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FULL ARTICLE

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Ameliorating effect of glutathione-enriched herbal formulation (glothione) on alloxan-induced experimental diabetic model by modulating oxidative stress and pathogenesis

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Abstract

Diabetes Mellitus is a common metabolic or endocrine disorder that occurs as a result of insufficient amounts of insulin secretion or defect in the action of insulin produced from pancreatic beta cells. Antioxidant containing food items are very effective in reducing complications that arise due to diabetes, indicating that it may be beneficial for treating metabolic disorders. In this study, we used some essential nutrients enriched with glutathione, named as Glothione (GN), and evaluated the antidiabetic effect of GN in alloxan-induced diabetic rats. The treatment with GN showed significant reduction in the blood glucose level, HbA1c level, and liver markers like SGOT, SGPT, and ALP and shows increased antioxidant status and decreased inflammatory markers. Histopathological analysis of pancreas and liver tissue showed that there were no abnormalities in the rat after the administration of GN. Thus, antioxidant-enriched formulation of GN can be used as a potent hypoglycaemic drug.

Practical applications

Glothione is a glutathione-enriched formulation that contains essential nutrients required for the normal functioning of the body. Recent trends in lifestyle and food habits have been noted to cause health risks and subject the body through physical and physiological stress––hence the importance of antioxidant-rich foods. Antioxidants are capable of boosting metabolism and other physiological processes. Thus, the consumption of GN can enhance the antioxidant status within the body. GN does not contain any chemical ingredients so it will not cause any side effects. It has a strong antidiabetic effect and is also able to control a number of diseases.

KEYWORDS

antioxidants, diabetes mellitus, glothione, oxidative stress

1 | INTRODUCTION

The existence of life depends upon many factors, food being one of the most valuable factor (Katz, Friedman, & Lucan, 2015). Food is a source of energy and should sustain life rather than be a cause of disease. Due to the over use of food additives like preservatives, artificial colors, taste makers etc., it has become a major cause of diseases which results from the disruption of normal homeostasis of the body

(Ayala, Munoz, & Arguelles, 2014). Modern lifestyle includes excessive consumption of junk food, which in turn, produces an excess of free radicals. Ultimately, this will lead to many chronic diseases like diabetes, liver diseases, cancer, heart attack (Prentice & Jebb, 2003).

Diabetes Mellitus (DM) is a complex chronic condition that affects the metabolism of carbohydrate, protein, and fat. It is also characterized by elevated blood glucose level (Hyperglycaemia) that leads to the development of both microvascular and macrovascular complications (Patel, Kumar, Prasad, Sairam, & Hemalatha, 2011; Rambhade, Chakraborty, Patil, & Rambhade, 2010) by abnormal secretion of insulin, action of insulin or both. Insulin regulates the circulating blood glucose level and helps the cells to take up glucose from the blood and convert them to pyruvate through glycolysis (Fowler, 2008). In carbohydrate metabolism, insulin promotes the transportation of glucose across muscles and adipocyte cell membrane, regulates the synthesis of glycogen, glucose from non-carbohydrate source in the liver and inhibit the breakdown of glycogen, thereby resulting in the reduction of blood glucose level (Piero, 2006; Piero, Nazaro, & Njangi, 2014).

In 2015, DM was reported to affect approximately 415 million people worldwide. It will steeply increase year by year up to 642 million in 2040 (Ogurtsova et al., 2017). The incidents of DM is low in Asia when compared to North America and Europe, but when compared to other developed countries, India is in an alarming state. Nowadays, DM and its associated complications are reported to cause the death of six people every minute (Lenzen, 2008). Treatment of DM is complicated due to the severe side effects caused by standard drugs. Hence, a much more preferred way of treatment is the consumption of healthy antioxidant and nutrient-rich foods.

Antioxidants can reduce the rate of oxidation of other molecules which is the reason why the medical field is focusing on the antioxidant therapy not only for the diabetic treatments but also for numerous diseases. Antioxidants defend the body against the oxidative stress-induced inflammation, apoptosis, and keep beta cells in its normal state. Other studies have already pointed out the ability of antioxidants to supress the complications of diabetes and regain insulin sensitivity (Deepa, Subbulakshmi, & Krishnamoorthy, 2018). GN is a novel formulation which is enriched with antioxidants and nutrients, so we assumed that the components in GN will help to prevent or repair the damage in pancreas and liver. Therefore, the present study, was focusing on the antidiabetic effect of an innovative glutathione-enriched formulation, hereinafter referred to as Glothione (GN) on alloxan-induced diabetic animal model.

2 | MATERIALS AND METHODS

2.1 | Chemicals

All the chemicals used for the study were high quality analytical grade reagents. Glucose Kits, SGOT, SGPT, ALP from Ray Biotics India Pvt Ltd. And ELISA Kits purchased from sigma- Aldrich USA, HbA1c from crystal chem USA.

2.2 | Preparation of glutathione enriched formulation "glothione"

Glothione is a glutathione-enriched antioxidant formulation. It contains, 500 mg of L-Glutathione, 100 mg of Alpha Lipoic acid, 100 mg of Rose Hip Extract, 75 mg of Grape seed extract, 6 mg of curcumin, 12.8 mg of Vitamin B2, 1.12mg of Vitamin B1, and 90 mg of L-selenomethionine.

2.3 | Animals

Adult male Wistar rats (weighing ± 150 g) were used for the study. They were kept in a controlled environment, temperature (24–26°C), humidity (55%–60%), and photoperiod (12:12 hr light-dark cycle). A commercial laboratory diet (Amrut laboratory Animals feeds, Maharashtra, India) and tap water were provided ad libitum. The animals received humane care, in compliance with the host institutional (IISc., Bangalore) animal ethics committee CPCSEA (CAF/600/2018).

