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SHORT COMMUNICATION

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# Pharmacodynamic interaction of fenugreek with insulin and glimepiride in streptozotocin-induced oxidative stress in *Sprague Dawley* rats

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## ABSTRACT

**This study was aimed to assess the effect of fenugreek-insulin-glimepiride interaction on oxidative stress in streptozotocin-induced diabetic rats. A total of 56 male Sprague-Dawley rats were randomly divided into 7 groups (n=8); group 1: served as Sham, group 2: Diabetic control, groups 3, 4 and 5: served as individual treatment group, groups 6 and 7: treated with combination of insulin-fenugreek and glimepiride-fenugreek, respectively. Serum creatinine levels of the rats were estimated at 4th and 8th weeks during treatment. Thiobarbituric Acid Reactive Substances (TBARS), Glutathione (GSH) and protein carbonyls were estimated in the kidney homogenate, and relative kidney weights were measured at the end of the experiment. Present study indicated that the levels of TBARS and protein carbonyls significantly increased in group 2 and decreased in groups 3 to 7. On the other hand, groups 6 and 7 showed significantly lowered values compared to the individual treatment groups. The concentration of GSH was significantly decreased in group 2 and significantly increased in groups 3 to 7, and group 7 showed significantly higher concentration among all the treated groups. The serum creatinine concentration in group 2 was significantly higher and all treatment groups (3 to 7) showed significantly lowered values at 4th and 8th wks after treatment. The individual treatment groups (3, 4 & 5), antagonised the significant alteration in the antioxidant parameters, and their combination was revealed synergism by improving the oxidative status in diabetic rats.**

## Keywords

Fenugreek, Glimepiride, Insulin, Oxidative stress, Protein carbonyls, TBARS

## ARTICLE HISTORY

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## INTRODUCTION

Diabetes Mellitus (DM) is characterized by high blood sugar levels resulting from the defects in insulin secretion from β-cells of pancreas (Adams, 2013). About 30-40% of type-1 diabetic patients develop nephropathy, which is characterized by microalbuminuria, glomerular hypertrophy and sclerosis, followed by proteinuria, and finally renal failure (Mungikar, 2006; Gunzler et al., 2013).

Fenugreek (*Trigonella foenum graecum*) is known to have several pharmacological effects such as hypoglycemic, hypocholesterolemic, and anti-oxidant (Ramesh et al., 2010; Abeed and Mashkor, 2014). Glimepiride, a second generation sulfonyluric agent, has antioxidant and hypolipidemic actions (Kakadiya et al., 2010). The activity of allopathic drugs can be altered or in some cases toxicity is reduced when these drugs interact with herbal products. However, the information regarding interaction of fenugreek (herbal product) with glumepiride is poorly studied. Therefore, the present study was designed to evaluate the possibility of interaction of fenugreek seed powder with insulin and glimepiride in diabetic *Sprague Dawley* rats.

## MATERIALS AND METHODS

**Animals:** Fifty six male *Sprague Dawley* rats aging 3 months and weighing between 200-250 gms were procured for the study. Feed (in the form of pellet as per National Institute of Nutrition feed standard) and water were provided *ad libitum*. Animals were kept in

polypropylene cages and maintained with 12 h dark/light cycle at College Animal House. Accustomised period of 2 wks was observed before the onset of experiment

**Drugs:** Three drugs were used for the experiments- (i) Streptozotocin (SRL Pvt. Ltd., Mumbai) was dissolved in 0.5 M sodium citrate, pH 4.5, (ii) Glimepiride (Ranbaxy, India). Administration of Glimepiride was done at 0.5% w/v in carboxy methyl cellulose sodium salt as suspension, (iii) Insulin (Insuman Basal-Aventis).

**Herb:** Fenugreek (*Trigonella foenum* graecum) seeds were dried, powdered and administered at 0.5% w/v in carboxy methyl cellulose sodium salt as suspension.

**Chemicals:** All the chemicals used for biochemical analyses were of analytical grade, and were procured from Qualigens Pvt. Ltd., SRL Pvt. Ltd., Mumbai.

**Induction of diabetes and initiation of herb/drug treatment:** After an acclimatization period of 2 wks, the rats were randomly divided into 7 groups (n=8 in each group). Serum samples from the rats were collected for glucose estimation. Subsequently, group 1 was kept as Sham, and the remaining 6 groups were induced diabetes with streptozotocin (40 mg/kg body weight) by intraperitoneal (i/p) injection. To prevent hypoglycemia, the rats were provided glucose water for 24 h. After 72 h of induction with streptozotocin, blood samples were collected for serum glucose estimation. Rats with blood glucose concentration >250 mg/dL were taken for the study (n=8). Treatment protocols were initiated from day 2 till 8$^{th}$ wks.

