International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Evaluation of Antioxidant Role of *Trigonella Foenum Graecum* in Alloxan Induced Diabetic Wistar Albino Rats

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Abstract: ***Diabetes mellitus is currently a serious global health concern. It has been demonstrated that diabetes mellitus is characterised by elevated lipid peroxidation. Oxidative stress results in increased production of ROS. This research work was undertaken to evaluate the antioxidative potential of Trigonella foenum - graecum in diabetic rats.*** **Trigonella foenum - graecum** ***is commonly known as Fenugreek. Diabetes was induced by an intraperitoneal injection of 150 mg/kg body weight of alloxan monohydrate dissolved in 0.9% normal saline. Alloxan is used to induce type 1 diabetes in Wistar albino rats. Alloxan is a toxic chemical substance that particularly destroys the β - cells of the islets of Langerhans in the pancreas. Diabetic rats were treated with an aqueous extract of Trigonella foenum - graecum by oral gavage for 21 days. Hyperglycemia, an inherent feature of diabetes mellitus, lowers antioxidant defense and, as a result, promotes the development of oxidative stress. The diabetic rat produced a significant increase in lipid peroxidation (TBARS) levels in blood and kidney, indicating an increase in the generation of free radicals, and the antioxidant enzymes glutathione s - transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) were observed to be reduced in the diabetic group as compared to the control group. Diabetic rats treated with an aqueous extract of Trigonella foenum - graecum showed a decrease in TBARS levels and increased the levels of antioxidant enzymes in the blood and kidney. According to the results of this investigation, fenugreek decreases the generation of reactive oxygen species in diabetic rats and increases scavenging enzymatic activity.***

Keywords: Alloxan, antioxidant effect, Wistar albino rats, Fenugreek

1. Introduction

Diabetes mellitus (DM) is a chronic, noncommunicable illness that is one of the most serious public health issues due to its high prevalence and risk of chronic consequences (Arroyave *et al,* 2020). There are various pathogenetic mechanisms that serve a vital part in the onset of diabetes since a few of them include the death of pancreatic beta cells, resulting in insulin shortage, and individuals who are resistant to insulin action (Rojas *et al,* 2018). Due to defect in the secretion or action of insulin on target tissues, hyperglycemia causes many defects to the body. Diabetes affects every organ in the body, and also compromises the ability of the immune system to fight infection (Kowluru, 2023).

Oxidative stress, characterised by an absence of balance among the oxidative and anti - oxidative systems of the body's cells and tissues, causes an excess of oxidative free radicals and reactive oxygen species (ROS) (Rani *et al*, 2016). Aerobic organisms have included antioxidant systems that comprise both enzymatic and non - enzymatic antioxidants that are typically efficient at mitigating the adverse effects of ROS. However, in pathological conditions, the antioxidant systems can be overwhelmed (Birben *et al*, 2012). The Lipid peroxidation (oxidative damage) in rat kidney is increased by experimentally induced hyperglycaemia. Antioxidants are synthetic or organic substances that can prevent or postpone some forms of cell damage. Many foods, including fruits and vegetables, contain antioxidants. Several synthetic antioxidants have been widely employed in recent decades. Many recent investigations have found that persistent usage of various artificial antioxidants have been associated with side - affects that affects health at the long term (Sindhi *et al*, 2013). As a result, the identification of safer natural antioxidants is presently critically required.

Scavenging or detoxification of excess ROS is achieved by an efficient antioxidative system comprising of the enzymic antioxidants. The enzymic antioxidants include Glutathione s - transferase (GST), Glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT). Ethnobotanical research has looked into various kinds of plant preparations used as therapeutic agents for diabetes - related problems and other issues (Skalli *et al*.2019). Trigonella foenum - graecum (Fenugreek) is an annual dicotyledonous aromatic plant which belongs to Fabaceae family. It is a medicinal plant also known as "Methi", It is widely used as a condiment in food preparations and it is a constituent of the Indian spicy 'curry' (Ebrahimghochi *et al*.2018; Rashid *et al*.2018). *Trigonella foenum - graecum* (TFG) is considered as one of the oldest medicinal plants and its health - promoting effects have been cited in Ayurveda and traditional Chinese medicine. The seeds have a characteristic rhomboid outline, pungent odor and slightly bitter - sweet taste (Basu *et al*.2018). Its leaves and seeds are widely used throughout the world. The seeds can be used as an antidiabetic agent due to their hypoglycemic properties and can reduce lipid peroxidation levels. It was previously used to treat a variety of illnesses, including diabetes and obesity. It possesses antioxidant, antihyperlipidemic, antibacterial, antifungal, anti - inflammatory, and galactagogic properties (Nagulapalli Venkata *et al*, 2017). TFG is widely used to treat diabetes mellitus, the present study was aimed to investigate the effect of Trigonella on lipid peroxidation and antioxidant enzyme levels in the blood and kidney tissue of diabetic rats.

