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Research Article

# COMBINED EFFECTS *GYMNEMA SYLVESTRE* AND GLIBENCLAMIDE ON ALLOXAN INDUCED DIABETIC MICE

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**ABSTRACT**

The present investigation was designed to investigate the combined effects of *Gymnema sylvestre* (GS) and glibenclamide (GLB) in the regulation of alloxan induced diabetes mellitus and tissue lipid peroxidation in mice. Ethanolic extract of GS (600 mg/kg body weight) and GLB (500 µg/kg body weight) were treated either alone or in combination for 15 days in alloxan (200 mg/kg body weight) induced swiss albino mice, following which alterations in glucose, total cholesterol (TC), triglyceride (TG) and serum glutamate oxaloacetate transaminase (SGOT) levels as well as lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) in liver, kidney, heart, muscle, testis and brain tissues were examined. Administration of alloxan increased LPO, glucose level and other serum parameters significantly which were reversed by the administration of GS or GLB alone or their combination. Test drugs also showed a significant increase in the levels of SOD, CAT and GSH in all the tissues which were declined by alloxan treatment. However, when both the drugs were administered simultaneously, the beneficial effects were more pronounced as compared to their individual effects. Therefore, our findings suggest a supplementation of GS extract along with glibenclamide for better diabetic control.

**Keywords:** *Gymnema sylvestre*, Glibenclamide, Mice, Antioxidant, Combined effects.

## INTRODUCTION

Diabetes mellitus, a well known metabolic disorder is characterized by high blood sugar level and impaired metabolism of carbohydrate, protein and fat.[1] It is also well understood that oxidative stress plays a major role in the pathogenesis of diabetes mellitus (DM) and its associated complications. Mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, but the main factors involved are activation of transcription factors, advanced glycation end products and protein kinase C.[2]

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) or other free radicals. ROS are small highly reactive molecules which are constantly formed in the human body. They are produced in the mitochondria due to incomplete reduction of molecular oxygen in electron transport chain.[3] In healthy physiological condition, normally equilibrium is maintained between oxidants that are generated inside the body and their detoxification by the antioxidant defense system of the cells.[4] However, excess production of ROS leads to the impairment of equilibrium between pro-oxidants and antioxidant systems.

Presently, the conventional treatment of diabetes mellitus includes use of insulin, metformin and glibenclamide. Despite the availability of these known antidiabetic medicines in the pharmaceutical market, drugs from medicinal plants have been used with success to treat diabetes.[5] Allopathic medicines are found to cause various side effects especially the development of resistance after a certain period of time.[6] In fact, many traditional plants are used throughout the world for the treatment of DM because they are considered to be less toxic and free from side effects.

*Gymnema sylvestre* (GS) is one such medicinal plant that belongs to Asclepiadaceae family. It is a slow growing perennial, medicinal woody climber found in central and southern India and tropical Africa. It is commonly used as an antihyperglycemic and antioxidative drug. In addition, it possesses antimicrobial, antihypercholesterolemic and hepatoprotective activities. The leaves of GS contain gymnemic acid which is responsible for its antidiabetic acitivity. GS is well known for its antidiabetic activity and is believed to have some unique features like stimulation of insulin secretion from pancreas, regeneration of β cells and prevention of the absorption of glucose.[7,8]

Glibenclamide (GLB) is an oral antihyperglycemic agent belonging to sulphonylurea class of antidiabetic medicine, primarily as an adjunct in diet to lower blood glucose level in patients with DM. It acts by stimulating β cell of the pancreas to release insulin. It also acts by increasing peripheral glucose utilization and decreasing hepatic gluconeogenesis. In fact, reports are already available on its use against alloxan induced diabetic mice.[9,10]

We presumed that a combination of a conventional drug and a herbal preparation might prove to be more effective. Therefore, the aim of the present investigation was to work out the efficacy of combined administration of GS and GLB on alloxan induced diabetic mice. Although, both these drugs (GS and GLB) are well known for their hypoglycemic activity, till date, nothing was known on their synergistic effects, if any. The present investigation is an attempt on this direction.

## MATERIALS AND METHODS

### Material

The test drug glibenclamide (Aventis, India) was obtained from a registered local pharmacy shop, sodium dodecyl sulphate (SDS), Ellman's reagent, tricholoro acetic acid (TCA), tris buffer were purchased from E merck Ltd, Mumbai, India, while thiobarbituric acid (TBA), ethylene diamine tetra acetic acid (EDTA) and meta-phosphoric acid were purchased from Hi-media, Mumbai.

### Plant material

The dried leaf powder of GS (5gm) was soaked in 200 ml of 70% ethanol and was allowed to stand for 24 h and then filtered. It was then evaporated at 37ºC. The dried powder so obtained was stored at -4ºC until use.