**Experimental Design:** Prior to the experiment, the protocol was approved by the Institutional Animal Ethics Committee (IAEC), India with approval number 6/I/10. After induction of diabetes, the rats of all groups were maintained as per the following drug and herb treatment schedule for 8 wks.

*Group 1: Sham,*
*Group 2: Diabetic control (Streptozotocin at 40 mg/kg bwt i/p),*
*Group 3: Treatment with Insulin dosed at 4 U/kg bwt,*
*Group 4: Treatment with Glimepiride dosed at 4 mg/kg bwt orally,*
*Group 5: Treatment with Fenugreek seed powder treatment dosed at 1 g/kg bwt orally,*
*Group 6: Treatment with Insulin + Fenugreek seed powder,*
*Group 7: Treatment with Glimepiride + Fenugreek seed powder.*
*Treatmant was given once daily for 8 wks in group 3 to 8.*

**Blood and sample collection:** The blood samples were collected through retro-orbital plexus on 4$^{th}$ and 8$^{th}$ wks during treatment. The sera were separated, which was used for analysis of creatinine concentration. Six rats from each group were sacrified by cervical decapitation at the end of 8$^{th}$ wk, and kidney samples from the rats were collected for estimation of GSH (Moron et al., 1979), TBARS (Balasubramanian et al., 1988) and protein carbonyls (Levine et al., 1990), from the kidney homogenates. Weights of kidney were also recorded. The protein concentration was estimated by Lowry method (Lowry et al., 1951) using Bovine serum albumin.

## RESULTS AND DISCUSSION

Oxidative stress in DM adversely affects the physiological and biochemical processes of cell, and among those islets cells of pancreas are more prone to be damaged due to the lowest intrinsic antioxidant defense mechanism. Chonic hyperglycemia and oxidative stress might be resulted due to the multiple biochemical pathways, and the mechanism of action could damage the vascular, renal and retinal tissues (Fiorentino et al., 2013).

**Table 1:** Serum creatinine concentration (mg/dL) in different groups of rats.

| Group | 4$^{th}$ wk | 8$^{th}$ wk |
|---|---|---|
| 1. Non-diabetic control | 0.540±0.004$^{aA}$ | 0.645±0.004$^{aB}$ |
| 2. DM control | 0.977±0.005$^{fA}$ | 1.105±0.002$^{dB}$ |
| 3. DM + Insulin | 0.739±0.003$^{cA}$ | 0.827±0.002$^{cB}$ |
| 4. DM + Glimepiride (GM) | 0.756±0.003$^{dA}$ | 0.833±0.003$^{cB}$ |
| 5. DM + Fenugreek (FG) | 0.786±0.003$^{eA}$ | 0.831±0.004$^{cB}$ |
| 6. DM + Insulin + FG | 0.721±0.001$^{bA}$ | 0.811±0.002$^{bB}$ |
| 7. DM + GM + FG | 0.715±0.002$^{bA}$ | 0.811±0.003$^{bB}$ |

DM: Diabetes mellitus, wk: week
Means with different alphabets as superscripts differ significantly ($P$<0.05); Capital alphabets for horizontal comparison and small alphabets for vertical comparison
Values are mean±standard error (n=8).

**Table 2:** Anti-oxidants parameters and relative weights of kidney of different groups of rats.

| Group | TBARS | GSH | Protein carbonyls | Relative weights |
|---|---|---|---|---|
| 1. Non-diabetic control | 3.23±0.05$^{a}$ | 29.74±0.19$^{f}$ | 1.54±0.11$^{a}$ | 0.58±0.02$^{a}$ |
| 2. Diabetic mellitus (DM) control | 8.39±0.12$^{d}$ | 15.28±0.24$^{a}$ | 4.62±0.10$^{f}$ | 1.80±0.05$^{e}$ |
| 3. DM + Insulin | 6.66±0.12$^{c}$ | 24.33±0.27$^{b}$ | 3.49±0.11$^{de}$ | 0.89±0.01$^{d}$ |
| 4. DM + Glimepiride (GM) | 6.26±0.21$^{c}$ | 24.91±0.17$^{bc}$ | 3.25±0.09$^{cd}$ | 0.76±0.04$^{bc}$ |
| 5. DM + Fenugreek (FG) | 6.40±0.23$^{c}$ | 25.52±0.32$^{cd}$ | 3.70±0.11$^{e}$ | 0.82±0.02$^{cd}$ |
| 6. DM + Insulin + FG | 4.73±0.19$^{b}$ | 26.13±0.32$^{d}$ | 2.89±0.12$^{bc}$ | 0.70±0.02$^{b}$ |
| 7. DM + GM + FG | 4.46±0.13$^{b}$ | 27.20±0.17$^{e}$ | 2.55±0.28$^{b}$ | 0.71±0.03$^{b}$ |

Values are Mean±SE (n=6); One way ANOVA (SPSS); TBARS=nmol/mg, GSH=umole/mg; Protein carbonyls=nmole/mg; relative kidney weight =% of bwt; Means with different alphabets as superscripts differred significantly ($P$<0.05).

