

# Effect of Glutathione Enriched Polyherbal Formulation on Streptozotocin Induced Diabetic Model by Regulating Oxidative Stress and PKC Pathway

Sheethal S<sup>1</sup>, Ratheesh M<sup>2,\*</sup>, Svenia P Jose<sup>2</sup>, Sandya S<sup>3</sup>

<sup>1</sup>Department of Chemistry, St. Thomas College, Palai, Kottayam, Kerala, INDIA.

<sup>2</sup>Department of Biochemistry, St. Thomas College, Palai, Kottayam, Kerala, INDIA.

<sup>3</sup>Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore, Karnataka, INDIA.

## ABSTRACT

**Background:** Increasing evidence shows that oxidative stress is one of the root causes of metabolic disorders like diabetes. Glucose oxidation and activation of various metabolic pathways lead to a disproportionate generation of free radicals. This will significantly reduce the antioxidant status in the body. **Objectives:** In the present study, we aimed to evaluate the effect of a novel glutathione enriched polyherbal formulation on a streptozotocin induced diabetic model. **Materials and Methods:** Diabetes was induced by a single intraperitoneal injection of streptozotocin. After 3 days of injection, Glibenclamide (5mg/kg), and glutathione enriched polyherbal formulation were given orally for 28 days. Fasting blood glucose and body weight changes were measured at specific intervals. For the study, antioxidant enzymes, lipid peroxidation products, nitrite, liver enzyme markers, gene expression of GLUT-2, and PKC levels were evaluated. Histopathological analysis was also done. **Results:** The result shows that glutathione enriched polyherbal formulation treated rats significantly reduced their blood glucose and maintained their body weight. As a result, the GLUT-2 expression was reduced, which prevented the activation of PKC. Moreover, oxidative stress was reduced by improving antioxidants like SOD, CAT, GPx, and GSH by inhibiting the lipid peroxidation process. In addition, hepatic damage was also prevented by protecting the liver cells, and thereby shielding the excessive leakage of SGOT, SGPT, and ALP enzymes. The histopathological analysis of the liver gives more support to other data. **Conclusion:** Findings show that glutathione-enriched polyherbal formulations have a powerful anti-diabetic effect by inhibiting oxidative stress and thus blocking PKC activation.

**Keywords:** Diabetes, Antioxidants, Oxidative stress, PKC, Glucose transporter.

## Correspondence:

**Dr. Ratheesh M**

Department of Biochemistry, St. Thomas College, Palai, Kottayam-686574, Kerala, INDIA.

Email id: biochemistrystcp@gmail.com

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## INTRODUCTION

Oxygen is considered as the primary driver of life's finite nature but in certain circumstances, this oxygen itself can threaten life. As a normal life process, lots of free radicals like reactive oxygen species (ROS) are produced in the body, and neutralise it with the help of antioxidants.<sup>[1]</sup> The disturbance between the generation of free radicals and its scavenging by antioxidants due to so many different reasons like drug action, toxic reactions, ageing etc can lead to the condition called oxidative stress.<sup>[2]</sup> Highly reactive oxygen species have the ability to damage the cellular constituents and other molecules which may result in the loss of function or structural aberrations. Hence, the oxidative stress could

implicated the pathogenesis of a wide range of diseases, especially, diabetes.<sup>[3]</sup> Diabetes is a lifestyle disorder and all the different types of diabetes are mainly characterised by hyperglycemia with a relative or complete absence of insulin secretion or its action.<sup>[4]</sup>

Maintenance of glucose homeostasis is a vital process in the body. So, when the glucose level increases, it will be transported to the cells via glucose transporters (GLUT). Among the various transporters, GLUT 2 plays a key role and it is primarily expressed in the major organs such as pancreas, kidney, liver, intestine, and central nervous system. Glycolysis is the initial process by which the glucose oxidation takes place. During diabetes, due to the excessive accumulation of glucose in blood give rise to the condition known as hyperglycemia. As a result, there will be an uncontrolled production of free radicals by the mitochondria, resulting in the suppression of the antioxidant defence system in the body. These radicals will damage the DNA, leading to the development of various microvascular, and macrovascular complications of diabetes.<sup>[5,6]</sup> In order to repair the DNA damage,



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the DNA repairing enzyme poly-ADP-ribose polymerase-1 (PARP-1) will be activated, and then it will block the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the glycolytic pathway. Thus, it results in the accumulation of the upstream glycolytic intermediates, and exerts its negative impact by stimulating pro-oxidant pathways.<sup>[7,8]</sup> The raise in the glycolytic intermediate dihydroxyacetone-3-phosphate (DHAP) will be reduced to glycerol-3-phosphate and subsequently undergo the *de novo* synthesis of diacylglycerol (DAG) and activate PKC pathway, one of the major molecular pathways in diabetes. Activation of PKC can affect a series of alterations in the cell signalling that may ultimately contribute to the direct ROS production or indirect activation of other molecular pathways leading to an oxidative stress environment.<sup>[9]</sup>

