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Antioxidant Activity of *Gymnema sylvestre* Leaf Extract and Glibenclamide in Alloxan Treated Albino Rats

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Abstract: Diabetes represents a state of increased oxidative stress, which is mainly based on evidence of increased lipid peroxidation or indirect evidence of reduced antioxidant enzymes in animal models. The aim of this study was to evaluate the potential activity of the aqueous extract of *Gymnema sylvestre* leaf extract at a dose of 300 mg/kg b wt on lipid peroxidation and antioxidant enzymes in control and alloxan-induced diabetic rats, and to compare it with the standard hypoglycemic drug, glibenclamide (3 mg/kg b wt). *Gymnema sylvestre* (GS) and glibenclamide (GBC) were administered orally for 21 days, and the effects on lipid peroxidation markers, including thiobarbituric acid reactive substances (TBARS) and antioxidant enzymes such as glutathione-S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) activities in the blood and kidney were studied. The experimental findings suggested that *Gymnema sylvestre* considerably recovered the lipid peroxidation (TBARS) indicators to normal and increased antioxidant levels in the blood and kidney, thus confirming that the aqueous extract of *Gymnema sylvestre* leaves helps protect against oxidative stress and its complications. Reference drugs also showed a significant increase in the levels of GST, GPx, SOD, and CAT and decreased lipid peroxidation in the blood and kidney. GBC was found to be more effective in reducing oxidative stress.

Keywords: Alloxan, Antioxidant enzymes, *Gymnema sylvestre*, Lipid peroxidation, Oxidative stress, Glibenclamide

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Introduction

One of the most significant causes of oxidative stress, which underpins the main problems in diabetes mellitus (DM) patients, is hyperglycemia. Alloxan, a hazardous drug that primarily kills pancreatic beta cells and causes type 1 diabetes, was actually used in this investigation to induce

diabetes. Hyperglycemia has been shown to promote the generation of an excessive amount of reactive oxygen species (ROS) due to the high rate of glucose oxidation in mitochondria (Bhatti *et al.*, 2022). Free radicals and ROS are produced as a result of numerous processes. Smoking, canned

foods or fried foods, anxiety, and changes in lifestyle are the main causes (Kaur *et al.*, 2019).

The reactive oxygen species (ROS) and reactive nitrogen species (RNS) are harmful to the organisms. At lower doses they participate in a variety of biological functions, including oxidant control, mitogenic reactions, cytoplasmic signaling processes, and immune system functioning. whereas at an elevated range, these reactive species cause oxidative stress (Phaniendra *et al.*, 2015). High levels of ROS and inhibition of the antioxidant defence system may accelerate the development of insulin resistance, oxidative injury, lipid peroxidation, and cell damage. To lessen or avoid ROS-directed oxidative damage, the human body has evolved an antioxidant defence mechanism that uses free radical scavenging, metal chelating, and enzymatic activities to neutralise the reactive species as soon as they have formed. Antioxidants often protect tissues from oxidative harm.

Antioxidants can lower the amount of reactive species in the biological system by either limiting the process of activity of free radical-producing enzymes or by increasing the expression as well as their activity of antioxidant enzymes such as glutathione s-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) (Aziz *et al.*, 2019).

Currently, a variety of pharmaceutical treatments are used to treat diabetes-related oxidative stress. However, there are a number of side effects and repercussions associated with these drugs that may be managed with exercise, a good diet, and natural therapies.

Patients with diabetes have become increasingly dependent on plant-based therapies in recent years. Due to its relative availability, perceived effectiveness, low cost, and lack of negative side effects, it has been estimated that almost two-thirds of individuals rely on one type of plant-based medication for their treatment. Additionally, this is connected to their active constituents, which, in addition to their

antioxidant potential, have certain distinguishing qualities, including the ability to regenerate pancreas cells, produce insulin, and combat the issue of insulin resistance. One of these plants is *Gymnema sylvestre* (GS), which is a member of the Asclepiadaceae family.

