Trigonella foenum-graecum seeds extract plays a beneficial role on brain antioxidant and oxidative status in alloxan-induced Wistar rats

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Abstract

Context: Trigonella foenum-graecum (TriFG) exhibits increased scavenger enzymatic activities and reduces the production of reactive oxygen species in diabetic rats.

Objective: The present study was aimed to investigate the effect of TriFG on lipid peroxidation levels and antioxidant status in brain tissue of rats exposed to alloxan.

Materials and Methods: Healthy male rats (180 ± 10 g) were allocated into five groups. Animals in group 1 maintained on normal tap water served as controls and rats in groups 2, 3, 4, and 5 were treated as experimental groups. Rats in group 2 were intraperitoneally injected with alloxan (120 mg/kg BW) and treated as diabetic rats, whereas rats in groups 3 and 4 were maintained on same experimental regimen as that of rats in groups 1 and 2, respectively, and in addition, they were orally gavaged with herbal extracts of TriFG (0.25 g/kg BW). Diabetic rats treated with glibenclamide in group 5 were used as positive controls.

Results and Discussion: Significant ($P < 0.001$) increase in the antioxidant enzymes with a significant ($P < 0.001$) decrease in the lipid peroxidation levels were observed in the brain tissue of diabetic rats treated with TriFG extract as compared to diabetic and glibenclamide-treated rats. No significant changes were observed in pro- and antioxidant levels in brain tissue of rats treated with TriFG extract alone when compared to normal rats. In diabetic rats, brain mitochondrial and cytosolic enzymes like succinate dehydrogenase, glutamate dehydrogenase, and glucose-6-phosphate dehydrogenase activity levels were significantly ($P < 0.05$) decreased with reversely increased was observed in lactate dehydrogenase activity ($P < 0.05$).

Conclusions: The findings of the present study suggested that TriFG, through its antioxidant properties, protects brain tissue by mitigating oxidative stress induced by alloxan-exposed rats. TriFG extract significantly increased the antioxidant and oxidative properties in diabetic rats when compared with the control group rats.
Introduction
Diabetes mellitus (DM) is a major health problem all over the world today. It is a metabolic disorder of carbohydrate, fat, and protein metabolism characterized by the elevation of both fasting and post-prandial blood glucose levels. DM has been shown to be a state of increased lipid peroxidation (LPx). LPx of cellular structures, a free radical-induced activity, is thought to play an important role in ageing, atherosclerosis, and late complications of DM (Klein et al., 2015). An impaired radical scavenger function has been linked to the altered activity of enzymes (catalase, superoxide dismutase, and glutathione peroxidase) and non-enzymatic (glutathione) free radical scavengers. The increased production of reactive oxygen species (ROS) has been attributed to protein glycation and/or glucose auto-oxidation due to a hyperglycaemic environment. It has been suggested that during DM almost all vital organs, including brain, are adversely affected (Nedzvetsky et al., 2012). The neurological complications of DM in the central nervous system are now receiving greater attention. The cognitive deficits, along with morphological and neurochemical alterations, illustrate that the neurological complications of diabetes are not limited to peripheral neuropathies. Moreover, DM also contributes to cerebrovascular complications, reductions in cerebral blood flow, disruption of the blood-brain barrier, and cerebral oedema (Prasad et al., 2014). All of these neurochemical and neurophysiological changes ultimately contribute to the long-term complications associated with diabetes, including morphological abnormalities, cognitive impairments, and increased vulnerability to the pathophysiological event (Sandeep et al., 2004).

The central complications of hyperglycaemia also include the damage of neuronal circuits and the damage is more augmented due to hyperglycaemic-induced oxidative stress. Oxidative stress, leading to an increased production of ROS, as well as LPx, is increased in diabetes and also by stress in euglycemic animals. Similarly, the oxidative damage in rat brain is increased by experimentally induced hyperglycaemia. Under experimental conditions, hyperglycaemia dramatically increases neuronal alterations and glial cell damage caused by temporary ischaemia. Several lines of evidence indicated that the modified oxidative state induced by chronic hyperglycaemia may contribute to nerve tissue damage; free radical species impair the central nervous system, attacking neurons and Schwann cells and the peripheral nerves. Because of its high polyunsaturated lipid content, Schwann cells and axons are particularly sensitive to oxygen when exposed free radicals impending the damage. LPx may increase cell membrane rigidity and impair cell function, which lead to many metabolic disorders. It has been claimed that synthetic oral hypoglycaemic agents and insulin that are used in the management of DM often lead to side effects (Yamamoto et al., 2001; Tolman and Chandramouli, 2003; Weijers, 2015). Hence, the demands for safer and more effective agents were required to treat diabetes.