Due to the high-risk factors involved in diabetes, it has become a global burden and reached epidemic proportion, and the global incidence likely to rise from 424 million in 2017 to 629 million by 2045 global.<sup>[10]</sup> Therefore, there is an urgent need for the development of innovative medicines for diabetes treatment, and management. A different and effective technique for discovering novel therapeutic agents is to conduct scientific research on plants that have been recognised for their medicinal significance. Apart from that the recent research studies shows that the external antioxidant supplementation especially glutathione can inhibit the oxidative stress condition by improving antioxidant level in the body and thereby it can prevent various diabetic complications.<sup>[11]</sup> Therefore, the aim of present study is to delineate changes in oxidative stress, and PKC/GLUT-2 pathway by the treatment of novel glutathione enriched polyherbal formulation in streptozotocin induced diabetic rats.

## MATERIALS AND METHODS

### Chemicals

Streptozotocin, citrate monohydrate, sodium citrate were purchased from Sigma-Aldrich, St. Louis, MO, USA. SGOT, SGPT, ALP were purchased from Agappe Diagnostic Ltd. PKC ELISA Kit was procured from Sigma Aldrich, USA. GLUT- 2 primers were purchased from Eurofins, India. All drugs were stored at the recommended temperature.

### Preparation of Glutathione enriched polyherbal formulation

The polyherbal formulation was prepared by combining Glutathione-100mg, Leaf powder of *Murraya koenigii*- 85mg, Grape seed extract- 50mg, *Capsicum frutescens* powder- 30mg, Fresh turmeric powder- 25mg, Vitamin C- 10mg.

### Animals

Adult male Wistar rats, weighing 175g-200g were used for this study. The animals were kept in a ventilated room under standard environment condition with temperature of 24-26°C, humidity

of 55-60% and photoperiod of 12:12hr light-dark cycle. They were provided with standard pellet diet (Amrut Laboratory Animals feeds, Maharashtra, India), and water *ad libitum*. Animals were undergo acclimatisation for one week before the initiation of experiments. All the experiments were conducted according to the guidelines of the animal ethics committee (CAF/Ethics/823/2021) of the host institute.

### Experimental induction of diabetes

Streptozotocin (STZ) at the dose of 60mg/Kg b wt was injected intraperitoneally in overnight fasted rats for a single time. It was dissolved in 0.1 M ice cold citrate buffer (pH 4.5). In order to prevent the sudden hypoglycemic shock, 5% glucose solution was given after the STZ injection. The fasting blood glucose level was measured after 72 hr and the animals having glucose level 200 mg/dL or above were selected for the study.

The rats were randomly selected and divided into 4 groups containing 6 rats in each groups. Group I was treated as normal control fed with normal saline (N). Group II was treated as STZ induced diabetic control group (DC). Group III was STZ induced animals treated with glibenclamide (DC+GB) 5mg/Kg b.wt, orally. Group IV was STZ induced animals treated with glutathione enriched polyherbal formulation (DC+GEF) 400mg/Kg b.wt., orally.

During the 28 days of experimental period, changes in the body weight and fasting blood glucose level were measured on day 0, 7, 14, 21, and 28. At the end of the study, all the rats were fasted, and sacrificed by euthanasia. Blood was collected and separated the serum. Kidney and liver tissues were dissected and fixed in formalin and also stored at -80°C for histopathological and other biochemical analysis respectively.

### Oral glucose tolerance test (OGTT)

The OGTT was performed at the end of 28<sup>th</sup> day experiment after the treatment with the samples. Normal and diabetic control were fed with distilled water and GEF and GB was supplemented as mentioned above. Glucose (2 g/kg) was given after 30min of sample administration. Blood was taken from the tail vein of the rats at 0, 30, 60, 90, and 120 min for measuring the glucose levels using glucometer.

### Biochemical analysis

#### Estimation of liver enzyme markers

SGOT, SGPT, ALP were measured by using the diagnostic kits according to the manufacturers instruction (Agappe Diagnostic Ltd India).

### Analysis of antioxidant activities

Antioxidant activity of superoxide dismutase (SOD) was measured by following the method of Kakkar *et al*, 1984, and SOD

**Table 1: Primer sequences.**

Gene	Forward primer	Reverse primer
GLUT-2	5'GTCAGAAGACAAGATCACCGGA3'	5'AGGTGCATTGATCACACCGA3'
GADPH	5'GCAGGTCAGGTCCACCACTGAC 3'	5'CGGAGTCAACGGATTGGTCGTAT3'

activity is defined as the enzyme concentration required to inhibit the chromogen production by 50% in one minute under the assay conditions.<sup>[12]</sup> The catalase (CAT) enzyme was assayed by the method of Maehly and Chance, 1954 based on the oxidation of hydrogen peroxide.<sup>[13]</sup> The unit of enzyme activity was expressed as  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  oxidised/min/mg protein. The evaluation of glutathione peroxidase (GPx) activity was done by the method of Agergaard and Jensen, 1982, and the enzyme activity is defined as  $\mu\text{moles}$  of NADPH oxidised/min/mg protein using 0.25mM of  $\text{H}_2\text{O}_2$  as substrate.<sup>[14]</sup> Reduced glutathione (GSH) was measured spectrophotometrically as described by Benke and Murphy, 1974, reduced glutathione was estimated, and expressed in nmol per gram tissue.<sup>[15]</sup>

