Group III), the mobility of sperm also decreased in hyperglycaemic mice but mortality increased in hyperglycaemic mice. In treated mice (Group III), the sperm count significantly increased, sperm mobility also increased but sperm mortality decreased significantly. There was a slight decrease in weight of testis (0.478±0.008gm to 0.33±0.006 gram) respectively when compared to control mice. The value improved after supplementation of *Moringa oleifera* leaf powder (0.33±0.006 gm to 0.415±0.005gm). Similar trend was recorded for Epididymal weight i.e. slight decrease in the weight of epididymis of diabetic mice (from 0.1444±0.003 gm to 0.0521±0.004) and a gradual increase was noticed in the weight from 0.00521±0.004gm to 0.1055±0.001gm after 21 days treatment.

Keywords: Hyperglycaemia, *Moringa oleifera* leaf powder, sperm count, testis, epididymis, *Mus musculus*

I. Introduction

Reproductive disorders in hyperglycaemic males have been studied in the present work. Different experiments have demonstrated several kinds of male reproductive dysfunctions in hyperglycaemia and associated Diabetes Mellitus both in structure and physiology (Handelsman *et al.*, 1985; O'Neill *et al.*, 2009). Hyperglycaemia has been identified as one of the major factors affecting male reproductive functions at manifold levels including its detrimental effects on endocrine control of spermatogenesis and or by impairing erection and ejaculation (Petroianu *et al.*, 2009). Shrilatha and Muralidharan (2007) have reported that the early oxidative stress may cause by the release of free radicals leading to metabolic diseases like hyperglycaemia and Diabetes mellitus. It might cause stress in testis and Epididymal sperm and may lead to the progression of genotoxicity. Ricci *et al.* (2007) found that insulin-dependent diabetes is accompanied by reduced semen volume and decreased vitality whereas increased mortality of the spermatozoa, but no change in seminal viscosity. The high level of blood sugar may affect sperm quality and therefore decreases male fertility and the potentials. Reports indicate higher rates of infertility and poor reproductive outcomes among hyperglycaemias in comparison to healthy men (Joao *et al.*, 2009).

Herbal therapy can alleviate male infertility, irrespective of the etiology of such diseases (Anthony *et al.*, 2006). A large number of plants have been tested for the possible fertility regulatory properties (Bhatia *et al.*, 2010). Some medicinal plants are extensively used as aphrodisiac to relieve sexual dysfunction or as fertility enhancing agents. They provide a boost of nutritional value thereby improving sexual performance and libido (Yakubu *et al.*, 2007; Sumalatha *et al.*, 2010).

Moringa oleifera Lam is a medicinally important plant, belonging to family *Moringaceae*. The plant is well recognized in India, Pakistan, Bangladesh and Afghanistan as a folkloric medicine (Mughal *et al.*, 1992). It is a small or medium sized tree up to 10 m tall, with thick, soft, corky, deeply fissured bark, growing mainly in semiarid, tropical and subtropical areas. Different parts of the tree have been used in the traditional system of medicine. In India, it is revealed that the *M. oleifera* leaves is being used traditionally as an aphrodisiac (Lalas and Tsaknis, 2002). The leaves are used for its protective effect by decreasing liver lipid peroxides, as an antimicrobial agent (Faizi *et al.*, 1998). The leaves are also reported as a potent antioxidant activity (Ghasi *et al.*,

2000). The leaf juice is believed to control glucose levels and also applied to reduce glandular swelling (Makonnen *et al.*, 1997). The stem bark is used as abortifacient (Nath and Sethi, 1992).

II. Materials and methods

Experimental animals: Three month old Male Swiss Albino mice (Body weight: 25 ± 5 g) obtained from CDRI Lucknow and were maintained at the Animal House of University Dept. of Zoology, Bhagalpur. They were kept in stainless steel cages in a temperature and humidity controlled condition with 12 h light/dark cycle. Food and water were given to the animals *ad libitum*. Animals were kept as accepted principles for laboratory animal use and care as per the guidelines of CPCSEA. The mice were of 12 weeks of age and acclimatized for one week before the experiment.

Leaf powder: Powder product of *Moringa oleifera* Lam. leaves were obtained from Sanjeevani Herbals, Salem, Tamil Nadu, which is a Government approved supplier of scientific grade articles. The powder is a spray dried product of Moringa leaves, standard in quality.

Induction of hyperglycaemia: Experimental animals were kept on fast for 18 h prior to induction of hyperglycaemia. It was induced by intra-peritoneal administration of Alloxan monohydrate (Rodriguez *et al.*, 1999). The 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).