GS is an Indian native plant species that has long been used to treat and control diabetes. Its leaves, known as "Gurmar" in India, are widely renowned for their ability to stifle the sweet taste (Thakur *et al.*, 2012). Its name literally refers to "sugar destroyer", which explains its ability to diminish hunger in general and cravings for sweets in particular, or the experience of sweetness may even be reduced or eliminated (as a result of chewing leaves, which impairs the capacity to recognise the sweet taste). In cases of glycosuria and other urinary illnesses. Gymnemic acid, which anaesthetizes the taste receptors that perceive sweetness and lessens the desire for sweet meals, is the cause of this effect. The leaves are used to cure coughs and fevers. The powdered leaf is used as a weight reduction aid in food additives because it is thought to help reduce cholesterol levels and help with sugar cravings.

The antidiabetic activity of GS is due to the presence of gymnemic acid in its leaves. GS is widely recognised for its antidiabetic effect and is thought to have several special properties, such as stimulating the pancreas' production of the hormone insulin, regenerating beta cells, and preventing the absorption of glucose (Sharma *et al.*, 2014). Additionally, this plant's leaves include cardiac glycosides, anthraquinones, saponins, and other compounds (Patel, 2017). Furthermore, tannin, quinones, flavonoids, and phenols were shown to be present in this plant (Senthilkumar, 2015).

Glibenclamide (GBC) is an oral antihyperglycemic drug from the sulphonylurea family of diabetic medications, largely as a dietary supplement to reduce blood glucose levels in DM patients. It works by stimulating a pancreatic beta cell to produce insulin. The most frequent adverse effect of glibenclamide therapy is hypoglycemia,

which is also associated with an increased risk of cardiovascular illnesses. It is evident that the availability of hypoglycemic medications used for problems caused by diabetes is one of the major causes of death.

The intention of the current study was to establish the individual efficacy of the administration of GS and GBC to alloxan-induced diabetic rats. Although the hypoglycemic effects of these two medications (GS and GBC) have been documented in several studies, this research primarily aimed to understand the effects of antioxidants (oxidative stress) on the blood and kidneys of diabetic and treated (GS and GBC) rats.

Materials and Methods

Chemicals and reagents:

Alloxan monohydrate was purchased from Nyniappa Naicken Street, Chennai Chemicals, Chennai, India. Alloxan monohydrate was freshly dissolved in 0.9% saline before being given intraperitoneally (IP). The biochemical kits and all other materials were of analytical quality.

Collection and preparation of GS leaf extract:

The GS leaves were collected locally. GS leaves were cleaned meticulously. The leaves were dried in shade. An electric blender was used to make air-dried leaves into a fine powder, which was then sieved. A flask containing around 30 g of leaf powder was filled with 360 ml of distilled water and left in a sterile environment for 24 h. The liquid extract was filtered, and then it was kept in a water bath at 80 to 90°C until it was totally dried out. For the subsequent study, the dried powder was collected and stored at 4°C (Desai *et al.*, 2019).

Acute Toxicity:

According to Organization for Economic Co-operation and Development (OECD) guideline 425, five healthy adult rats were randomly selected for this study. The four-hour-fasted rats were fed the herbal extract of aqueous GS leaf extract (300 mg/kg b wt). Initially, a test dose was administered to one animal. Test doses of 300,

1000, 1500, and 2000 were gradually administered to additional animals after the animal had endured survival for 48 h. In order to determine whether there were any physical indicators of toxic exposure, rats were examined for any change in fur loss, breathing pattern, trembling hands, convulsions, odd faeces (diarrhoea), weariness, severe discomfort, stress, and illness. The animals were then observed for the following 14 days (Gopinathan and Naveenraj, 2014).

Experimental animals:

We obtained Wistar albino rats (b wt 200-240 g) from the Mass Biotech Animal Unit in Pulipakkam, Chengalpet. Experimental rats were housed in polypropylene rat cages that were maintained in sterile environments with a 12 h light/dark cycle, ambient temperatures between 22°C and 24°C, and humidity between 30 and 70%. Each animal received standard laboratory food made up of 70% carbohydrates, 25% proteins, and 5% fats, as well as an endless supply of water. Prior to the experiment, the animals had a seven-day acclimatization period in a familiar environment. The Institutional Animal Ethics Committee (IAEC) granted permission for the experimental technique (Ethical clearance number: CPCSEA Regn. No.: 2084/PO/RcBt/S/19/CPCSEA).