### Determination of lipid peroxidation

Lipid peroxidation in the liver tissue was evaluated based on the method of Ohakawa *et al.*, 1979 by quantifying the thiobarbituric acid reactive substances (TBARS).<sup>[16]</sup> It was measured spectrophotometrically at 532 nm and expressed as mmol MDA/g tissue.

### Analysis of nitrite in rat serum

Nitrite was measured using griess reagent which contains 1% of sulfanilamide with 0.1% Naphthylethylenediamine. Equal volume of samples and griess reagent was added and incubated for 10 min. The absorbance was measured at 540nm. The concentrations was obtained from the standard graph.

### Enzyme linked immuno-sorbent assay (ELISA)

PKC was measured by using commercially available rat PKC ELISA kit and the assay was done according to the manufacturer's protocols.

### Total RNA isolation and RT-PCR analysis

GLUT-2 gene expression was measured by reverse transcription-polymerase chain reaction (RT-PCR). For the isolation of total RNA, frozen liver tissues were thawed, and homogenised using Trizol followed by chloroform extraction. Nucleic acid is then precipitated with isopropanol and the pellet was washed using ethanol and re-constituted in molecular grade water. The isolated RNA was quantified and equal volume was reverse transcribed separately in two step with the help of instruction given in the RT-PCR kit. PCR products were subjected for separation by electrophoresis on 1.5% agarose gel containing ethidium bromide, visualised under a UV-trans-illuminator. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was kept as an

internal control for RNA loading. Table 1 shows the sequence of primers used in the study.

### Histopathological analysis

For the histopathological analysis, liver tissues were dissected out, and fixed in 10% buffered formalin. Tissues were then embedded in paraffin blocks and sectioned with approximately 3–5  $\mu\text{m}$  of thickness. These sections were stained with hematoxylin-eosin (H&E). The histological anomalies in the tissue sections were examined under a light microscope and photographs were taken.

### Statistical Analysis

Statistical analysis was performed using the programme SPSS/PC+, version 20.0 (SPSS Inc., Chicago, IL, USA). All the data were evaluated by utilising one-way analysis of variance (ANOVA) for comparing significant differences among groups. Pair fed comparisons between the groups was made by Duncan's multiple range tests.  $P < 0.05$  was considered as statistically significant.

## RESULTS

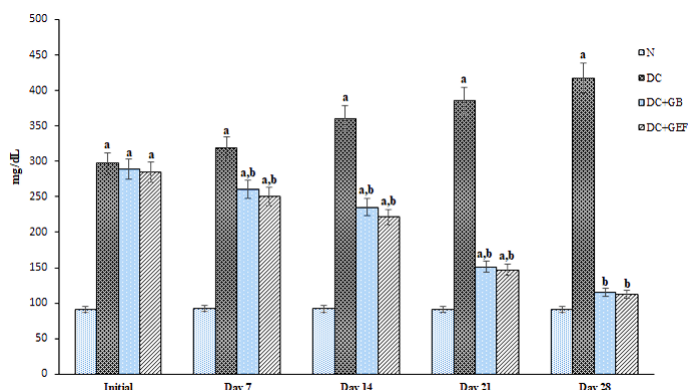
### Anti-hyperglycemic effect of GEF

The results show that STZ injection significantly induced a diabetogenic response. As a result, diabetic control rats exhibited significant elevation of blood glucose up to 28 days as compared to the normal control group. However, the rise in blood glucose was significantly ( $p < 0.05$ ) controlled by GEF supplementation to near normal as compared to diabetic control group. Figure 1 shows the blood glucose levels of control and experimental groups.

### Effect of GEF on body weight

The body weight assessment of rats shows that except the normal control, body weight was reduced in all the other three group of animals on 7<sup>th</sup>, and 14<sup>th</sup> day. On 21<sup>st</sup> day to the end of the experimental duration, diabetic control group had a marked reduction in the body weight as compared with the normal rats. The continuous oral administration of GEF resulted in remarkable increase in the body weight of around 31.8% increase on 28<sup>th</sup> day as compared to diabetic and GB administered group of rats. Results are represented in the Table 2.

The glucose tolerance test shows that the blood glucose level was increased to the peak after the supplementation of glucose but as the time goes the level was gradually decreased. As compared to normal, in diabetic control rats there was no considerable fall in the glucose level observed till 180 min. On the other



**Figure 1:** Effect of GEF on blood glucose (mg/dl) level. a- Statistical difference with control group at  $p < 0.05$ . b-Statistical difference with diabetic treated rats at  $p < 0.05$ .

hand, GEF and GB treated group reduced the glucose level but antihyperglycemic action was significantly showed by GEF treated group from 60 min, and reduced consistently up to 180 min. The results are depicted in the Table 3.