Traditional medicinal plants have been extensively used for the treatment of diabetes and therein exist as a hidden wealth of potentially useful natural products for diabetes control (Gray and Flatt, 1997). Trigonella foenum-graecum (TriFG) is commonly known as fenugreek. It is an annual herb that belongs to the family Fabaceae and its active ingredient is Trigonelline. Fenugreek seeds are used
as a traditional remedy for the treatment of diabetes (Miraldi et al., 2001; Basch et al., 2003). Supplementation of fenugreek seed powder in the diet leads to a reduction in biomarkers of oxidative damage in alloxan diabetic rats (Ravikumar and Anuradha, 1999). These fenugreek seeds have been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal values in various animal model systems (Raju et al., 2001). In addition to its antidiabetic properties, Trigonella also has antioxidant properties (Genet et al., 2002). However, its antioxidant potential in the brain tissue of diabetic condition is poorly understood. Considering the facts that (a) DM at least in part targets pro- and/or antioxidant status in brain and (b) TriFG is widely used to treat DM, the present study was aimed to investigate the effect of TriFG on antioxidant enzyme levels and LPx in the brain tissue of diabetic rats.

Materials and Methods

Extraction preparation

The seeds of TriFG were purchased from local market, dried in shade, and powdered. The powder was used for the extraction of antidiabetic principle/s using ethanol as solvent. The active principles of the above-mentioned seeds were extracted into ethanol. The powdered material was soaked in water in a glass jar for 2 days at room temperature and the solvent was filtered. This process was repeated three to four times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi, rotavapor R-114 and finally freeze dried. This extract was administered orally to rats to know whether they have any influence on the antioxidant status in the brain tissue of diabetic rats.

Experimental design

Group 1 - Normal untreated rats—Normal (N)
Group 2 - Diabetic untreated rats—Diabetic(D)
Group 3 - Normal rats treated—N + TriFG with 0.25 g/kg/BW of TriFG
Group 4 - Diabetic rats treated—D + TriFG with 0.25 g/kg/BW of TriFG
Group 5 - Diabetic rats treated—D + Glibenclamide with 0.2 g/kg/ BW of Glibenclamide

The animals were killed after the last treatment by cervical dislocation and the brain tissues were isolated. These tissues were washed with ice-cold saline and immediately stored at −80°C for biochemical analysis and enzymatic assays.

Enzymatic assays

The antioxidant enzymes that include glutathione-S-transferase (GST), catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and LPx levels were estimated with respective procedures as follows. GST activity in the cytosol fraction of the brain was assayed by using 1-chloro-2,4-dinitro benzene (at 340 nm) as described by Habig et al. (1974). Catalase activity was assayed by the method of Chance and Maehly (1955) and SOD activity in brain homogenate was determined according to the method of Minami and Yoshikawa (1979). GPx activity was measured by the method described by Rotruck et al. (1973); the level of LPx in the brain was measured in terms of malondialdehyde (a product of LPx content and determined by using the thiobarbituric acid reagent (TBAR). The reactivity of TBAR is determined with minor modifications of the method adopted by Hiroshi et al. (1979).

Brain tissue homogenates (10% W/W) were prepared in 0.25 mol/L ice-cold sucrose solution. The mitochondrial and cytosol fractions were separated by centrifugation and used for biochemical analysis. Succinate dehydrogenase (SDH) (E.C. 1.3.99.1) activity was estimated by the method of Nachlas et al. (1960). Lactate dehydrogenase (LDH) (E.C. 1.1.1.27) activity was estimated by the method of Srikanthan and Krishnamurthy (1955). Glucose-6-phosphate dehydrogenase (G-6-PDH) (E.C. 1.1.1.49) activity was assayed by the method of Bergmeyer and Bruns (1965). Glutamate dehydrogenase (GDH) (E.C. 1.4.1.3) activity was determined by the method of Lee and Lardy (1965).

The enzymatic values of antioxidative enzymes and oxidative enzymes of control and experimental values were calculated to determine the significance of the effect of the treatment on the experimental group of animals.