Induction of Diabetes:

Alloxan monohydrate was used to elicit the development of diabetes mellitus in Wistar albino rats. The other three groups were alloxan-induced, with the control group being the exception. Alloxan was given to all three groups except the control group. Alloxan monohydrate, 150 mg/kg b wt, diluted in 0.9% normal saline, was injected intraperitoneally to induce diabetes in overnight-starved rats. This diabetes experiment was conducted according to the methods of Etuk *et al.* (2023), Mahajan *et al.* (2018) and Preetha *et al.* (2013). The animals were given a 5% glucose solution to drink right away to stop temporary hypoglycemia (Shah and Khan, 2014). After 2 days (48 h), the identification of body weight loss,

polyuria, glycosuria, polydipsia, and polyphagia in diabetic animals served as confirmation. Treatment with GS leaf extracts and GBC was started 48 h after an injection with alloxan. The treatment began on the third day following the alloxan injection, which is considered the first day of treatment. It lasted for 21 days.

Experimental Design:

Rats were randomly divided into four groups (each group consisting of six rats) and treated as follow:

Group 1: The rats of this group received no treatment and employed as control.

Group 2: A single intraperitoneal injection of alloxan monohydrate at 150 mg/kg of b wt was given.

Group 3: Alloxan-induced diabetic rats received 300 mg/kg b wt of GS leaf extract orally for 21 days.

Group 4: Alloxan-induced diabetic rats received 3 mg/kg b wt of GBC (Kanthlal *et al.*, 2014) orally for 21 days.

Collection of blood and tissue sampling:

Rats were kept under deep ether anaesthesia on the 22nd day, and blood was collected via the retroorbital plexus (Ramachandran *et al.*, 2012). Animals were killed, and the kidneys were removed. The samples thus collected were used to test antioxidant enzymes and lipid peroxidation (TBARS).

Evaluation of TBARS and antioxidant enzyme activity in the blood and kidney:

The state of lipid peroxidation was analyzed by calculating TBARS based on the procedure of Ohkawa *et al.* (1979). Glutathione s-transferase (GST) activity was examined by the technique of Habig *et al.* (1974). Glutathione peroxidase (GPx) was determined by the process of Rotruck *et al.* (1973). Superoxide dismutase (SOD) activity was evaluated using the method of Beauchamp and Fridovich (1973). The activity of catalase (CAT)

was assessed via the technique of Chance and Maehly (1955).

Statistical analysis:

The results are presented as mean $\pm$ standard deviation (SD). Differences between means were calculated using a student t-test followed by one-way analysis of variance (ANOVA). Differences between groups at $p < 0.05$ were considered statistically significant.

Results

The acute toxicity investigation conducted in this study demonstrated that *Gymnema sylvestre* leaf extract is non-toxic.

The study's results, such as lipid peroxidation (TBARS) antioxidant enzyme levels in the blood and kidneys of the control group, diabetic group, and treated groups with GS leaf extract, were compared to GBC medication.

Evaluation of lipid peroxidation in the blood and kidney:

Alloxan-induced diabetic animals had significantly ($p < 0.01$) higher levels of TBARS in blood and kidney tissues, showing the oxidative destruction of fats. Repeated administration of GS leaf extract treatment for 21 consecutive days led to a significant decrease in oxidative stress in the blood and kidneys of diabetic rats, as depicted by the reduced lipid peroxidation level ($p < 0.01$). GBC administration has also shown significant ($p < 0.01$) decline in lipid peroxidation as compared to alloxan-induced rats in both blood and kidney tissues (Tables 1, 2).

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

When compared to the control group, the diabetic group's TBARS percentage changes in the blood and kidney increased to 259% and 212%, respectively. In contrast to the diabetic group, the TBARS level in the blood and kidneys was lowered by 80%, 72%, and 74% and 69%, respectively, after treatment with GS extract and GBC (Fig. 1).