### Antioxidant activity of GEF

Figures 2a and 2b represented the antioxidant activities of the enzymatic antioxidants CAT, SOD, GPx, and the non-enzymatic antioxidant GSH respectively. The results showed that there was a significant reduction in these antioxidants in liver tissue of STZ induced diabetic rats when compared with the normal control rats. However, measuring the activity of these antioxidants in diabetic rat treated with GEF gives a contradictory result of significantly increased CAT, SOD, GPx, and GSH antioxidant activities. As evident from the results, GEF is more efficient than the standard drug in enhancing the antioxidant activities.

### Effect of GEF on Lipid peroxidation level

Lipid peroxidation was estimated by measuring the changes in the level of TBARS. The result obtained from the study shows that the lipid peroxidation was significantly higher in diabetic rats as there was a large amount of free radicals generated due to hyperglycemia. In contrast, GEF supplemented rats showed considerable decrease in TBARS level ( $p < 0.05$ ) which indicates that the lipid peroxidation process was inhibited by GEF (Figure 3).

### Assessment of GEF on liver enzyme marker

To investigate whether GEF has protective effect on liver damage in diabetic rats, we determined the level of liver enzyme markers such as SGOT, SGPT, and ALP. When comparing the STZ induced diabetic rats with normal rats, SGOT, SGPT, and ALP levels was remarkably increased. On the other hand, there was a substantial rise in these marker enzymes were significantly ( $p < 0.05$ ) declined by the treatment of GEF and reverted back to normal level. The results are represented in the Figure 4.

### Inhibitory effect of GEF on PKC

High level of glucose in blood will activate the PKC pathway. So, in order to study the molecular mechanism involved in diabetes, we had evaluated the PKC level in the liver tissue. In diabetic rats, due to the elevated level of blood glucose, PKC level was found to be significantly higher ( $p < 0.05$ ) when compared with the normal control. In contrast, the rats treated with GEF attenuate the level of PKC as compared with the diabetic control group (Figure 5).

### Effect of GEF on Nitrite level

The results showed that due to severe oxidative stress, Nitrite level was raised in diabetic rats whereas the treatment with GEF considerably lowered, and almost maintained normal level of Nitrite. The Figure 6 depicted the nitric oxide level in treated and untreated rats (Figure 6).

### GLUT-2 gene expression in liver

To study the physiological role of GLUT-2 transporter during diabetes, we examine the gene expression of GLUT-2 by RT-PCR. As presented in the Figure 7, diabetic control group exhibited increased GLUT-2 mRNA expression whereas the supplementation of GEF to the diabetic rats control the over expression of this gene. Therefore, there was only a reduced expression of GLUT-2 in the GEF treated group to regulate the excessive utilisation of glucose.

### Histopathological evaluation of liver tissue

The H&E staining done for the histopathological analysis of liver tissue clearly gives a confirmation for all the aforementioned biochemical analysis. Normal section of liver showed the regular

**Table 2:** Effect of GEF on changes in the body weight of rats.

Groups	Body weight (g)				
	Baseline	Day 7	Day 14	Day 21	Day 28
N	176± 0.85	184±1.23	194±1.22	215±2.91	236±3.10
DC	175±1.09	180±1.57	163±1.62 <sup>a</sup>	151±0.93 <sup>a</sup>	137±2.44 <sup>a</sup>
DC+GB	178±0.55	182±1.28	193±1.43 <sup>b</sup>	207±2.39 <sup>a,b</sup>	224±3.58 <sup>a,b</sup>
DC+GEF	176±0.85	183±1.66	193±1.56 <sup>b</sup>	210±3.32 <sup>b</sup>	232±3.40 <sup>b</sup>

Values are expressed in mean ±SEM; n=6 in each group. a- Statistical difference with control group at  $P < 0.05$ . b-Statistical difference with diabetic treated rats at  $P < 0.05$ .



**Table 3: Effect of GEF on oral glucose tolerance of rats.**

Groups	Concentration of blood glucose (mg/dL)				
	0 min	30 min	60 min	90 min	120 min
N	92.1± 1.98	142.4±2.34	130.5±1.85	111.7±3.34	94.6±2.02
DC	233.3±4.05 <sup>a</sup>	321.8±4.17 <sup>a</sup>	287.2±2.10 <sup>a</sup>	261.9±2.83 <sup>a</sup>	243.1±3.73 <sup>a</sup>
DC+GB	217.3±3.96 <sup>a,b</sup>	310.6±5.89 <sup>a</sup>	225.4±4.34 <sup>a,b</sup>	140.9±2.62 <sup>a,b</sup>	120.9±2.58 <sup>a,b</sup>
DC+GEF	221.1±4.15 <sup>a,b</sup>	309.2±5.97 <sup>a</sup>	217.2±5.03 <sup>a,b</sup>	135.4±3.02 <sup>a,b</sup>	116.9±2.45 <sup>a,b</sup>