Experimental design: Experimental animals were divided into three groups each having 6 animals. Group-I (Control), Group-II (Diabetic control), Group-III (Diabetic control mice fed with leaf powder of *Moringa oleifera* Lam.). The total experimental protocol was maintained for 3 weeks i.e. 21 days after induction of hyperglycaemia (Nambiar and Seshadri, 2001).

III. Histological observation

After 21 days of experiment, mice were sacrificed and their organs were removed and paraffinised, Haematoxylin - Eosine stained sections of testis and epididymis were observed under light microscope. (Pears, 1985) on 10 X and 40 X magnification.

Organ Weight Measurement

Mice were sacrificed by cervical dislocation at the end of the experiment. The entire male reproductive organs were exposed and both the left and right testis were dissected and weighed together.

Sperm Counting and Head Morphology

The left and the right cauda epididymis were incised and the sperm were allowed to swim for 15 min. Solution of 1: 10 dilution is made by adding 90 ml of water to 10 ml of sperm suspension. Sperm counts were done by using haemocytometer. For head morphology study, the sperms were collected from epididymis and vas deference. The suspension was smeared, dried and fixed with fixative (three volume of absolute methanol and one volume of glacial acetic acid), stained with haematoxylin for 15 mins and washed then stained with 1% eosine for 10 mins, washed and left to dry at room temp (Wyrobek, 1979).

Calculations

Sperm count = dilution \times (count in 5 squares) $\times 0.05 \times 10^6$

Sperm motility = $\frac{\text{Motile sperm}}{\text{Motile + non-motile sperms}} \times 100$

IV. Results and Discussion

Hyperglycaemia is associated with reproductive impairments in both males and females. Male reproductive alterations have been widely reported in individuals suffering with diabetes. (Murray *et al.*, 1983; Seethalakshmi *et al.* 1987, Scarano *et al.* 2006). In men affected by insulin-dependent diabetes, sperm have severe structural defects (Baccetti *et al.* 2002), significantly lower motility, and lower ability to penetrate mice eggs (Urner and Sakkas, 1996).

The administration of doses of Alloxan Monohydrate to male mice induces a decrease in testicular testosterone production (Arikawe *et al.* 2006). The present study thus confirms that in hyperglycaemia (Group II), almost all sperm parameters had a statistically significant reduction in comparison with controls (Group I) and also demonstrated that spermatozoa of hyperglycaemic mice recovered significantly in mice of Group III which were under treatment with Moringa. This experimental design thus establishes and confirms the role of Moringa as a potent agent for Hyperglycaemia. The sperm count decreased from 18.36 ± 0.044 to 8.36 ± 0.041 (table 1) in Group II, whereas after Moringa leaf powder administration for 21 days treatment significantly increased the sperm count i.e. 16.11 ± 0.148 (table 1). The same pattern was found in sperm motility, where the sperm motility decreased in diabetic animal (Group II) from $82.2 \pm 0.629\%$ to $53.1 \pm 0.809\%$ (table 1), it significantly increased to $73.6 \pm 0.650\%$ among animals of Group III.

In the present experiment, hyperglycaemia induced sperm abnormalities in mice have been compared with control mice and subsequent repair mechanism with Moringa application. It has been established that Insulin signalling is important for spermatogenesis, sperm maturation and capacitation, and insulin deficient mice

showed decreased sperm quality, decreased steroidogenesis and sperm maturation as also reported by Kim and Moley, 2008. Bucholtz *et al.*, 2000 opined that Insulin is known to influence the hypothalamic-pituitary axis. Spaliviero *et al.*, 2004 suggested that low plasma insulin levels can significantly decrease testosterone formation, which is known to limit the process of spermatogenesis. Similar observations have been recorded in the present experiment justifying the above views.

The hyperglycaemic mice (Group II) have serious effect on their sperm morphology, sperm count, and testicular weight and Epididymal weight (Table: 1 and 2). Weight of testes largely depends on the mass of the various spermatogenic cells. Hence, the depletion in the spermatogenic elements might be the possible cause of the reduction in the testes weight. This observation finds supports from observations of different workers as Sherins and Hawards, (1978); Takihara *et al.*, (1987); Mathur *et al.*(2001, 2003, 2005) and Sharma *et al.* (2008). Wyrobek *et al.*, (1983) suggested that several kinds of mutation can lead to abnormal sperm morphology.