Statistical analysis

The mean, standard deviation (SD) were carried out according to Steel and Torrie (1960) using basic programming techniques on SPSS for different parameters. The P-value of more than 0.01 was considered as not significant.

Results

General toxicity

No clinical signs of toxicity were observed in any of the control and experimental groups. None of the animals were excluded from the present study.
TriFG effect on blood glucose levels

The blood glucose levels in the control and experimental groups were checked at different time intervals. The blood glucose level was significantly increased in diabetic untreated rats. Oral administration of plant extract of TriFG and glibenclamide to diabetic rats significantly reversed all these changes to near normal levels. TriFG seeds powder for diabetic animals has been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal blood glucose values (Figure 1).

Effect of TriFG on LPx and antioxidant status

The changes in LPx and antioxidant status in the brain tissue of the control and experimental groups were given in Table 1. In alloxan-treated diabetic rats, the levels of TBARS in the brain tissue were significantly increased (77.27) as compared to control rats. On the other hand, oral gavage of TriFG (7.272) and glibenclamide treatment (20.00) compared to diabetic rats significantly decreased. The levels of lipid peroxidative marker in the brain tissue where the effect is more in the TriFG-exposed diabetic rats (−14.66). Whereas no significant change was observed in the LPx levels in the brain of normal rats exposed to TriFG (Table 1). Alloxan treatment significantly reduced the activity levels of antioxidant enzymes such as SOD (−37.293) and catalase (−70.19), GST (−82.251) and reduced glutathione levels (−59.647) in the brain of rats when compared to controls. Exposure of plant extracts of TriFG significantly increased the levels of antioxidant enzymes such as SOD (−4.962), catalase (−9.294), GST (−75.541) and reduced glutathione levels (−25.433) in the brain of rats as compared to controls. No significant changes were observed in the brain tissue of normal rats exposed to plant extract alone.

The effects of TriFG extract on oxidative enzymes in brain tissues are given in Table 2. The activity levels of mitochondrial marker enzyme SDH were significantly (P < 0.05) decreased in renal and hepatic tissues of diabetic rats when compared to normal rats. The SDH activity was significantly (P < 0.05) increased in TriFG extract treated diabetic rats when compared to diabetic rats. Brain tissue homogenate LDH activities were significantly (P < 0.05) increased in diabetic rats when compared to normal control rats, in contrast TriFG extract to diabetic rats significantly decreased (P < 0.05) was observed in LDH activity levels when compared to diabetic rats. In diabetic rats brain tissue GDH activity was significantly (P < 0.05) decreased as compared to the normal control rats, interestingly in TriFG extract treated diabetic rats GDH activity was significantly (P < 0.05) increased when compared to diabetic rats. Brain G-6-PDH activity was significantly (P < 0.05) decreased in diabetic rats when compared to normal control. However, in TriFG extract-treated diabetic rats, G-6-PDH activity was significantly (P < 0.05) increased when compared with diabetic control rats.

When TriFG alone treated to normal control, no significant change was observed when compared with normal control rats. When compared to Aloe vera extract, glibenclamide treatment showed the similar results regarding restoration of biochemical, antioxidant enzyme changes against diabetic-induced depletion.

Discussion

The present findings of the present study demonstrate that an imbalance in the oxidative status of the nervous tissue leads to increased generation of ROS as a consequence of perturbations in the brain tissue of alloxan-treated rats; and on the other hand, surprisingly, oral administration of TriFG reversed the alloxan-induced effects in the brain tissue of rats. It is well known that under normal conditions, the generation of free radicals or of active species in the brain, as in other tissues, is maintained at extremely low levels (Chan, 1996). Earlier studies have demonstrated a defective metabolism of lipid peroxides in other tissues of diabetic animal (Pari and Latha, 2004). Hyperglycaemia has been shown to generate free radicals from auto-oxidation of glucose, the formation of advanced glycated end products and increased polyol pathway, with a concomitant increase in cellular LPx and damage of membrane in diabetes. One of the consequences of LPx degenerative processes can result in enzyme activity changes. This increased lipid peroxidation during diabetes disturbs the anatomical integrity of the membrane, leading to the inhibition of several membrane-bound enzymes. Previously, studies reported that the mouse cerebral enzymes pump Na+K+ ATPase activity inhibition of by ultraviolet C (UV-C) generated OH – and a peroxy (ROO−) radical is mediated via LPx induced disturbances of membrane integrity (Jammé et al., 1995). The decreased activity of Na+K+ ATPase observed in diabetic brain tissue may be due to the membrane fluidity and peroxidative damage induced by increased lipid peroxidative status. The statuses of elevated ROS may also affect the membrane-linked enzyme activity through modifications of membrane fluidity because the activity of most membrane-bound enzymes are dependent on the membrane fluidity. The antioxidant enzyme changes against diabetic-induced depletion.
Table 1. Effect of plant extracts of TriFG on lipid peroxidation, activities of enzymatic and non-enzymatic antioxidants status in the brain tissue of diabetic rats.