Values are expressed in mean ±SEM; n=6 in each group. a- Statistical difference with control group at  $p < 0.05$ . b-Statistical difference with diabetic treated rats at  $p < 0.05$ .

arrangement of hepatocytes (H) with central vein (CV), portal triads which consist of portal vein (PV), hepatic artery (HA) and bile duct (BD), and kupffer cells (KC) were presented. Sinusoidal spaces (SS) also appear normal. Degenerated hepatocytes (DH) with infiltrated inflammatory cells (IC) were observed in diabetic control rat liver tissue. Moreover, sinusoidal space was more dilated, and cytoplasmic vacuoles (CyV) were also appeared. The arrangement of cells in GEF treated group was refurbished to the similar structure of normal section with normal hepatocytes, portal triads, and sinusoidal space (Figure 8).

## DISCUSSION

A chronic disorder like diabetes can have a negative impact on the body due to the instigation of several stress-sensitive signalling pathways. As a result, a bewildering set of changes can occur at both micro-, and macrovascular levels. In the present study, the effect of glutathione enriched polyherbal formulation on oxidative stress, and PKC activation was assessed by using streptozotocin induced animal model.

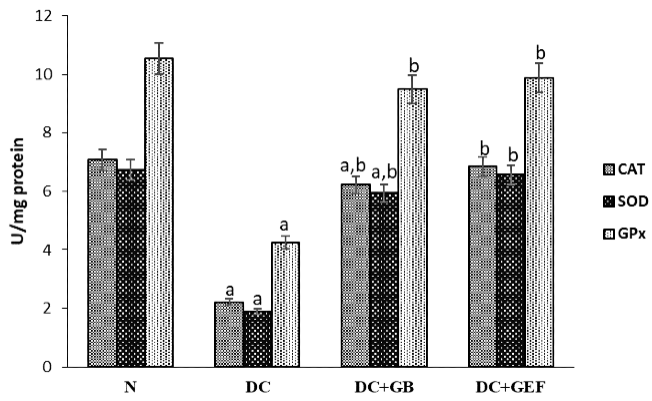
During hyperglycemic conditions, the metabolic pathways become imbalanced and the cells cannot use glucose as their energy source due to the lack of insulin production, or action. Conveniently, the cells start using proteins. Normally, insulin is responsible for regulating the synthesis of protein, and proteolysis. Streptozotocin mainly works by inducing damage to the pancreatic beta cells and reducing insulin secretion. As a result, an uncontrolled flux of glucose will be observed in the blood. So, during diabetic conditions, in the absence of insulin, body weight will be lost due to the reduction in protein storage.<sup>[17,18]</sup> The results obtained from the present study were similar to the observations made in previous studies where diabetic rats lost their body weight, but on the other hand, the oral administration of GEF to the diabetic rats significantly improved their body weight. Hence, this observation suggests that the GEF has the ability to normalise glucose metabolism, and inhibit proteolysis in skeletal muscles, which helps to maintain body weight in a normal manner.

In order to balance the equilibrium of glucose metabolism in the body, glucose transporters (GLUTs) play an important role, and they are expressed differently in various cells. Among the different GLUTs, GLUT-2 is the major glucose transporter in the liver cells. GLUT-2 regulates the majority of glucose uptake in hepatocytes,

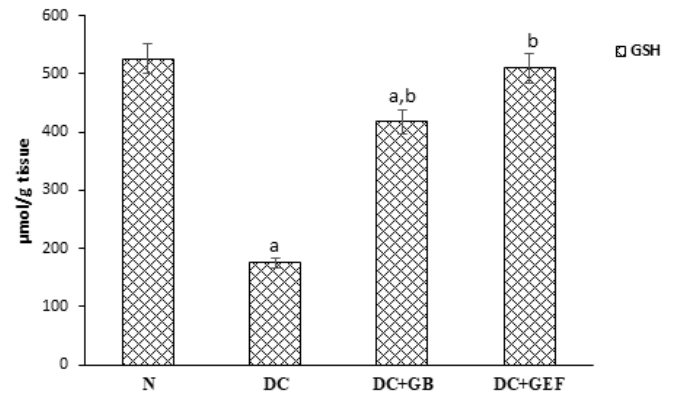
which is influenced by the amount of glucose in the bloodstream. Once it enters into the cell, glucose is immediately phosphorylated by the enzyme glucokinase to glucose-6-phosphate, which is then subjected to the further steps of glycolysis, or stored as glycogen.<sup>[19]</sup> As a result, the transport of glucose into the cell via the GLUT-2 transporter is regarded as an important rate-limiting step in carbohydrate metabolism.<sup>[20]</sup> Studies show that the GLUT-2 transporters cannot be replaced with another isoform of GLUT because of their unique and specific qualities.<sup>[21]</sup> Therefore, if a drug had the ability to translocate GLUT-2 properly, and improve insulin sensitivity, it could be more effective in the treatment of diabetes.<sup>[22]</sup> According to our study, the GEF administrated group showed a diminution in the blood glucose level as a result. We observed a reduced expression of GLUT-2, which implies that there was proper uptake of glucose into the hepatocytes through GLUT-2. The OGTT result of the present study is represented as supportive data because we found an excellent improvement in the glucose utilisation by GEF treated rats, which is an indication of progression in glucose homeostasis.