The administration of *M. oleifera* L. powder in the treated male mice i.e. Group III showed significantly higher testes weight gaining from 0.33 ± 0.006 to 0.415 ± 0.005 and epididymis weight recovering from 0.052 ± 0.004 to 0.106 ± 0.001 (Table-2) in comparison to animals of Group II. It may be due to its leaves as they are excellent source of Vitamin B, Calcium, Protein and Potassium. Beta-carotene and other phytochemicals with known powerful antioxidant ability – Kaempferol, Quercetin, Rutin and Caffeoylquinic acids; powerful antioxidant vitamins - C, E, and A and essential micronutrients with antioxidant activity - Selenium and Zinc as explained by Fuglie, (1999); Jaiswal *et al.*, (2009) and Vongsak *et al.*, (2013).

D'cruz and Mathur (2005) proved that the sperm cytoplasm contained very low concentrations of scavenging enzymes therefore an increase in the antioxidant enzyme system levels by Moringa treatment can favour the reproductive process and also enhances spermatogenesis. Sudha *et al* (2010) also found that methanolic extract of Moringa does not affect sexual behaviour or serum androgen level but enhances seminiferous tubules, testis and Epididymal weight and seminal vesicles in the male rats.

Distinct changes have been observed in sperm structure among mice of Group I when compared to animals of Group II (Fig2). Photomicrographs showing headless sperm, round head sperm and coiled tail sperm in abundance as well as banana head sperm and amorphous head sperm, few swollen head sperm confirm the effect of hyperglycaemia among mice inducing such changes.

HE stained section of Alloxan monohydrate induced hyperglycaemic mice showed significant alterations in the histological structural patterns in the testis. Abnormalities in testicular tissues are intense intercellular spaces, irregular diameter of seminiferous tubules, and high amount of necrotic cell in the lumen compared with controls (fig 5). In addition they showed that the Epididymal sperm motility is also decreased in diabetic mice (fig5). Similar changes accompanied by the accumulation of immature cells within the tubular lumen were also observed in rats under the influence of Alloxan monohydrate induced diabetic mice (Cameron;1985) The release of immature germ cells within the tubular lumen in Alloxan monohydrate treated animals reported here represents a degenerative process. The restoration of the morphological features of the seminiferous tubules in the *Moringa oleifera* leaf powder at fixed dose for three weeks in different groups of mice indicated an apparent reversibility. This is noticed by the presence of abundant spermatid in their seminiferous tubules and the thickened Epididymal epithelial lining (Figure -5) compared to the control group. Lumen formation which is also an indication of the degree of spermatogenesis was highly seen in sections in mice treated by Moringa oleifera leaf powder.

On the basis of above discussed data and facts it can be concluded that the Moringa oleifera powder significantly reduce the alteration arisen in reproductive ability and associated structures in the Alloxan monohydrate induced male diabetic mice.

Table-1: Diabetes Induced Male Reproductive Changes and their treatment with *Moringa oleifera* leaf powder

Sperm Parameter	Group of mice		
	GROUP I	GROUP II	GROUP III
Sperm count (million /mm ³)	18.36±0.044	8.36±0.041**	16.11±0.148**
Sperm motility (%)	82.2±0.629	53.1±0.809**	73.6±0.650*
Sperm mortality (%)	17.8±0.629	46.9±0.809**	26.4±0.650*

N=10 Values are given as mean ±SEM for groups of ten mice.

Values are statistically *significant (p<0.05); ** highly significant (p<0.01).

Table-2: Diabetes Induced Male Reproductive Changes and their treatment with *Moringa oleifera* leaf Powder for three weeks on organ weight testis (Gram).

Organ weight	Group I	Group II	Group III
Testis weight	0.478±0.008	0.33±0.006**	0.415±0.005**
Epididymis weight	0.144±0.003	0.052±0.004**	0.106±0.001*

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

Fig. 1: Different abnormalities of sperm morphology.