Table 1: Effect of GS leaves extract and GBC on GST, GPx, SOD, CAT, and TBARS levels in blood

S. No.	PARAMETERS	GROUP 1 (control group)	GROUP 2 (Diabetic group)	GROUP 3 (GS treated group)	GROUP 4 (GBC treated group)
1	GST	0.35± 0.105	0.088±0.044**	0.328±0.107##	0.355±0.103##
2	GPx	0.433±0.139	0.066±0.027**	0.438±0.126##	0.431±0.117##
3	SOD	0.338±0.070	0.146±0.025**	0.31±0.067##	0.341±0.082##
4	CAT	0.815 ±0.135	0.451±0.057**	0.818±0.149##	0.82±0.131##
5	TBARS	0.063 ±0.021	0.226 ±0.099**	0.046±0.020##	0.058±0.021##

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 compared to control group and ##p < 0.01 when compared to diabetic group. GST: μ mol CDNB /min/mg; GPx - μ mol/min/mg; SOD: μ mol/mg/min; CAT: μ mol H₂O₂/min/mg; TBARS: μ mole/mg.

Table 2: Effect of GS leaves extract and GBC drug on GST, GPx, SOD, CAT, and TBARS levels in kidney

S. No.	PARAMETERS	GROUP 1 (control group)	GROUP 2 (Diabetic group)	GROUP 3 (GS treated group)	GROUP 4 (GBC treated group)
1	GST	0.248± 0.094	0.038± 0.014 **	0.201±0.100 #	0.218±0.092 ##
2	GPx	0.528±0.081	0.061±0.021 **	0.486±0.081 ##	0.511±0.080 ##
3	SOD	0.363±0.076	0.041±0.023 **	0.335±0.0781##	0.358±0.079 ##
4	CAT	0.65 ± 0.04	0.345±0.168 **	0.60±0.037 ##	0.631±0.038 ##
5	TBARS	0.086±0.023	0.268±0.120 **	0.068±0.026 ##	0.083±0.021 ##

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 compared to control group and #p< 0.05, ##p<0.01 when compared to diabetic group. GST: μ mol CDNB/min/mg; GPx - μ mol/min/mg; SOD: μ mol/mg/min; CAT: μ mol H₂O₂/min/mg; TBARS: μ mol/mg.

Analysis of antioxidant enzymes in blood:

Alloxan-induced diabetes caused a significant downregulation of antioxidant enzyme activities (p < 0.01) in blood and kidney. Treating the rats with GS leaf extract caused a significant increase (p < 0.01) in the antioxidant enzymes GST, GPx, SOD, and CAT in comparison with the diabetic group. GBC-administered group rats also showed (p < 0.01) an increased level of all the enzymatic activities with that of the diabetic group (Table 1).

Percentage change of antioxidant enzymes in blood:

The percentage changes of diabetic group GST, GPx, SOD, and CAT in blood were reduced to 75%, 85%, 57%, and 45%, respectively, when compared to the control group. The percentage change in GS leaf extract-treated groups of GST, GPx, SOD, and CAT was increased to 273%, 564%, 112%, and 81%, and GBC-treated groups also increased 303%, 553%, 134%, and 82%, respectively, in the blood compared to the diabetic group (Fig. 2).