The presence of high glucose will activate distinctive metabolic signalling pathways like the DAG-induced PKC pathway. Hyperglycemia triggers the formation of DAG by de novo synthesis and from phospholipase D and phospholipase C. DAG is commonly known to be the physiological activator of PKC and, consequently, it activates NADPH oxidase, which is capable of producing excessive ROS.<sup>[23]</sup> Activation of PKC is linked with various changes in metabolic processes and ultimately results in an oxidative environment.<sup>[24]</sup> In our study, we showed that the concentration of PKC was significantly reduced in the diabetic animals after the treatment with GEF, and it may be due to the reduction in blood glucose concentration, which inhibits the activation of DAG.

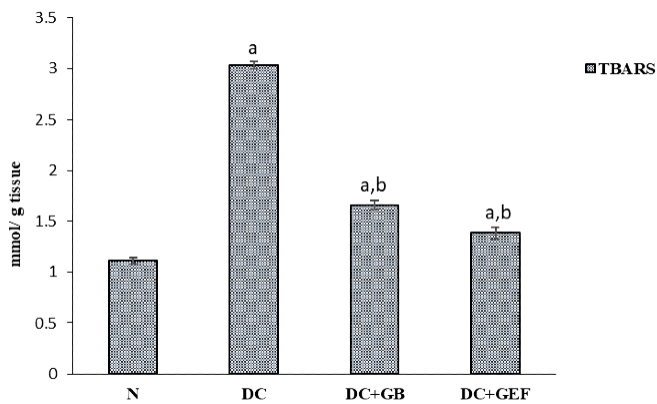
The hyperglycemic condition is experimentally induced by STZ, which imparts its effect by DNA alkylation, and is also a potent donor of intracellular NO. This NO radical then reacts with the superoxide radical, resulting in nitrosative stress.<sup>[25]</sup> Furthermore, the hyperglycemic condition promotes the generation of more, and more free radicals via multi-dimensional processes. Overall, this will disrupt the body's antioxidant defence system, impart severe oxidative stress, and weaken the cellular functions. The enzymatic antioxidants SOD, CAT, and GPx, and the non-enzymatic



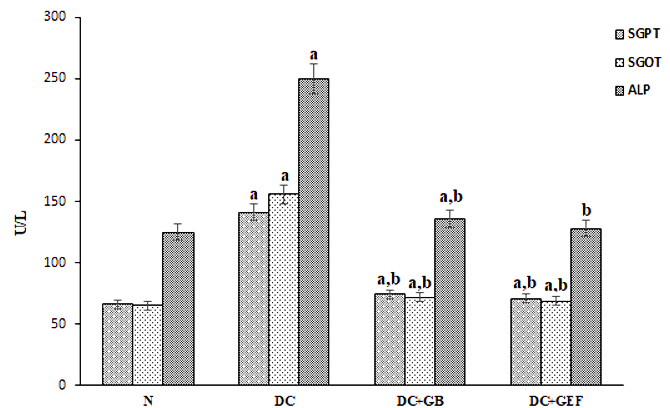
**Figure 2a:** Effect of GEF on the enzymatic antioxidant activity. 'a'-Statistical difference with normal group at  $p \leq 0.05$ . 'b'-Statistical difference with diabetic treated rats at  $p < 0.05$ . SOD: U- enzyme concentration required to inhibit chromogen production by 50% in 1 min/mg protein. Catalase: U- $\mu\text{mol H}_2\text{O}_2$  decomposed/min/ mg protein. GPx: U- $\mu\text{mol NADPH}$  oxidised / min/ mg protein.



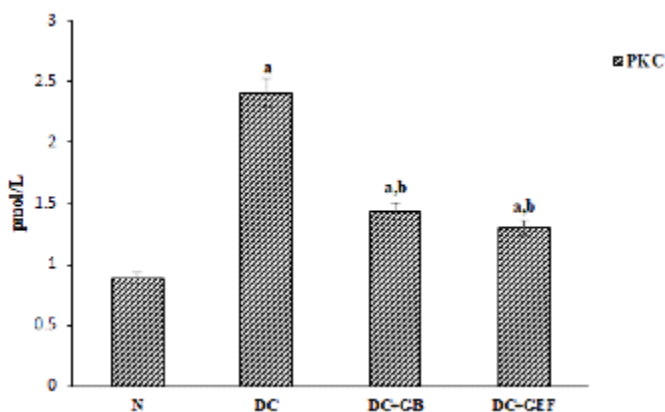
**Figure 2b:** Effect of GEF on the non-enzymatic antioxidant GSH level. 'a'-Statistical difference with normal group at  $P \leq 0.05$ . 'b'-Statistical difference with diabetic treated rats at  $P < 0.05$ . GSH: U-  $\mu\text{mol/g}$  tissue.