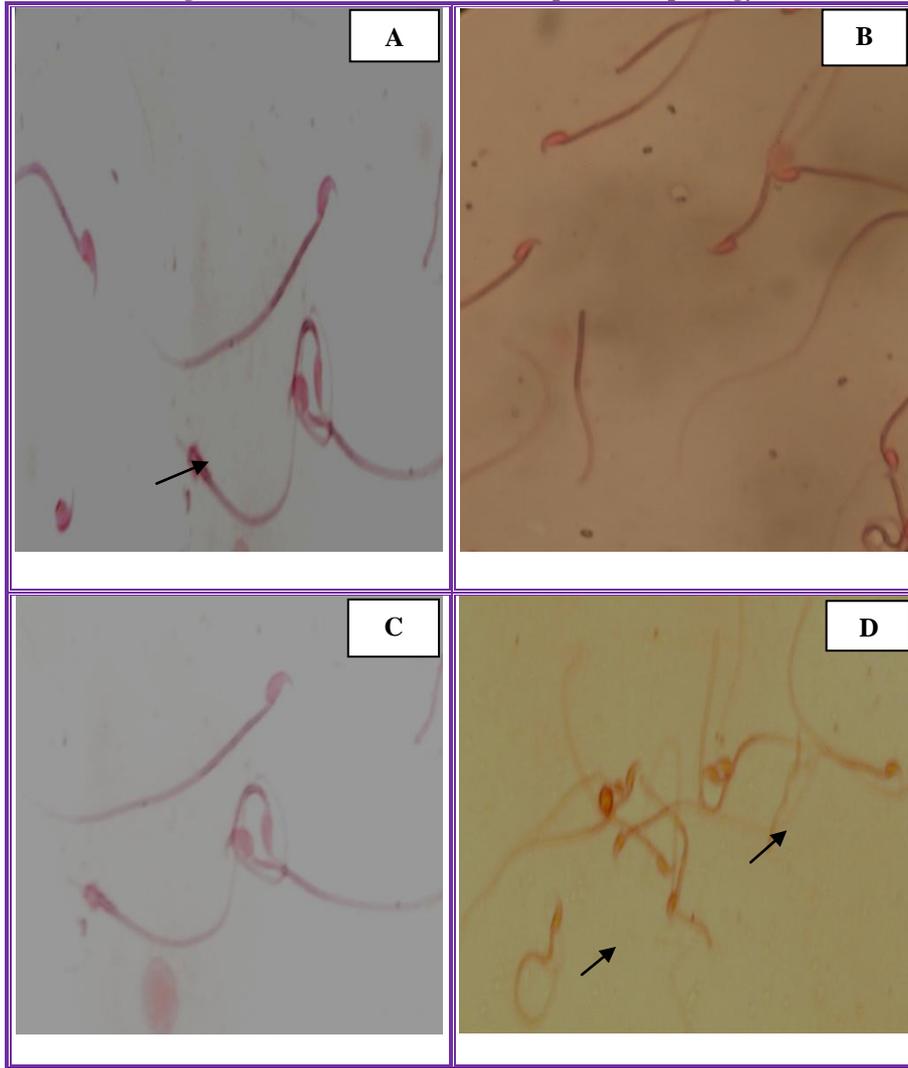


Fig. 2: Photographs showing structures of Sperms among Hyperglycaemic mice. A-Normal sperm, B-Headless sperm; C- Round head sperm; D-Coiled tail defect, Double tail Sperm, Tail bent defect,(40x)

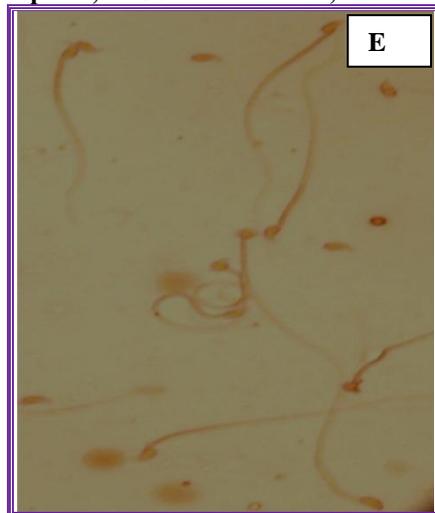


Fig. 3: Photographs showing Sperm structure abnormalities induced by hyperglycaemia in Swiss Albino Mice. E-Round headed sperm,(40x)