| Groups          | TBARS (mM/100 g tissue) | Catalase (μmoles of H₂O₂ consumed/min/mg) | SOD (units A SOD/mg protein) | GPX (μg of GSH consumed/min/mg/protein) | GST (μmoles of CDMB-GSH/conjugate formed/min/mg/protein) | Reduced glutathione (mg/100 g tissue) |
|-----------------|-------------------------|------------------------------------------|-----------------------------|----------------------------------------|----------------------------------------------------------|--------------------------------------|
| Normal (N)      | 1.10 ± 0.08†            | 3.12 ± 0.21†                             | 6.65 ± 0.38†                | 3.41 ± 0.21†                           | 1.04 ± 0.13†                                             | 4.62 ± 0.28†                         |
| Diabetic (D)    | 1.95 ± 0.05 (77.272)    | 0.93 ± 0.05 (−70.19)                     | 4.17 ± 0.3 (−37.293)        | 1.01 ± 0.04 (−70.381)                  | 0.82 ± 0.02 (−82.251)                                    | 35.19 ± 3.31†                         |
| N + TriFG       | 1.03 ± 0.06 (−6.363)    | 4.00 ± 0.23 (28.205)                     | 6.03 ± 0.28 (−9.02)         | 3.8 ± 0.19 (11.436)                    | 4.9 ± 0.28 (6.060)                                      | 14.2 ± 2.14†                          |
| D + TriFG       | 1.18 ± 0.07 (−9.272)    | 2.83 ± 0.29 (−9.294)                     | 6.32 ± 0.2 (−4.962)         | 2.91 ± 0.1 (−14.662)                   | 1.13 ± 0.12 (−75.541)                                   | 26.24 ± 2.12†                          |
| N + TriFG       | 1.03 ± 0.06 (−6.363)    | 4.00 ± 0.23 (28.205)                     | 6.03 ± 0.28 (−9.02)         | 3.8 ± 0.19 (11.436)                    | 4.9 ± 0.28 (6.060)                                      | 14.2 ± 2.14†                          |
| D + TriFG       | 1.18 ± 0.07 (−9.272)    | 2.83 ± 0.29 (−9.294)                     | 6.32 ± 0.2 (−4.962)         | 2.91 ± 0.1 (−14.662)                   | 1.13 ± 0.12 (−75.541)                                   | 26.24 ± 2.12†                          |
| D + Glibenclamide | 1.32 ± 0.09            | 1.98 ± 0.13 (−36.538)                    | 5.32 ± 0.3 (20.00)          | 1.99 ± 0.12 (−77.489)                  | 25.5 ± 2.10 (−27.536)                                   | 35.19 ± 3.31†                         |

Values are mean ± SD of six rats in each group and values not sharing common superscript letter are significantly different at P < 0.05.
Enzyme activities in brain tissue of control and experimental group rats.

| Enzyme       | SDH          | LDH          | GDH          | G-6-PDH       |
|--------------|--------------|--------------|--------------|---------------|
| Normal (N)   | 0.591±0.06   | 0.412±0.04   | 0.631±0.06   | 0.534±0.05    |
| Diabetic(D)  | 0.362±0.03 (-38.64) | 0.542±0.04 (32.19) | 0.361±0.03 (-42.85) | 0.328±0.03 (-38.11) |
| N + TriFG    | 0.609±0.06 (2.22)  | 0.419±0.04 (2.19)    | 0.649±0.06 (3.04)    | 0.548±0.05 (3.39)   |
| D + TriFG    | 0.459**±0.03 (-22.20) | 0.389**±0.03 (-5.12) | 0.502**±0.05 (-20.31) | 0.491**±0.05 (-7.34) |
| D + Glibenclamide | 0.448**±0.02 (-24.06) | 0.372**±0.03 (-9.76) | 0.482**±0.04 (-23.80) | 0.481**±0.04 (-9.24) |

Values are mean ± SD of six individual rats in each group. Values in the parenthesis are % change from that of the control. *P < 0.01 as compared with the control group, **P <0.01 as compared with the diabetic control group.