### Experimental Animals

Swiss albino mice, weighing 30±2 g were used. They were housed in polypropylene cages in a standard photoperiod (14 h light: 10h dark) and temperature (27±1ºC) controlled room with the provision of laboratory feed (Gold Mahur feed, Hindustan Lever Limited, Mumbai, India) and water ad libitum. Standard ethical guidelines of the Committee for the Purpose of Control and Supervision on Experiments in Animals (CPCSEA), Ministry of Environment and Forest, Government of India, were followed. The approval of the departmental ethical committee for handling and maintenance for experimental animals was also obtained before starting the investigation. Thirty five adult healthy male mice were divided into following 5 groups of seven each.

**Group 1:** Received distilled water (0.1ml DDW) and served as control.

**Group 2:** Injected (IP) with single dose of alloxan at 200 mg/kg body weight[11] served as diabetic control.

**Group 3:** Injected with alloxan and given GLB orally at 500 µg/kg body weight[12] for 15 days.

**Group 4:** Injected with alloxan and given GS orally at 600 mg/kg body weight[13] for 15 days.

**Group 5:** Injected with alloxan and given GLB along with GS at same doses for 15 days.

The extract, drug and vehicle were administered between 11.00 to 12.00 hr of the day to avoid circadian variation, if any. On 16$^{th}$ day, all overnight fasted animals were sacrificed by cervical dislocation. Blood from each animal was collected and serum was isolated for the estimation of glucose, triglyceride and total cholesterol. Liver, kidney, heart, muscle, brain and testis were excised and processed for the estimation of different biochemical parameters.

**Parameters**

For the estimation of lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and protein content, particular tissue was homogenized in 10% (w/v) ice cold phosphate buffered saline (PBS) (pH 7.4, 0.1 M/L), centrifuged at 15,000g for 30 min and supernatant was processed for different assays as done before.[14]

Serum glucose was measured by glucose oxidase / peroxidase method based on the protocol of Trinder.[15] For measuring the amount of serum total cholesterol (TC), triglyceride (TG) and serum glutamate oxaloacetate transaminase (SGOT), different commercial kits were used.

LPO was determined by the method of Ohkawa et al,[16] SOD by using the protocol of Marklund and Marklund,[17] catalase activity by the method of Aebi,[18] and estimation of GSH content was done by the protocol of Ellman.[19]

**Statistical analysis**

Data are expressed as mean ± SE. Statistical evaluation of the data was made using analysis of variance (ANOVA), followed by student's t-test. *P* values of 5 % and less were considered to be significant.

**RESULT**

Alloxan administration brought a significant increase in liver LPO ($p< 0.001$) with a concomitant decrease in SOD, CAT and GSH ($p< 0.001$for all). By the administration of the test drugs individually the effects exerted by alloxan were reversed. Following the co-administration of both the drugs the toxic effects were also reversed significantly ($p< 0.001$ for all the indices), but with higher percent change as compared to the effects of individual drug treatment. The percent change of the combined treatment as compared to alloxan treated animals were 57% decrease for LPO and increase of 232%, 79% and 180% for SOD, CAT and GSH respectively.

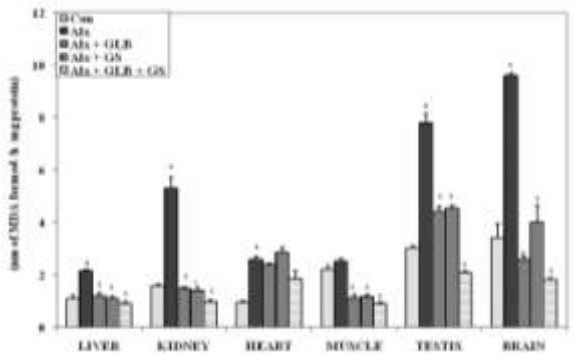


**Fig. 1: *G. sylvestre* (GS) leaf extract and glibenclamide (GLB) in alloxan-induced diabetic mice on LPO (nM MDA h$^{-1}$ mg protein $^{-1}$) in liver, kidney, heart, muscle, testis and brain.**

Data are in mean ± SEM (n = 7). [a], $p< 0.001$, as compared to the respective control values. [x], $p< 0.001$, as compared to the respective values in alloxan treated group.

For all other tissues also similar pattern was found, because by the co-administration of both the drugs the percent improvement was much better as compared to the individual treatments. The percent changes for LPO, SOD, CAT and GSH in kidney were 82↓%, 393↑%, 119↑% & 100↑% respectively ; for heart tissue it was 29↓%, 75↑%, 329↑% & 31↑% respectively; for muscle tissue it was 73↓%, 104↑%, 181↑% & 217↑% respectively and for brain tissue it was 81↓%, 132↑%, 174↑% & 225↑% respectively as compared to the effects of alloxan treated animals.