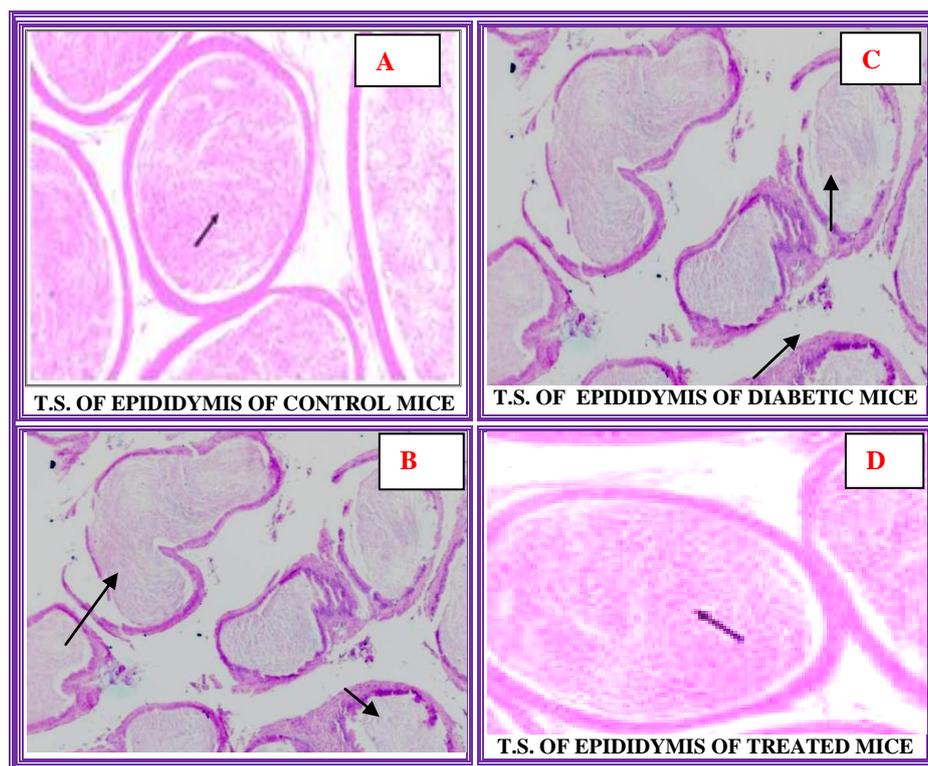


Fig. 4: Microphotographs showing histological changes in the tissue architecture. The epithelial lining is degenerating in diabetic group as well as the density of sperm in lumen is lessen.

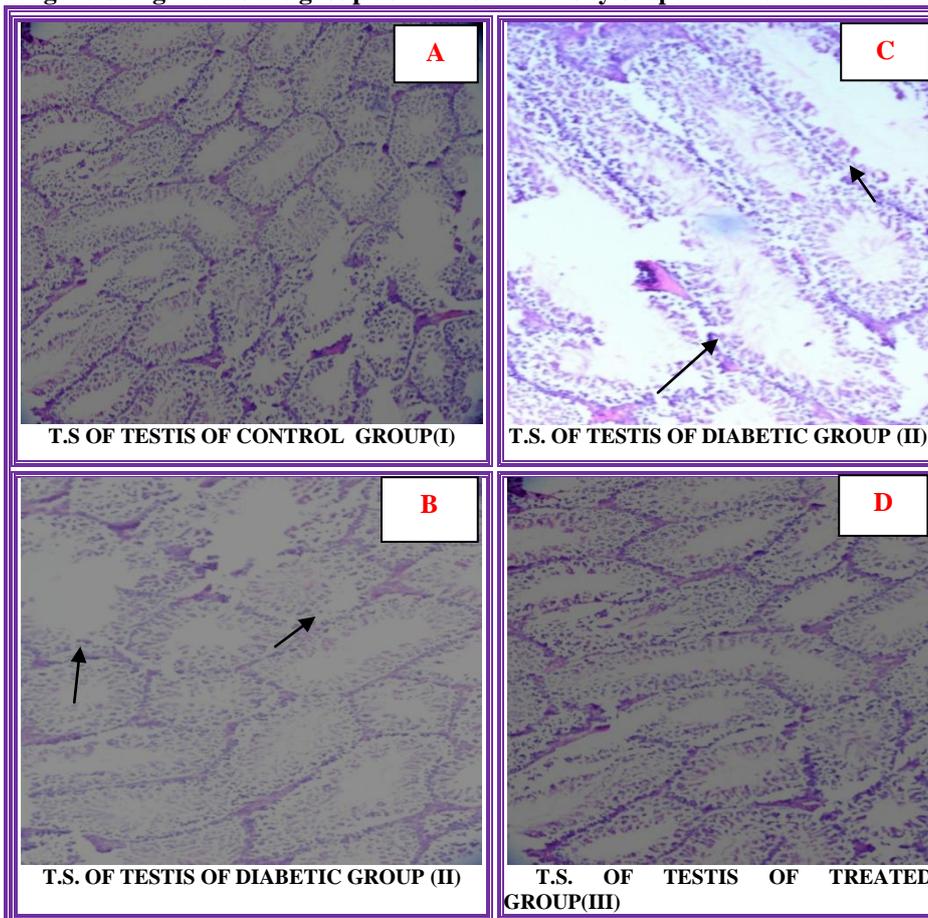


Fig. 5: Photomicrographs of T.S. of hyperglycaemic Testis A-Control, B- 14 days, References

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