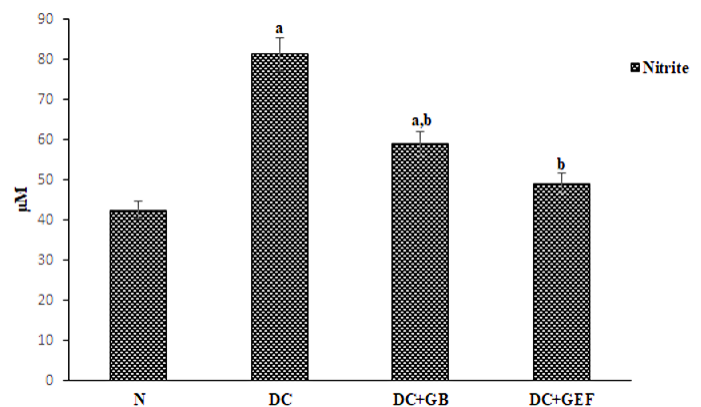
**Figure 3:** Effect of GEF on lipid peroxidation level. 'a'-Statistical difference with normal group at  $p \leq 0.05$ . 'b'-Statistical difference with diabetic treated rats at  $p < 0.05$ .



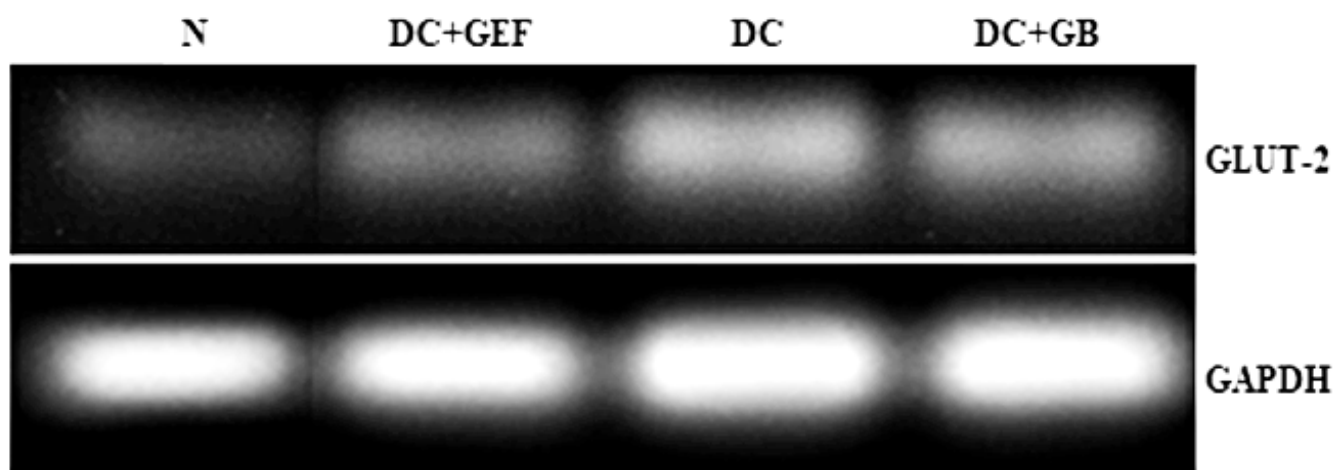
**Figure 4:** Effect of GEF on liver enzyme markers. The values are expressed as mean  $\pm$  SEM of six rats in each group. U: SGOT-  $\mu\text{mol}$  of oxaloacetate liberated /min/mg protein. U: SGPT-  $\mu\text{mol}$  of pyruvate formed /min/mg protein. U: ALP- amount of enzyme to decompose 1  $\mu\text{mole}$  of P-NPP/minute at  $25^\circ\text{C}$ . a-Statistical difference with control group at  $p < 0.05$ . b-Statistical difference with diabetic treated rats at  $p < 0.05$ .