2. Materials and methods

Chemicals and reagents
Alloxan monohydrate has been bought from Chennai Chemicals, Nyniappa Naicken Street, Chennai - 003. Before being injected intraperitoneally (IP), alloxan monohydrate was newly dissolved in 0.9% saline. All of the items, including the biochemical kits, were of analytical quality.

Collection and making of TFG seed powder
The seeds of Trigonella foenum - graecum were bought in a neighbourhood marketplace in Chennai. Fenugreek has been washed to remove any stones or contaminants. In the shade, the seeds were dreid. Air - dried seeds have been ground into a fine powder using an electric blender and sieved.

Techniques for extracting plants
A flask containing about 30g of seed powder was dissolved in 360 ml of distilled water and kept in a sterile setting for 24 hours. The liquid extract was filtered, and then it was heated at 80 to 90 °C until it was totally dried out. The dry powder was collected and kept at 4°C for the upcoming investigation (Desai *et al*., 2019).

Study on Acute Toxicity
Five healthy adult rats were chosen at random for this investigation based on Organisation for Economic Co - operation and Development (OECD) guideline 425 (Zarei *et al*, 2023). The rats were administered a four - hour fasting herbal extract of aqueous *Trigonella foenum graecum* seed extract (300 mg/kg b. wt.). A test dosage was initially given to one animal. After the animal had survived for 48 hours, test dosages of 300, 1000, 1500, and 2000 were gradually delivered to other animals. Rats were inspected for any changes in fur loss, breathing pattern, shaky hands, convulsions, unusual faeces (diarrhoea), fatigue, severe discomfort, stress, and sickness to evaluate whether there were any physical symptoms of hazardous exposure. The animals were then monitored for the next 14 days.

Experimental animals
The Mass Biotech Animal Unit at Pulipakkam, Chengalpet, provided Wistar albino rats gauging 200 to 240 g. The animals were acclimatised in a familiar habitat for seven days before to the trial. Rats were housed in polypropylene rat cages in sterile conditions with a 12 - hour light/dark cycle, ambient temperatures ranging from 22°C to 24°C, and humidity levels ranging from 30 to 70% before the tests began. Each animal was fed a conventional laboratory diet consisting of 70% carbs, 25% proteins, and 5% fats, as well as an unlimited supply of water. The experimental approach was approved by the Institutional Animal Ethics Committee (IAEC). Ethical clearance number: 2084/PO/RcBt/S/19/CPCSEA.

Induction of Diabetes
In Wistar albino rats, alloxan monohydrate was utilised to induce diabetes mellitus. Except for the control group, alloxan was induced in two groups. To develop diabetes in overnight - starved rats, 150 mg/kg body weight (b. wt) alloxan monohydrate dissolved in 0.9% normal saline was given intraperitoneally. This diabetes experiment was carried out using the techniques of (Yella *et al*, 2019; Umar *et al*, 2018). To prevent transient hypoglycemia, the animals were given a 5% glucose solution to drink straight after (Saifi *et al*, 2016). The findings of body weight loss, polyuria, glycosuria, polydipsia, and polyphagia in diabetic rats after 2 days (48 hours) were noted. Treatment with *Trigonella foenum graecum* (Fenugreek) began 48 hours after an alloxan injection. which is considered the first day of treatment and it was continued for 21 days.

3. Experimental Design

Rats were placed into three groups at random, with each group consisting of six animals (n=6).
Group 1: The rats in this group are healthy rats.
Group 2: The diabetic group received a single intraperitoneal injection of alloxan monohydrate at 150 mg/kg body weight.
Group 3: Alloxan - induced diabetic rats were given 300 mg/kg body weight *Trigonella foenum graecum* seed extract (Anarthe *et al*, 2014) orally for 21 days.

Collection of blood and tissue sampling
On the 22nd day, rats were put under deep ether anaesthesia and blood was obtained via the retroorbital plexus (Anarthe *et al*, 2014). Animals were slaughtered, and their kidneys were extracted. The obtained samples were utilised to assess antioxidant enzymes and lipid peroxidation (TBARS).

