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Effect of combination of fenugreek with insulin and Glimepiride on male reproductive system in Streptozotocin-induced diabetic rats

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Present study was conducted to assess the effect of fenugreek, insulin and Glimepiride alone and their combination on male reproductive system in diabetic rat. Fifty six male Sprague Dawley rats of uniform age were randomly divided into seven groups. Group 1 and 2 kept as non-diabetic control and Streptozotocin (40 mg/kg i/p single dose)-induced diabetic control; Group 3, 4 and 5 were given treatment with insulin, Glimepiride and fenugreek seed powder, respectively; Group 6 and 7 were given combination of insulin + fenugreek seed powder and Glimepiride + fenugreek seed powder, respectively. At the end of experiment, testes were collected, weighed and used for histopathology and estimation of GSH, TBARS and epididymus was collected for estimation of total sperm count. The concentration of TBARS was increased, while GSH and relative testis weights were decreased in diabetic rats. The histology of testis revealed marked changes in diabetic rats and mild changes in combination treatment groups. The treatment groups showed significant improvement as compared to diabetic rats, while the combination groups showed greatest improvement. The study revealed that addition of fenugreek seed powder to insulin and Glimepiride had positive interaction in improving the male reproductive parameters in streptozotocin-induced diabetic Sprague Dawley rats.

Keywords: Diabetes, Fenugreek, Glimepiride, Oxidative stress, Testis,

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Introduction

Diabetes mellitus (DM) is a rapidly growing metabolic disorder characterized by hyperglycaemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular disease. The prolonged exposure of tissues to hyperglycaemia causes most diabetic complications. Over 2,00,000 people die each year because of DM-related complications. The prolonged exposure of tissues to hyperglycaemia causes most diabetic complications as confirmed by The Diabetes Control and Complications Trial[1] and United Kingdom Prospective Diabetes Study[2]. Hyperglycaemia is a connector between diabetes and diabetic complications[3,4]. DM in male rats is often accompanied by a marked decrease in reproductive functions, including reduced accessory sex organ weight[5], reduced sexual behavior[6,7], degenerative changes in the seminiferous tubules[8], decrease in semen volume and sperm motility[9] and infertility. The testicular testosterone concentration[10] and luteinizing hormone receptor levels were decreased in type 1 DM.

Glimepiride is a second generation sulfonylurea agent that reduces blood glucose concentrations to satisfactory levels with once-daily dosing. It has insuliomimetic action and possesses antioxidant activity. Various constituents of fenugreek are responsible for its hypoglycaemic action, antioxidant activity[11,12] and steroidogenic activity[13].

Herbal products are considered natural and safe and used as over the counter medicines but they require attention for potential risk as they are pharmacologically active. These herbal products may interact with allopathic drugs resulting in altered activity and toxicity. Current information on herb-drug interactions is scanty. Hence an experiment was designed to study the interaction of fenugreek with insulin and Glimepiride in Streptozotocin-induced diabetic rats.

Materials and Methods

Chemicals

Glimepiride (gratis sample from Ranbaxy, India) was administered as suspension in freshly prepared 0.5 % w/v carboxy methyl cellulose sodium salt. Insulin (Aventis) and Streptozotocin (SRL, Mumbai) were purchased. All the chemicals (for preparation of reagents and buffers) were procured from Qualigens Pvt Ltd, Mumbai and SRL Pvt Ltd, Mumbai.

Herb preparation

Fenugreek (*Trigonella foenum-graecum* L.) seeds were purchased from local market, shade dried, powdered and administered as suspension

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freshly prepared in 0.5 % w/v carboxy methyl cellulose sodium salt.

Animals

Fifty six male Sprague Dawley rats of uniform age (3 months) and weight were procured from National Center for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad for the study. Feed and water was provided *ad libitum* throughout the experiment. Animals were housed in polypropylene cages in a well ventilated animal house with 12 h – 12 h light – dark cycles.

Induction of diabetes

After an acclimatization period of 2 weeks, rats were randomly divided into 7 groups of 8 rats in each and blood samples were collected and serum was separated for glucose estimation. Subsequently, group 1 was kept as normal control throughout the experimental period. Remaining 6 groups were induced diabetes by intraperitoneal injection of streptozotocin @ 40 mg/kg body weight. The rats were provided with glucose water for 24 h to prevent hypoglycaemia. Blood samples were collected after 72 h and serum was separated for glucose estimation. Rats with blood glucose value of >250 mg/dl (72 h after streptozotocin administration) were included in the study (n=8). Treatment protocols were initiated from day 2 post-confirmation of diabetes (day 5 post-streptozotocin administration) and were continued for 8 wks. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Experimental design

After induction of diabetes, all the groups were maintained as per the following drug and herb treatment schedule for 8 wks. Group – 1: Non-diabetic control; Group – 2: Streptozotocin (40 mg/kg i/p single dose)-induced diabetic control; Group – 3: Insulin (4 U/kg once daily for 8 wks) treatment in diabetic rats; Group – 4: Glimepiride (4 mg/kg orally once daily for 8 wks) treatment in diabetic rats; Group – 5: Fenugreek seed powder treatment (1 g/kg orally once daily for 8 wks) in diabetic rats; Group – 6: Insulin + Fenugreek seed powder treatment (once daily for 8 wks) in diabetic rats; Group – 7: Glimepiride + Fenugreek seed powder treatment (once daily for 8 wks) in diabetic rats.