Diabetic nephropathy is the leading cause for end-stage renal disease and cardiovascular deaths (Kamiyama et al., 2013). Reactive oxygen species (ROS) are generated due to hyperglycemia causing cellular damage and ultimately leads to secondary complications (Jaganjac et al., 2013). The present study was undertaken to investigate the oxidative stess biomarkers of lipid, protein, and glutathione metabolism in DM. The concentration of TBARS and protein carbonyls revealed a significant ($P$<0.05) increase in diabetes control rats (group 2) compared to control rats (group 1) and groups 3 to 7 showed a significant ($P$<0.05) decrease in their concentration compared to the rats of group 2. On the other hand, the groups 6 and 7 showed significant ($P$<0.05) decrease in their concentration compared to groups 3, 4 and 5. The concentration of GSH revealed a significant ($P$<0.05) decrease in diabetes control group (group 2) compared to control group (group 1) and a significantly ($P$<0.05) increased GSH concentration in groups 3 to 7 was observed compared to diabetes control group. Group 7 showed significantly ($P$<0.05) higher concentration among all the treated groups (**Table 2**). Hyperglycemia causes autoxidation of glucose, glycation of proteins, and activation of polyol metabolism. These changes accelerate the generation of ROS and increase oxidative chemical modification of lipids, DNA, and proteins in various tissues (Giacco and Brownlee, 2010). Moreover, these lipid peroxidation represents a close relationship with the hyperglycemia and oxidative stress in DM (Salgueiro et al., 2013).

TBARS are the most commonly used biomarker for lipid peroxidation in the tissue, and the high levels of TBARS in the plasma and tissues of diabetic animals are due to lipid peroxidation (Vijayakumar et al., 2006). Current study data confirmed Streptozotocin-induced diabetic nephropathy characterized by oxidative stress and were further confirmed by increased TBARS, protein carbonyls and decreased GSH levels in kidney tissue. Fenugreek and glimepiride treatment reduced the levels of TBARS, protein carbonyls and serum creatinine, and increased GSH levels. Previous study reported that antioxidant activity of fenugreek is caused mainly due to its higher levels of flavonoid contents (Norziah et al., 2015).

GSH is an endogenous anti oxidant molecule which detoxifies several exo and endogenous toxic compounds by reacting with singlet oxygen, superoxy, peroxy and hydroxyl molecule (Kumar and Reddy, 2012). These result were further supported by the elevated serum creatinine level which signifies impaired kidney function and the serum creatinine concentration of group 2 was significantly ($P$<0.05) higher than those of group 1 at 4$^{th}$ and 8$^{th}$ wks, respectively. The treatment groups (3 to 7) showed significant ($P$<0.05) decrease at the end of 4$^{th}$ and 8$^{th}$ wks compared to diabetic control rats (group 2). The groups 6 and 7 showed significant decrease among all the treated groups at the end of 4$^{th}$ and 8$^{th}$ wks (**Table 1**).

In the present study, the relative kidney weight (% of bwt) revealed a significant increase in diabetes control group (group 2) compared to control group (group 1), and groups 3 to 7 showed a significant decrease in their weight as compared to diabetes control group ( group 2). The groups 4, 5, 6 and 7 were comparable to that of group 1. The increase in kidney weight might be due to increased glucose utilization and subsequent enhancement of glycogen synthesis, lipogenesis and protein synthesis. These changes might lead to microvascular renal complications like increased synthesis of glycoproteins, followed by thickening of basement membrane and renal hypertrophy (Iannello et al., 2005). Fenugreek treatment prevented alterations in the kidney as reported earlier (Neveen et al., 2007).

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 patients as well as in induced diabetic experimental animals has adverse effects on all organs. Fenugreek has hypoglycemic and insulin stimulating action of 4-hydroxy isoleucine on the β cells of pancreas, and reduced ROS production. The antioxidant action of glimepiride was also reported earlier (Krauss et al., 2003).

## CONCLUSION

The pharmacodynamic interaction of fenugreek (herbal drug) with insulin and glimepiride can improve antioxidant parameters facilitated by reducing lipid peroxidation and increasing Glutathione (GSH) in streptozotocin-induced diabetic rats.

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