Evaluation of TBARS and antioxidant enzyme activity in the blood and kidney
The condition of lipid peroxidation was assessed using the Ohkawa *et al*. (1979) method of calculating TBARS (Thiobarbituric acid reactive substance). The methodology of Habig *et al*. (1974) was used to assess the activity of glutathione s - transferase (GST). The Rotruck *et al*. (1973) method was used to determine glutathione peroxidase (GPx). The activity of superoxide dismutase (SOD) was measured using the Beauchamp and Fridovich (1973) technique. Catalase (CAT) activity was measured using the Chance and Maehly (1955) approach.

Evaluations of statistics
The mean ± standard deviation (SD) is used to represent the findings. A student t - test was used to compute mean differences, followed by a one - way analysis of variance (ANOVA) followed by a Tukey post hoc multiple comparison test. Differences between groups at $p<0.05$ were considered statistically significant.

4. Results

This study's acute toxicity study revealed that *Trigonella foenum graecum* seed extract is non - toxic up to 2000 mg/kg body weight.

The study's findings, such as lipid peroxidation (TBARS), antioxidant enzyme levels in the blood and kidneys of the control, diabetic, and *Trigonella foenum graecum* extract - treated groups. The present study's findings were reported in the tables.

Effect of treatment on lipid peroxidation in the blood (serum) and kidney
According to the findings, the diabetic group's TBARS level in blood and kidney homogenate was significantly ($P<0.01$)

higher than the control group's. In contrast, when rats were treated with TFG aqueous extract for 21 days, the lipid peroxidation level measured as TBARS was significantly (P<0.01) lower in the blood and kidney homogenate of treated diabetic rats compared to untreated rats. The lipid peroxidation levels (LPO) of all groups were shown in Tables 1 and 2.

Percentage change of lipid peroxidation (TBARS) in blood and kidney

The diabetic group's TBARS percentage change of difference in the blood and kidney increased to 259% and 212%, respectively, as compared to the control group. In comparison to the diabetic group, TFG extract reduced TBARS levels in the blood and kidneys by 76% and 72%, respectively as shown in figure 1.

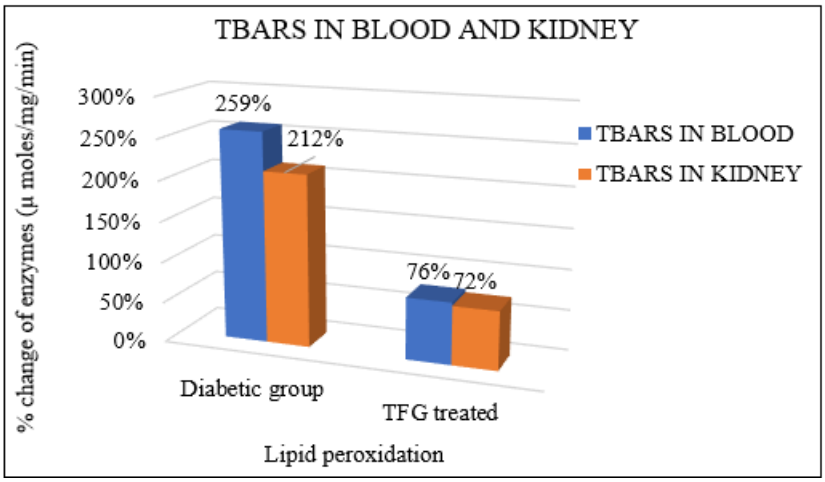

Figure 1: Percentage change of TBARS in blood and kidney

Effect of TFG treatments on antioxidants enzymes activities in blood and kidney

In the current investigation, in diabetic rats antioxidant enzyme activities such as glutathione s - transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) were significantly reduced (P< 0.01) in the blood and kidney of alloxan - exposed rats compared to control groups. Conversely, the activity of those antioxidant enzymes were considerably (P<0.01) normalised in diabetic rats supplemented with TFG aqueous extract compared to untreated diabetic group, indicating that the TFG aqueous extract has antioxidant characteristics, as shown in tables 1 and 2.