Sample collection and analysis

At the end of the experiment, 6 rats from each group were sacrificed and testes were collected weighed and stored at -20°C for further estimation of GSH[14], TBARS[15]. The protein content was estimated by Lowry method[16]. Testes samples were collected in formal saline for histopathology. Epididymus was collected for total sperm count.

Collection of epididymal sperm

The epididymal sperms were collected by cutting epididymis into small pieces and flushing the sperm in normal saline. The sperm collected was centrifuged at 225 × g for 10 min. The pellet was re-suspended in 2.0 mL of normal saline. An aliquot of sperm suspension was homogenized for few seconds, centrifuged at 800 × g for 10 min and used for analysis.

The epididymal sperm was obtained as described above and incubated at 37 °C. The epididymal fluid was then diluted to a volume of 5.0 mL of pre-warmed (37 °C) normal saline. The epididymal fluid was subjected to sperm count using Neubauer haemocytometer[17]. The epididymal fluid was drawn up to the 0.5 mark of WBC pipette (White Blood Cell pipette) and the semen diluting fluid (sodium bicarbonate 5 g, formalin 1 mL, distilled water 99.0 mL) was drawn up to '11' mark and subsequently mixed well. One drop was added to the haemocytometer chamber and allowed the sperms to settle by keeping haemocytometer in humid place (wet chamber) for 1 h. After incubation, the number of spermatozoa in the appropriate squares of the haemocytometer was counted under the microscope at 40 X. The sperm concentration refers to the number of spermatozoa/mL fluid and calculated using the following formula.

$$\text{Sperm count} = \frac{\text{No of spermatozoa counted} \times \text{dilution factor} \times \text{depth factor}}{\text{No of areas counted}}$$

Relative organ weights

Testes weights in comparison to body weights of different groups of rats were noted at the time of sacrifice. Relative organ weight was expressed as % of body weight.

$$\text{Relative organ weight} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

Statistical analysis

The data was subjected to statistical analysis by applying one way ANOVA using statistical package

for social sciences (SPSS) 15.0 version. The values were expressed as Mean + S E. Differences between means were tested using Duncan's multiple comparison test and significance level was set at 0.05.

Results

The relative weight of testes (% of body weight) revealed a significant ($p<0.05$) increase in group 1 as compared to group 2. The groups 3 to 7 showed a significant ($p<0.05$) decrease in relative testes weight as compared to group 1 and significant ($p<0.05$) increase as compared to group 2 (Table 1).

The concentration of TBARS (n moles MDA /mg protein) in testis revealed a significant ($p<0.05$) rise in group 2 as compared to group 1. The groups 3 to 7 showed a significant ($p<0.05$) decrease in TBARS concentration as compared to group 2. The values of groups 3 to 7 were comparable to that of group 1 (Table 1).

The concentration of GSH (µ moles/mg protein) in testis revealed a significant ($p<0.05$) decrease in group 2 as compared to group 1. The groups 3 to 7 showed a significant ($p<0.05$) increase in GSH concentration as compared to group 2. The groups 6 and 7 showed significantly ($p<0.05$) higher concentration among all the treated groups (Table 1).

The epididymal sperm count (millions/mL) showed a significant ($p<0.05$) decrease in group 2 as compared to group 1. The groups 3 to 7 showed a significant ($p<0.05$) increase in sperm count as compared to group 2, the group 6 and 7 showed significantly ($p<0.05$) higher concentration among all the treated groups (Table 1).

The testis section of group 2 showed marked congestion between seminiferous tubules, few seminiferous tubules showed discontinuation of epithelium and edematous fluid (Plate 1a). The sections of groups 3 (Plate 1b) 4 and 5 revealed mild congestion and edematous fluid (Plate 1c). The group 6 showed mild disrupted epithelium (Plate 1d), while group 7 (Plate 1e) and group 1 did not show any lesions of pathological significance.