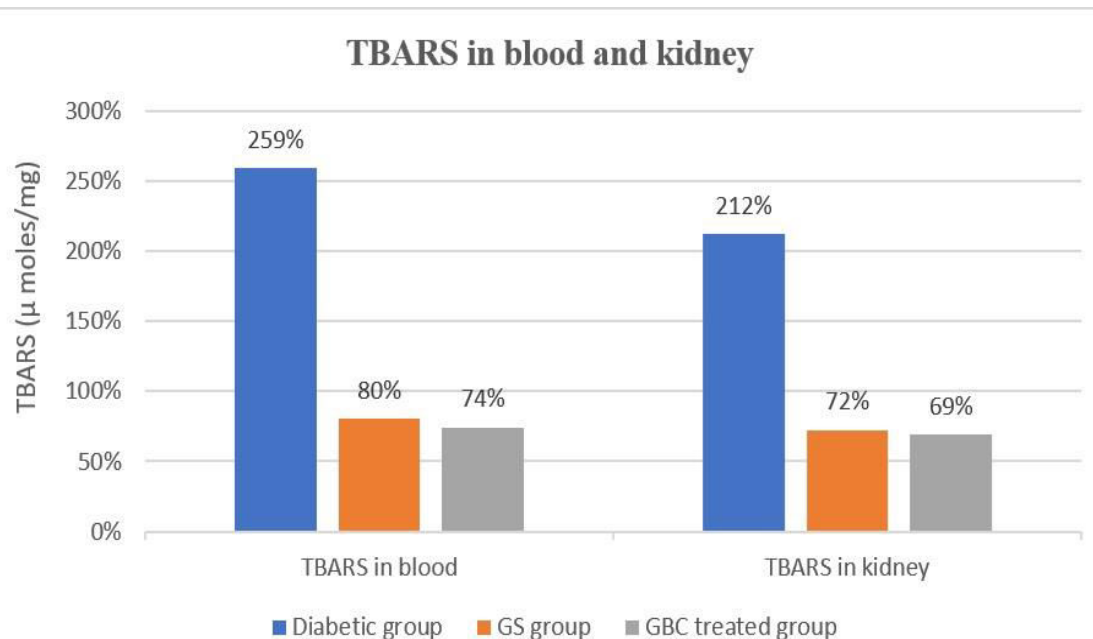


Fig. 1: Percentage change of TBARS in blood and kidney.

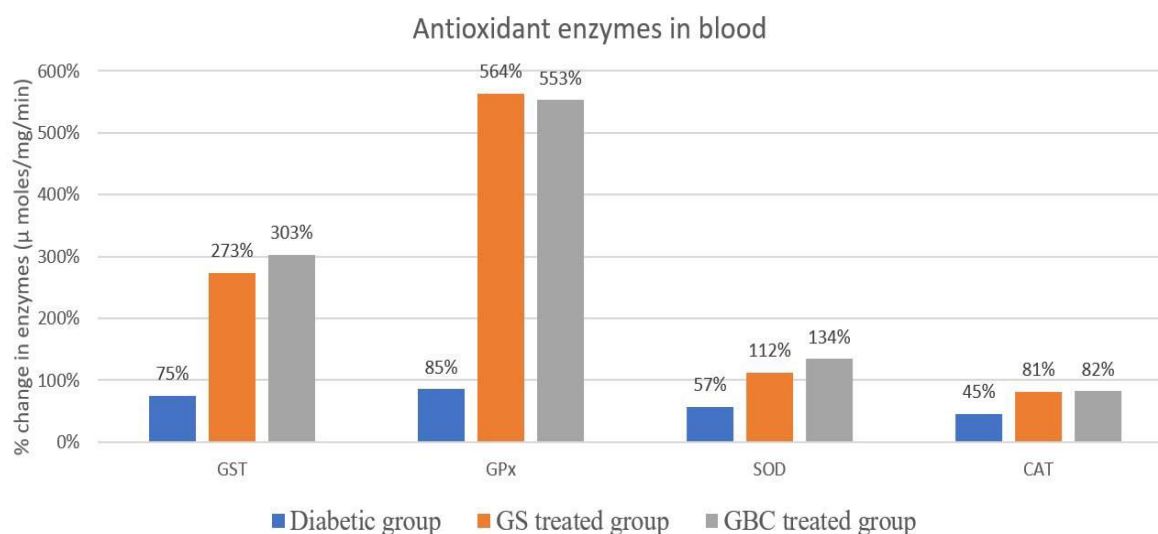


Fig. 2: Percentage change of enzymes in blood.

Analysis of antioxidant enzyme activities in kidney tissues:

There was a significant ($p < 0.01$) decrease in enzymes such as GST, GPx, SOD, and CAT levels in the diabetic group when compared to the control group. After administration of GS extract for 21 days, the enzymes GST ($P < 0.05$), GPx, SOD, and

CAT were increased, ($p < 0.01$), and GBC was significantly increased ($p < 0.01$) near normal compared to the diabetic group (Table 2).