Table 1: Effect of *Trigonella foenum graecum* seed extract on GST, GPx, SOD, CAT, and TBARS levels in blood

S. No	Parameters	Group 1 (control group)	Group 2 (Diabetic group)	Group 3 (*TFG* treated group
1	GST	0.35 ± 0.105	0.088±0.044**	0.34±0.098$^{\#\#}$
2	GPx	0.433±0.139	0.066±0.027**	0.446±0.121$^{\#\#}$
3	SOD	0.338±0.070	0.146±0.025**	0.33±0.069$^{\#\#}$
4	CAT	0.815 ±0.135	0.451±0.057**	0.806±0.122$^{\#\#}$
5	TBARS	0.063 ±0.021	0.226 ±0.099**	0.055±0.018$^{\#\#}$

Values are presented as mean ± SD (standard deviation), where n = 6, and their significance level was calculated using the student t - test, followed by a one - way ANOVA. The significance level is **p < 0.01 when compare to control group and $^{\#}$ $^{\#}$p<0.01 when compare to diabetic group.

Units

GST: µ moles CDNB /mg/min. **GPx:** µ moles / mg/ min/. **SOD:** µ moles /mg /min. **CAT:** µ moles H_2O_2 /mg/min. **TBARS:** µ mole/mg.

Table 2: The impact of *Trigonella foenum graecum* seed on GST, GPx, SOD, CAT, and TBARS levels in kidney

S. No	Parameters	Group 1 (control group)	Group 2 (Diabetic group)	Group 3 (treated group
1	GST	0.248± 0.08	0.038± 0.014 **	0.24±*0.084*$^{\#\#}$
2	GPx	0.528±0.081	0.061±0.021 **	0.503±*0.079*$^{\#\#}$
3	SOD	0.363±0.076	0.041±0.023 **	0.353±*0.076*$^{\#\#}$
4	CAT	0.65 ± 0.04	0.345±0.168 **	0.635±*0.043*$^{\#\#}$
5	TBARS	0.086±0.023	0.268±0.120 **	0.076±*0.026*$^{\#\#}$

Values are given as mean ± SD (standard deviation), where n = 6, and their significance level was calculated using the student t - test, followed by a one - way ANOVA. The significance level is **p < 0.01 when compare to control group and $^{\#\#}$ p<0.01 when compare to diabetic group.

Units

GST: µ moles CDNB /mg/min. **GPx:** µ moles / mg/ min/. **SOD:** µ moles /mg /min. **CAT:** µ moles H_2O_2 /mg/min. **TBARS:** µ mole/mg.

Percentage change of antioxidant enzymes in blood

When compared to the control group, the percentage changes in GST, GPx, SOD, and CAT in the diabetic group were 75%, 85%, 57%, and 45%, respectively. When compared to the diabetic group, the percentage change in TFG extract - treated groups of GST, GPx, SOD, and CAT was 286%, 532%, 126%, and 79%, respectively as shown in fig 2.

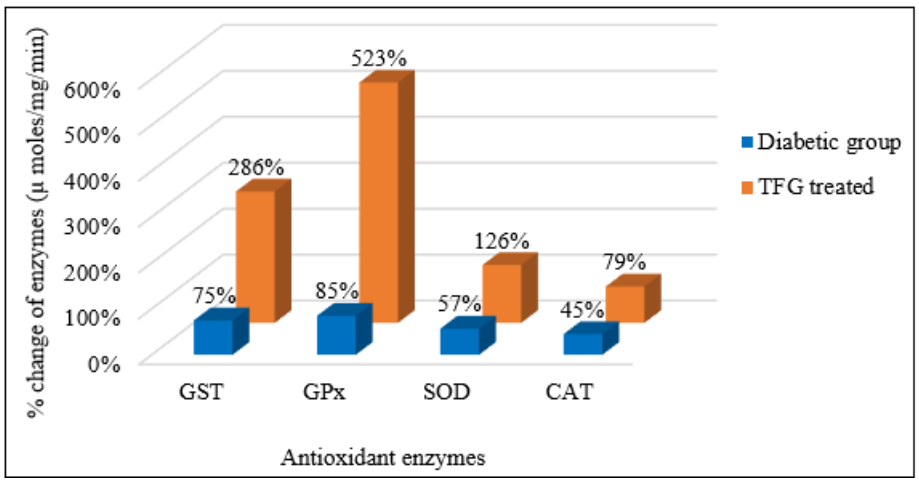

Figure 2: Percentage change of enzymes in blood

Percentage change of antioxidant enzymes in kidney

The percentage change in the diabetic groups GST, GPx, SOD, and CAT in the kidney was reduced to 85%, 88%, 89%, and 47% respectively, when compared to the control group. When compared to the diabetic group, TFG seed extract raised the proportion of enzymes such as GST, GPx, SOD, and CAT by 532%, 725%, 761%, and 84%, respectively as shown in fig 3.