Discussion

Insulin is well known as an anabolic hormone that plays a vital role in maintenance of body growth and overall body metabolism. Partial or complete insulin deficiency in diabetic humans as well as in induced diabetic experimental animals has adverse effects on all organs, including reproductive organs. Streptozotocin (STZ) reduced weights of testis and epididymis, testosterone production and sperm motility and count, suggesting a toxic effect of STZ in the structural and functional integrity of testicular tissues. STZ reduced testosterone production, suggesting a decrease in the function of both Leydig (testosterone producing cell) and sertoli (spermatogenesis) cells, which might be caused by a reduction in insulin secretion[18,19]. These changes are probably due to increased Reactive Oxygen Species (ROS) production by accelerated Advanced Glycation End Products (AGE) formation[20], polyol pathway, hexosamine and Protean Kinase C pathway. Mohasseb *et al*[21] reported that treatment with antioxidants had protective effect on testicular oxidative damage and germ cell apoptosis. Similar results were obtained in the present study and fenugreek improved the testes weights, sperm count in the groups 6 and 7 among treatment groups (3 to 7), thus suggests pharmacodynamics interactions exist with insulin and Glimepiride. Fenugreek seed extract evident to reduce the lipid peroxidation levels by increasing GSH levels due to its antioxidant action. Further, its extract showed hypoglycaemic and insulin

Table 1 — Effect of combination of fenugreek with insulin and Glimepiride on male reproductive system in streptozotocin-induced diabetic rats

S. No.	Groups	Relative organ weights (%)	TBARS concentration (n moles of MDA released/mg protein)	GSH concentration (n moles/mg protein)	Epididymal sperm count (million/mL)
1.	Non-diabetic control	0.89 ± 0.02^{c}	11.57 ± 0.19^{a}	40.96 ± 0.29^{d}	61.37 ± 1.24^{e}
2	Diabetic mellitus (DM) control	0.65 ± 0.02^{a}	20.63 ± 0.34^{c}	15.25 ± 0.18^{a}	29.91 ± 1.23^{a}
3	DM + Insulin	0.84 ± 0.02^{bc}	13.73 ± 0.19^{b}	33.40 ± 0.17^{b}	45.74 ± 1.12^{b}
4	DM + Glimepiride (GM)	0.76 ± 0.06^{b}	13.51 ± 0.12^{ab}	33.23 ± 0.20^{b}	44.78 ± 1.15^{b}
5	DM + Fenugreek (FG)	0.85 ± 0.03^{bc}	13.45 ± 0.20^{ab}	33.26 ± 0.24^{b}	45.91 ± 1.37^{bc}
6	DM + Insulin + FG	0.79 ± 0.02^{b}	12.27 ± 0.19^{ab}	36.17 ± 0.46^{c}	49.89 ± 1.26^{cd}
7	DM + GM + FG	0.76 ± 0.02^{b}	12.51 ± 0.25^{ab}	36.22 ± 0.56^{c}	49.45 ± 1.55^{d}

Values are Mean $\pm$ SE (n =6); One way ANOVA (SPSS)
Means with different alphabets as superscripts differ significantly ($P<0.05$)