Percentage change of antioxidant enzymes in kidney:

In the kidney, the percentage change of the diabetes groups GST, GPx, SOD, and CAT was

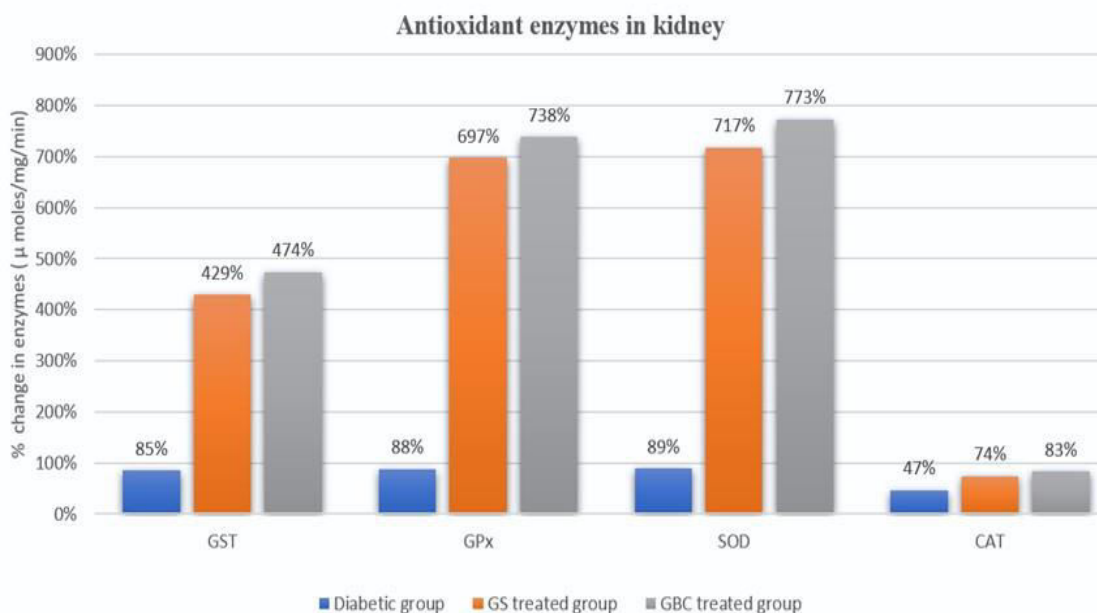


Fig. 3: Percentage change of enzymes in kidney.

lowered to 85%, 88%, 89%, and 47%, respectively. Compared to the control group, treatment with GS leaf extract increased the percentage of enzymes such as GST, GPx, SOD, and CAT by 429%, 697%, 717%, and 74%, respectively. In addition, treatment with GBC increased the percentage of enzymes in the kidney by 474%, 738%, 773%, and 83%, respectively, in comparison to the diabetic group (Fig. 3).

Discussion

In accordance with several studies, oxidative stress contributes to the excess production of free radicals and the depletion of free radical scavengers in experimental diabetes (Kawser Hossain *et al.*, 2016). Alloxan, a drug that is administered intraperitoneally at a specific dosage, may successfully cause hyperglycemia in experimental animals (Macdonald Ighodaro *et al.*, 2017).

The apparent symptoms of this medication, such as polyphagia, polydipsia, and polyuria with weight loss, can confirm that it has a diabetogenic effect, according to a study that found that alloxan suppressed insulin production via molecular pathways. Alloxan is a source of free radicals that

may also induce DNA damage and the consequent degeneration of beta cells once it is administered to animals (Orsolic *et al.*, 2021).