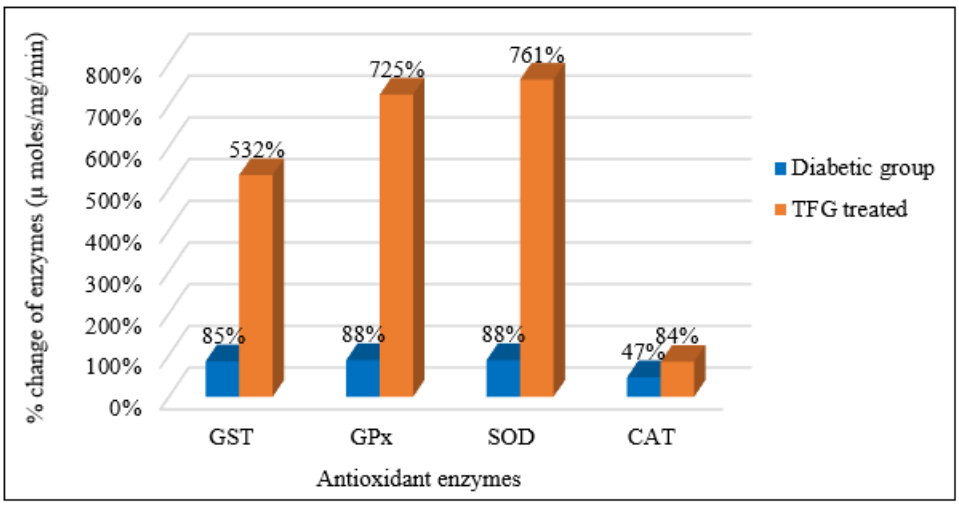

Figure 3: Percentage change of enzymes in kidney

5. Discussion

The current findings show that an imbalance in the oxidative state in blood and renal tissue leads to increased ROS formation as a result of issues in alloxan - induced rats and however, oral treatment of TFG restored the alloxan - induced effects in rat blood and renal tissue. It is well known that the accumulation of free radicals or reactive species in the kidney is maintained at extremely low levels under normal conditions and increases during pathological situations. In diabetes, hyperglycemia is known to produce radicals that result from auto - oxidation of glucose, the production of advanced glycated end products, as well as a rise in cellular LPO and membrane damage (Rahmani *et al*, 2023). Hyperglycemia is meant to lower antioxidative enzyme activity and enhance lipid peroxidation (LPO), as well as alterations in glutathione redox state (Naoom *et al*, 2023). Increased oxidative stress, which accord significantly to the the mechanism of diabetes - related problems, results from either increased ROS generation or decreased ROS scavenging ability. Multiple investigations have found changes in antioxidant enzymes under diabetes conditions (Goycheva *et al.*, 2023). The antioxidative defence system, such as SOD and catalase, revealed reduced activity in the kidney during diabetes, and the findings are consistent with previously published results (Weydert and Cullen, 2010: Alanazi *et al*, 2023).

In the current inquiry treatment with aqueous extracts of TFG shown considerable hypoglycemic action in diabetic rats. The

active component of TFG seeds has been discovered to be associated with the seeds' defaulted portion, which is high in fibre and contains steroidal saponins and proteins.

One of the most notable outcomes of the current study is that oral administration of TFG seed extract corrected alloxan - induced changes in the pro - and antioxidant status of rats' blood and kidney tissue. In the present investigation, diabetic rats were treated with plant extracts of TFG 300 mg/kg body weight, which lowered TBARS, a marker used to evaluate oxidative damage, and raised antioxidant enzyme levels. These findings are identical with those of Alwan *et al*, 2020: Pradeepkiran *et al*, 2020.

As a result, the high phenolic and flavonoid content of *Trigonella* may be responsible for the lower LPO level in the kidney tissue homogenate (Singh *et al*, 2020). Based on these findings, it is possible to assume that a reduction in the intrinsic defence system resulted in the formation of free radicals, as demonstrated by the high level of lipid peroxidation content and the other way around, Trigonella treatment greatly improved the antioxidant system and hence neutralised free radical production in alloxan - induced diabetic rats.

Treatment of Trigonella foenum graecum extract significantly boosted the activity of antioxidant enzymes in the blood and kidney of diabetic rats. The current study found that fenugreek seed extract significantly reduced oxidative damage in diabetic rats.

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