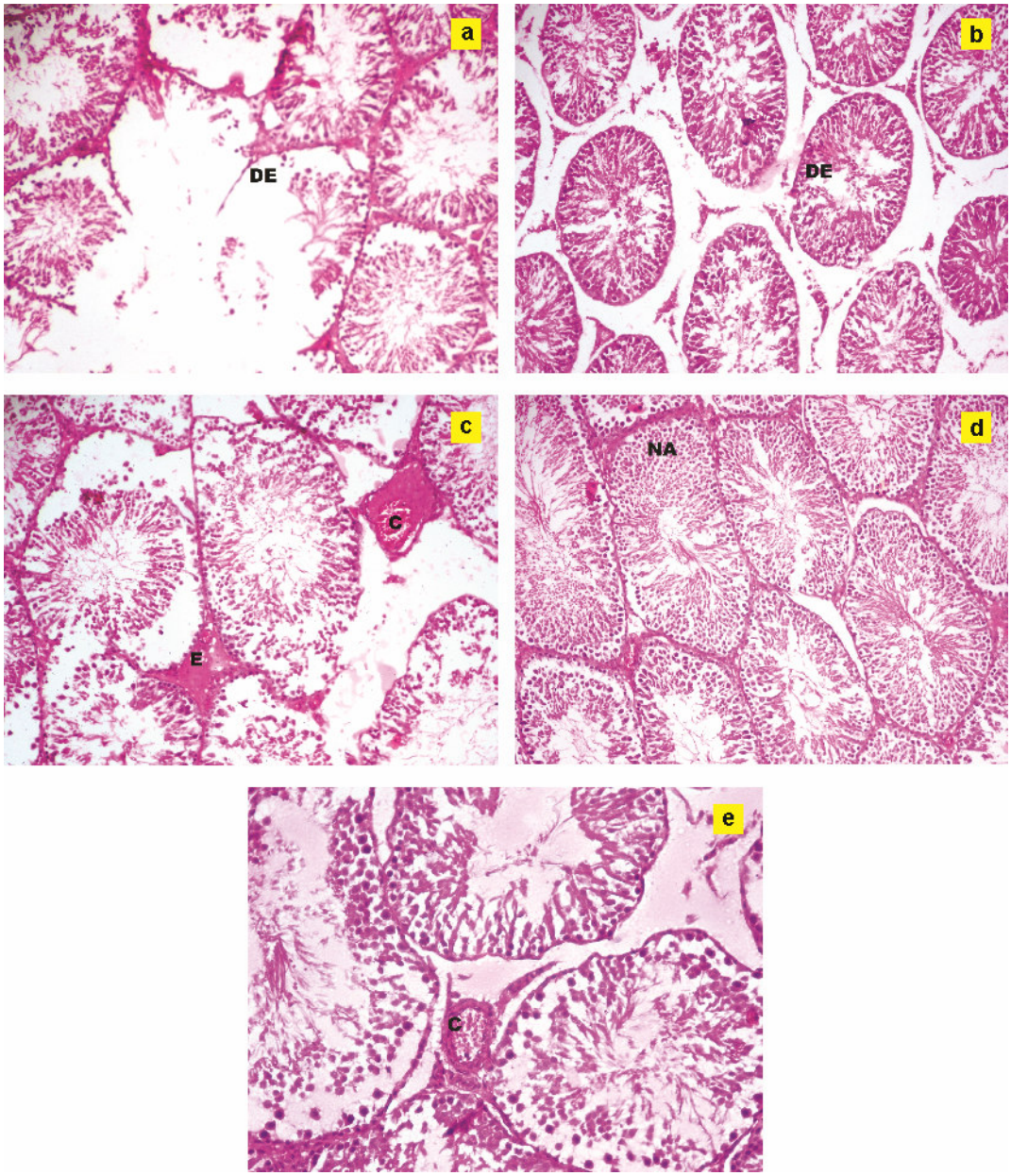

Plate 1– Photomicrograph of testis showing various stages of results in different groups: a. marked disruption of seminiferous tubular epithelium. H&E X 100 (Group 2); b. congestion and edema between seminiferous tubules. H&E X 100 (Group 3); c. congestion between seminiferous tubular epithelium. H&E X 200 (Group 5); d. mild disruption of seminiferous tubular epithelium. H&E X 100 (Group 6); e. normal architecture, H & E X 100 (Group 7).

stimulating action due to presence of 4-hydroxy isoleucine which acts on the β cells of pancreas and reduced free radical production[22]. The other compounds called trigonelline, galactomannan and trigoneosides also work together to provide benefits for blood sugar. The present study results indicated that fenugreek improved GSH levels in the groups 6 and 7 among treatment groups (3 to 7), thus again suggests that pharmacodynamics interactions exist with insulin and Glimeperide. Moreover, extract of fenugreek steroids upregulate steroidogenic enzymes in testis, thereby increasing testosterone levels[13]. Similar findings were reported by Irshaid and Mansi[23]. Glimepiride reduced sperm abnormality and increased testis weights and sperm count by its antioxidant action[24]. In the present study, results evident that Glimepiride had significantly reduced MDA (Malondialdehyde) levels suggesting a decrease

in reactive oxygen species levels and subsequent lipid peroxidation. Also Glimepiride had significant effect on the GSH levels and prevented GSH depletion thus, helped to maintain an antioxidant balance which is important for vascular protection against lipid peroxide. The antioxidant capacity of Glimepiride, might be through inhibition of cellular cyclo-oxygenase pathways[25], or up-regulate antioxidant enzyme genes like paraoxonase, superoxide dismutase and catalase gene through reducing the activation of the redox sensitive nuclear factor kappa-B (NF-be) or through that Glimepiride possessed agonistic activities for PPARγ[26]. Glimepiride increased levels of catalase, SOD, GPx and reduced lipid peroxidation in somatic and germinal cells[24]. The antioxidant action of Glimepiride was reported earlier also[27]. Further, this was supported by histopathological findings of testis, which showed marked congestion between seminiferous tubules, discontinuation of epithelium and edematous fluid in group 2. The sections of groups 3, 4 and 5 revealed mild congestion and edematous fluid in few tubules. The group 6 showed mild disrupted epithelium, while group 7 did not show any lesions of pathological significance.

Conclusion

The study revealed that addition of fenugreek seed powder to insulin and Glimepiride had positive interaction in improving the male reproductive parameters in streptozotocin-induced diabetic Sprague Dawley rats, which was evident from improvement in organ parameters in the groups that were treated using a combination of fenugreek with either insulin or Glimepiride as compared to individual agent-treated groups.

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