TBARS is a significant biomarker of oxidative stress and can be easily measured. The current investigation confirmed that the diabetic group who were not receiving treatment had elevated TBARS activity in the blood and kidneys. This statement is supported by studies of Sekiou *et al.* (2021). An increase in TBARS might be a sign of oxidative damage. Many studies have also shown that lipid peroxidation increases during the progression of the disease. Bandeira *et al.* (2013) revealed that the LPO status of target tissues is also related to the long-term consequences of hyperglycemia. The present study investigated both the blood and kidney LPO status by measuring TBARS levels. Vodošek Hojs *et al.* (2020) reported that increased TBARS levels in tissues are an indication of oxidative stress in degenerative illnesses such as diabetes mellitus and chronic renal disorders. Additionally, Betonico *et al.* (2016) highlighted in their study that diabetes mellitus is a primary factor in chronic renal disease. In the study conducted by Makni *et al.* (2011), lipid peroxidation significantly

increased in diabetic rats. Cells and tissues of the pancreas, kidney, and liver are destroyed by free radicals produced during the metabolism of alloxan. The kidneys become aberrant as a result of a specific level of injury. In comparison to the diabetic group, rats treated with GS leaf extract and GBC had lower blood and renal TBARS. This result coincided with the findings of Ramachandran *et al.* (2012) and Orumwense *et al.* (2022). Glibenclamide (GBC) has some protective effect against oxidative damage in hyperglycemic conditions. Similar to this, in research conducted by Ananthan *et al.* (2004), *Gymnema monantum* leaf extract was given to rats for 30 days, and the kidney were examined for the extent of decreased lipid peroxidation and an increase in antioxidant enzymes. According to a report by Pandarekandy *et al.* (2017), glibenclamide therapy significantly lowers blood sugar levels while simultaneously increasing oxidative stress. According to Kang *et al.* (2012), diabetic rats given the GS extract had lower lipid peroxidation levels, with serum levels falling by 31.7% and kidney levels falling by 9.1%. According to Kumar *et al.* (2017), when the diabetic rats were given GS treatment, there was a decrease in MDA in the plasma.

The biological system defends itself against oxidative stress by free radical-scavenging enzymes such as GST, GPx, SOD, and CAT (Jena *et al.*, 2023). The outcomes of this study point out that alloxan effectively causes oxidative stress in diabetic rats, as evidenced by the significantly lower levels of GST, GPx, SOD, and CAT in these groups. This study is in agreement with Umar *et al.* (2018). The levels of the antioxidant enzymes GST, GPx, SOD, and CAT in the diabetic rats' blood and kidneys improved when the rats were administered with 300 mg/kg of GS leaf extracts on a daily basis for 21 days as compared to the control group. This is brought on by the fact that the *Gymnema sylvestre* extract contains a number of secondary metabolites. These metabolites, which include oleanane saponins and dammarane saponins, contribute to enhancing the antioxidant impact (Laha and Paul, 2019). GS leaf extract significantly suggests the antioxidant potential of

attenuating oxidative stress associated with diabetes mellitus, which was likely mediated by increasing glutathione status in the tissues and free radical scavenging activities (Gopinathan and Naveenraj, 2014). The body's defense mechanism against oxidative damage caused by free radicals is comprised of antioxidant enzymes, which safeguard the cell membrane and other cellular components (Vona *et al.*, 2021). When diabetic rats are provided with GBC 3 mg/kg b wt for 21 days, it also raises the levels of antioxidant enzymes in blood and kidney.

Likewise, consistent with our observations, diabetic rats given GBC (3 mg/kg b.wt.) had substantial antioxidant enzyme restoration and a reduction in TBARS. This concept was also supported by Ibrahim *et al.* (2020). In the present investigation, GBC had a notable antioxidant impact on hyperglycemic rats. Similarly, a study conducted by Maruthupandian and Mohan (2011) stated that glibenclamide decreases the level of TBARS and increases the level of antioxidant enzymes.

Conclusion

Diabetes is a condition with an altered antioxidant status that may be causing increased lipid peroxidation in different organs. Therefore, medicines for diabetic therapy should not only focus on hypoglycemia alone but also be considerate of other pharmacological aspects for the overall management of diabetes, like reduced lipid peroxidative effects and restoration of antioxidant levels. Treatment with GS leaf extract also showed improvement in treating oxidative stress in alloxan-induced diabetics. Glibenclamide possesses hypoglycemic and antioxidant effects. The oral hypoglycemic drug GBC combats oxidative damage in diabetics. It could be observed in the TBARS activity of the renal tissues and serum of alloxan-induced groups. GBC's antioxidant effects are higher than those of GS extract.

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