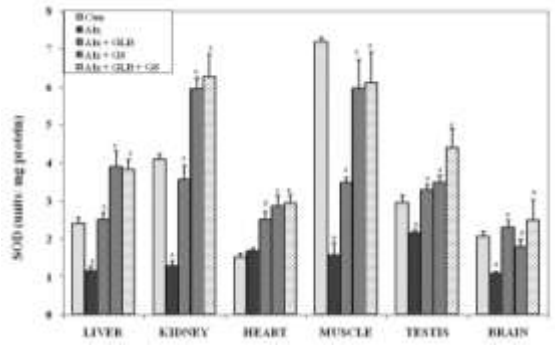


**Fig. 2: *G. sylvestre* (GS) leaf extract and glibenclamide (GLB) in alloxan-induced diabetic mice on SOD (units mg protein $^{-1}$) in liver, kidney, heart, muscle, testis & brain.**

Data are in mean ± SEM (n = 7). [a], $p< 0.001$, [b], $p< 0.01$ as compared to the respective control values. [x], $p< 0.001$, [y], $p< 0.01$ & [z], $p< 0.05$ as compared to the respective values in alloxan treated group.

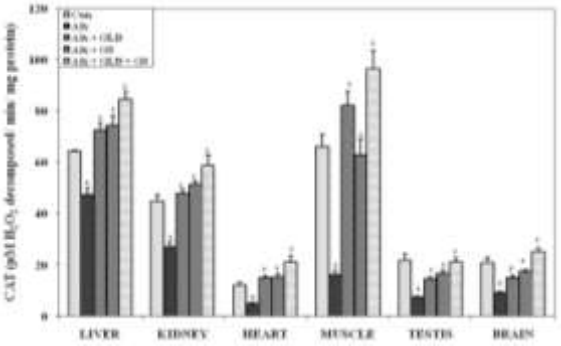


**Fig. 3: *G. sylvestre* (GS) leaf extract and glibenclamide (GLB) in alloxan-induced diabetic mice on CAT (µM $H_2O_2$ decomposed min $^{-1}$mg protein $^{-1}$) in liver, kidney, heart, muscle, testis & brain.**

Data are in mean ± SEM (n = 7). [a], $p< 0.001$, as compared to the respective control values. [x], $p< 0.001$, [y], $p< 0.01$ as compared to the respective values in alloxan treated group.

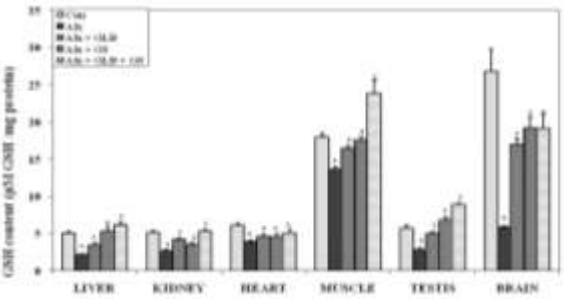


**Fig. 4: *G. sylvestre* (GS) leaf extract and glibenclamide (GLB) in alloxan-induced diabetic mice on GSH (µM GSH mg protein$^{-1}$) liver, kidney, heart, muscle, testis & brain.**

Data are in mean ± SEM (n = 7). [a], p < 0.001, as compared to the respective control values.[x], p< 0.001, [y], p< 0.01 & [z], p< 0.05 as compared to the respective values in alloxan treated group.

**Serum parameters**

A significant increase was noticed in the levels of glucose, triglyceride, total cholesterol and SGOT (p< 0.001) after the administration of alloxan, which was significantly decreased in GLB administered animals with a percent decrease of 33, 40, 38 & 24% for glucose, TC, TG & SGOT respectively. GS also decreased the above mentioned parameters with a percent decrease of 27, 32, 53 & 33% respectively. However, with respect to all the parameters the combined administration of the drugs was found to be more effective with greater percent decrease of 46, 40, 63 & 49% for glucose, TC, TG & SGOT respectively as compared to the respective value of alloxan treated animals.

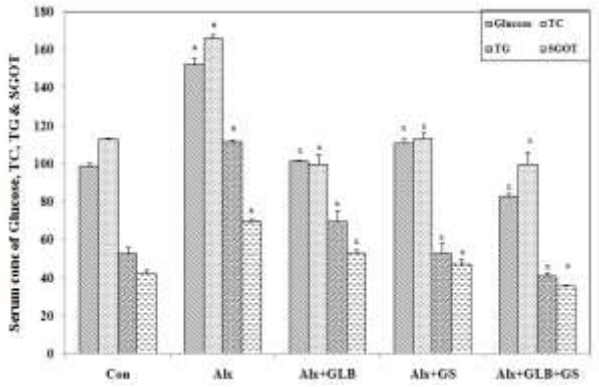


**Fig. 5: *G. sylvestre* (GS) leaf extract and glibenclamide (GLB) in alloxan-induced diabetic mice on serum Glucose (mg/dl), TC (mg/dl), TG (mg/dl) and SGOT (IU/L) concentrations.**

Data are in mean ± SEM (n = 7). [a], p< 0.001, as compared to the respective control values. [x], p<0.001, as compared to the respective values in alloxan treated group.