In the present study, treatment of diabetic rats with plant extracts of TriFG reduced the TBARS, a marker used to monitor LPs levels in the brain of rats. These results are consistent with the results of Kumar et al. (2011). The mechanism, by which Trigonella exerts its effects, is still not clear; however, it is well recognized that Trigonella also has antioxidant properties (Genet et al., 2002). It has been reported that the reducing power of bioactive compounds is associated with antioxidant activity.

Thus, the antioxidant properties of Trigonella can be attributed to its phenolic content. Moreover, the chemical composition of Trigonella indicates the presence of phenolic compounds such as tannins and flavonoids, which are known to possess antioxidant properties (Prieto et al., 1999). Therefore, the high phenolic and flavonoid contents in the Trigonella might be responsible for decreased LPs content in the brain tissue homogenate. Piecing these data, it can be concluded that a decrease in the intrinsic defense system induced generation of free radicals as evidenced by high LPs content; and on the other hand, oral treatment of Trigonella significantly enhanced antioxidant system thereby neutralized free radical generation in the brain tissue of alloxan-induced diabetic rats.

SDH is a vital enzyme of citric acid cycle that catalyzes the reversible oxidation of succinate to fumarate. In the present study, SDH activity is significantly decreased in diabetic rats, and it clearly indicates that the energy production through aerobic oxidation is decreased in diabetic rats. Similar to our studies, Shannugam et al. (2011) studies decreased the SDH activity in STZ-induced diabetic rats. Increased SDH activity in hepatic and renal tissues of Aloe vera extract-treated diabetic rats indicates that increased mitochondrial oxidative potential, and utilization of carbohydrates and fats, may be due to some compounds like flavonoids, carotenoids, and tannins present in the Aloe vera. In the present study, increased LDH activity in brain of diabetic rats indicates that the conversion of pyruvate to lactate increased due to less insulin availability in diabetes (El-Demerdash, 2005). In Trigonella extract treated diabetic rats LDH activity was increased by the antidiabetic compounds present in the Trigonella. In the present investigation, GDH activity significantly decreased in brain tissue of STZ-induced diabetic rats suggesting that affected the regulation of ammonia levels, also impairment of glutamate causes decrease the GDH activity (Diao et al., 2006). However, in Trigonella extract treated diabetic rats GDH activity significantly increased due to the elevation of glutamate content in the cells. G-6-PDH is an important enzyme in hexose monophosphate shunt, it is not only an alternate pathway for glucose oxidation but also produces pentose sugars and reduced NADP, which are much needed for the synthesis of nucleic acids, fatty acids, and amino acid (Xu et al., 2005). The G-6-PDH activity was decreased which slows down the pentose phosphate pathway in diabetic conditions (Abdel-Rahim et al., 1992). In our study, administration of Trigonella extract significantly increased the activity of G-6-PDH in diabetic rats when compared with the control group rats of brain oxidative status.

**Conclusions**

In diabetic rats brain the decreased antioxidant and increased oxidative stress observed. In TriFG diabetic treated rats antioxidants like SOD, CAT, GPx, GST and GSH was significantly improved. Oxidative enzymes like SDH, GDH, and G-6-PDH as significant decrease observed in alloxan-induced diabetic rats. The reversed action of Trigonella by the oral administration of TriFG seeds extract maintained and near to normal levels both in antioxidant and oxidative enzymes. The levels of TBARS and hydroperoxides were significantly increased in diabetic rats when compared to the normal and TriFG-treated rats. Oral administration of TriFG to diabetic rats significantly decreased the levels of lipid peroxidative markers and increased oxidative stress. The effects produced by TriFG were more significant than glibenclamide. The above observations show that the aqueous extract of TriFG possesses antioxidant and oxidative property, which could exert a beneficial role against pathological alterations caused by the presence of free radicals in diabetes. Moreover, the molecular-level investigation and phytochemical investigations may need to find the beneficial activities of plant extracts of TriFG is further needed. Our future studies to give more information to find the bioactive compounds which influences the antioxidant and antidiabetic properties of TriFG.

**Conflict of interest statement.** The authors declare that they have no competing interests.

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