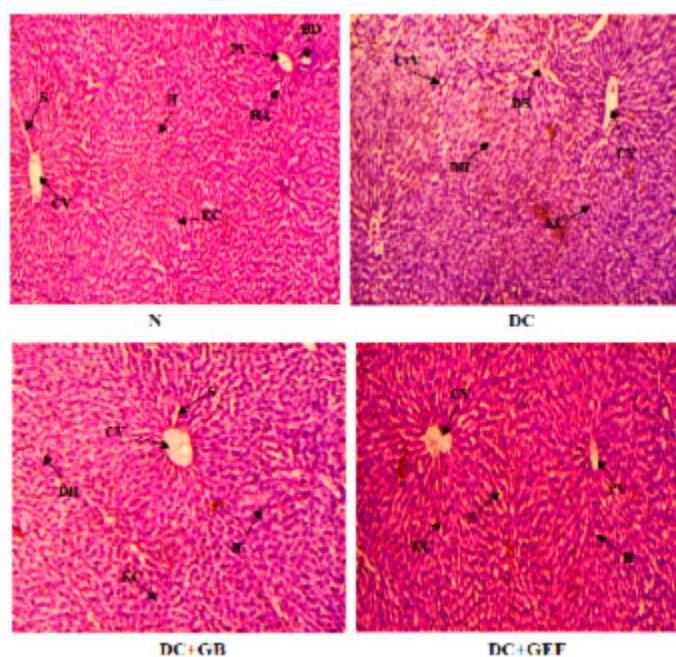
**Figure 5:** Effect of GEF on PKC level. a-Statistical difference with control group at  $p < 0.05$ . b-Statistical difference with diabetic treated rats at  $p < 0.05$ .



**Figure 6:** Effect of GEF on Nitrite level. 'a'-Statistical difference with normal group at  $p \leq 0.05$ . 'b'-Statistical difference with diabetic treated rats at  $p < 0.05$ .



**Figure 7:** Gene expression of GLUT-2 by RT-PCR.



**Figure 8:** Histopathology of rat Liver (H&E stain) (20X).

antioxidant GSH will strictly regulate the stress conditions in the body during a normal state. The overproduction of ROS usually causes an imbalance in the antioxidant status and results in the initiation of a lipid peroxidation process that produces products like malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE).<sup>[26,27]</sup> As a reflection of lipid peroxidation, there will be an advancement in the complications of diabetes due to the alteration in the fluidity gradient of the lipid bilayer which may hinder the function of many enzymes, and receptors.<sup>[28]</sup> In line with this, the present study shows that in diabetic rats the antioxidants significantly declined and, consequently, the lipid peroxidation products TBARS and Nitrite were increased, but the treatment with GEF apparently ameliorates the oxidative stress by enhancing antioxidant status, lowering Nitrite, and inhibiting

the lipid peroxidation process. Therefore, it represents the strong antioxidant scavenging potential of GEF against free radical induced damage.

Considering all the aforementioned mechanisms together to corroborate the reason behind the hepatic damage during diabetes, we evaluated the liver enzyme markers like SGOT, SGPT, and ALP. We found an increase in these enzymes in diabetic rats, which might be due to severe oxidative stress and inflammation that damage the hepatocytes, and change their overall activities, which leads to the leakage of liver enzymes into the blood.<sup>[29]</sup> As supporting evidence for this result, the histopathology of liver tissue clearly demonstrates the severe damage in the hepatocytes, indicating liver damage. Concomitantly, GEF supplementation protected the liver from free radical attack and maintained



its normal architecture, and function, so the levels of SGOT, SGPT, and ALP were found to be normal. This demonstrates the hepatoprotective action exerted by GEF during diabetic conditions.

## CONCLUSION

The present study revealed that the glutathione enriched polyherbal formulation has the ability to tolerate the glucose load and maintain its normal level in the blood without losing the body weight. The result obtained from the expression of GLUT-2 in GEF treated animals supports the above data. As a complex metabolic disorder, there involve many changes in the normal mechanism. One such important molecular pathway is PKC which activates due to the excessive accumulation of glucose in the blood during diabetic condition. Through our study we found that the PKC level was significantly reduced after the treatment using GEF. Moreover, we confirmed its potent antioxidant activity by reducing the oxidative stress, and lipid peroxidation. Therefore, the study clearly portray the potent anti-diabetic effect of the novel glutathione enriched polyherbal formulation. Hence, it could be a promising herbal anti-diabetogenic formulation that can be effectively used for the management of diabetes, and its related complications.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**GEF:** Glutathione enriched polyherbal formulation; **GB:** Glibenclamide; **STZ:** Streptozotocin; **OGTT:** Oral glucose tolerance test; **SGOT:** Serum glutamic oxaloacetic transaminase; **SGPT:** Serum glutamic pyruvic transaminase; **ALP:** Alkaline phosphatase; **GLUT-2:** Glucose transporter 2; **PKC:** Protein kinase C; **SOD:** Superoxide dismutase; **CAT:** Catalase; **GPx:** Glutathione peroxidase; **TBARS:** Thiobarbituric acid reactive substances; **ROS:** Reactive oxygen species; **DAG:** Diacylglycerol; **MDA:** Malondialdehyde.

## SUMMARY

Diabetes is one of the most common and leading diseases, so there is a need to develop an effective drug that targets the root cause of the disease. Oxidative stress is the primary reason that leads to the activation of various molecular pathways associated with diabetes. From the study, glutathione-enriched polyherbal formulation was found to be very effective in preventing stress-inducing free radicals and protecting against various cellular and molecular changes associated with diabetes. Thus, the study demonstrated that it has potent anti-diabetic activity.

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