**DISCUSSION**

Several important observations were made in our study supporting the beneficial effects of combined drug treatment against alloxan induced diabetes mellitus and associated problems.

Our results show that the treatment of alloxan to healthy mice raised the level of serum glucose, triglyceride and total cholesterol levels as well as lipid peroxidation in different test tissues as reported earlier by other workers.[20,21] On the other hand, by the administration of either GS or GLB or by their combined administration, there occurred a significant reduction in all the above mentioned indices. However, the simultaneous administration of both the drugs was found to be more helpful in reducing alloxan induced toxic effect as compared to their individual treatment. This was supported by enhanced cellular antioxidant levels in alloxan + GS + GLB treated animals.

In the present study we have observed the adverse effects of alloxan in different organs like liver, kidney, heart, muscle, testis and brain, similar to the reported deleterious effects on other organs by other workers.[22] We also evaluated the antioxidative activity of the drugs through the evaluation of key antioxidative enzymatic and non enzymatic parameters such as SOD, CAT and GSH, which were declined in the alloxan induced animals, as already reported earlier.[23,24] However, the administration of drugs reversed the effects exerted by alloxan. Interestingly, when both the drugs were administered together the beneficial effects were more pronounced as compared to that of individual drugs.

Oxidative stress is described as impairment of equilibrium between pro-oxidants and antioxidants system. The major cellular mechanism for eliminating the ROS is the availability of more antioxidant enzymes and small antioxidant molecules. The antioxidative enzymes include two forms of superoxide dismutase (SOD): cytosolic (Cu /Zn-SOD; SOD1) and mitochondrial (Mn-SOD; SOD2) that converts $O_2\bullet$- to $H_2O_2$ and catalase (CAT) that degrades $H_2O_2$ to water.[25] Another important enzyme is glutathione reductase (GR) which helps in balancing the redox status of the glutathione system by converting oxidized glutathione (GSSG) to reduced glutathione (GSH).[26] Therefore, in case of alloxan treated diabetic animals the levels of these antioxidative enzymes were found to be low, while on the administration of drugs there was a reverse effect clearly indicating the antioxidative properties of both the test drugs. Interestingly, the combined administration of both the drugs could improve the antioxidative activity of the cell in a better way.

Insulin dependent diabetes mellitus occurs due to autoimmune destruction of insulin producing β cells in the pancreas, resulting in low or no production of insulin, a hormone necessary for survival. The ethanolic extract of GS plays a major role in blood glucose homeostasis through increased serum insulin level by the regeneration of endocrine pancreas. Moreover, the leaves of GS contain gymnemic acid which is known to suppress transport of glucose from the intestine into the blood stream by binding to the receptor present in the intestine. As the structure of gymnemic acid molecule is similar to that of glucose molecule, these molecule bind to the receptor which is located on the taste buds of the tongue, thereby preventing their activation by sugar molecule and suppress the uptake of sugar. Other Possible mechanism for hypoglycemic effect of gymnemic acid from GS leaf could also be the secretion of more insulin from the pancreas, promoting the regeneration of islet cells, increasing glucose utilization by an insulin dependent pathway. Also the involvement of alkaloids, polypeptides and flavonoids present in GS, cannot be ruled out for its antidiabetic and antioxidative activity.[7,27]

GLB is known to work by binding to and activating the sulphonylurea receptor 1, the regulatory subunit of the ATP sensitive potassium channels ($K_{ATP}$) in pancreatic β cells. This inhibition causes cell membrane depolarization and opening voltage gated dependent $Ca^{++}$ channel, resulting in an increase in intracellular calcium in the β cells and subsequent stimulation of insulin release.[28] Besides this, GLB exerts its effect on other organs as well.[29] GLB in some way may also reduce the release of glucose from liver, which in turn is attributed to the blocking of key enzymes concerned either with glycogen to glucose conversion, or in the conversion of non-carbohydrate precursors to glucose through gluconeogenesis.[30] Thus the antihyperglycemic effects of the test plant extract and that of GLB are well established. However, the present findings clearly indicate the synergistic effects of these two drugs. This is because, when both drugs were administered simultaneously the preventive effects of the drugs were augmented leading to a much better control of hyperglycemia and associated peroxidative problems.

**CONCLUSION**

From the present observations it can be concluded that, whatever may be the mode of action, the combination of herbal and conventional test medicines may offer substantially more benefits as compared to their individual treatment in diabetes mellitus. However, it needs further investigation for the therapeutic use in diabetic patients.

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