2.3.1 | Experimental design

Rats were divided into four groups:

Group I: Normal control rats (N).

Group II: Alloxan-induced group (DC) (120 mg/kg. bwt).

Group III: Alloxan induced + Glibenclamide (DC + GB) (5 mg/kg. bwt).

Group IV: Alloxan induced + Glothione (DC + GN) (500 mg/kg. bwt).

Alloxan (120 mg/kg. bwt) was given intra-peritoneally and Glibenclamide and GN were administered by oral gavages (intragastrically). Experimental duration was 30 days. Rats were sacrificed after overnight fasting by euthanasia. For histological examination, pancreas and liver tissues were dissected and fixed in 10% formalin. Then the tissues were processed and embedded in paraffin. For studying various biochemical parameters, pancreatic tissue and blood were also collected.

2.4 | Analytical procedure

2.4.1 | Oral glucose tolerance test

After the overnight fasting glucose level in the blood was measured. This was evaluated by using test kit. After 1h of consuming the GN and GB, rats were received glucose solutions (2 g/kg). To measure the glucose concentration blood was taken from the tail-vein at 0 min, before and after 30, 60, 90, and 120 min of glucose intake. Serum glucose levels was estimated by measuring glucose on day 0, 5, 10, 15, 20, 25, and 30 days during the course of treatment.

2.4.2 | Assay for the activity of catalase

Estimation of the catalase activity was determined using the method explained by Maehly and Chance (1954). The enzyme activity was assayed by homogenizing tissue in Tris buffer and centrifuged at 4,000 rpm. Supernatant was collected and used for assay. Optical density was measured at 230 nm and activity of enzyme was expressed in units/mg of protein.

2.4.3 | Assay for the activity of superoxide dismutase

Superoxide dismutase (SOD) assay was done by the method described by Kakkar, Das, and Viswanathan (1984). For the homogenization of tissue Tris-EDTA buffer was used and then centrifuged it. Supernatant was collected and used for assay. Color intensity was measured at 560nm against butanol as blank. One unit of the enzyme activity is defined as the enzyme concentration required to inhibit the chromogen production by 50% in one minute under the assay conditions. Enzyme activity was expressed in unit/mg of protein.

2.4.4 | Assay for the activity of glutathione peroxidase

Assay activity of Glutathione Peroxidase (GPx) was done by the method of Lawrence and Burk (Lawrence et al., 1976) as modified by Agergaard and Jense (1982). Homogenization of tissue (10%) was done in 0.0125 M sucrose and centrifuged at 10,000 rpm for 30 min and the supernatant obtained was used for the assay. Optical density was measured at 340 nm at 20 s intervals for 3min. Enzyme activity was expressed in μmoles of NADPH oxidized/min/mg protein using 0.25 mM of H_2O_2 as substrate.

2.4.5 | Estimation of glutathione content (GSH)

Glutathione content was estimated by the method of Benke (Benke et al., 1974). Using 5 ml of precipitating solution tissue was homogenized. Then the tube was incubated for 5 min at room temperature and filtration was done using a course grade filter paper. To 0.2 ml filtrate, 3 ml of 0.3M phosphate solution and 1 ml 0.04% DTNB was added. These tubes were capped, mixed by inversion and contents were read at 412 nm within 4 min.

2.4.6 | Estimation of thiobarbituric acid reactive substance

Thiobarbituric Acid Reactive Substance (TBARS) level was estimated by using Ohkawa, Ohishi, and Yagi (1979) method. About 1 g tissue was taken and homogenized in phosphate buffer (0.12 M) pH 7.5, centrifuged and supernatant was taken. Absorbance was read at 532 nm kept butanol as blank. The lipid peroxidase levels were expressed as mmol of malonaldehyde produced.

2.4.7 | Estimation of blood parameters

The quantification of hemoglobin A1C (HbA1C) in whole blood by using hemoglobin A1C assay kit, Crystal Chem USA.

2.4.8 | Estimation of SGOT, SGPT, and ALP

SGOT, SGPT, ALP were measured by using the diagnostic kits from Ray Biotic Company, Pvt Ltd, India.

2.4.9 | Estimation of cytokines using ELISA method

It was done by the method of Engvall and Perlman (1971). Optical density for measuring IL-6 & TNF-α was 400 nm using a microplate reader.

2.4.10 | Histopathological study of liver and pancreas tissue

The complete pancreas and liver tissue were dissected and the sections (5 μm) were fixed in 10% formalin solution at room temperature. For the histopathological analysis, pancreas and liver tissues embedded in paraffin were stained using hematoxin-eosin (H&E). The tissue samples were then observed and took photographs under a light microscope for the visualization of structural anomalies. The damages in pancreas and liver were judged by two independent observers who is blinded to the experimental protocol.

2.4.11 | Statistical analysis

The result obtained from the study were analyzed using SPSS/PC+ (statistical program), version 11.0 (SPSS Inc, Chicago, IL, USA). One-way ANOVA was used for comparing the significant difference among groups. Pair fed comparisons between the groups was done by Duuncan's multiple range test. $p < .05$ was considered as significant.

3 | RESULTS

3.1 | Effect of GN on glucose tolerance

Oral Glucose Tolerance Test (OGTT) was determined by analyzing the glucose level at 0, 30, 60, 90, and 120 min. From the result it was clear that the diabetic control was unable to tolerate the fasting

glucose. There was no impaired glucose tolerance in GN and standard drug-treated rats as compared to the diabetic control group (Table 1). There was a significant reduction in the concentration of glucose at 120 min as compared to the standard drug-treated group.

3.2 | Effect of GN on blood glucose level

Blood glucose level in the alloxan-induced group was hiked in the 1st day in comparison with normal control rats. On the 7th and 14th day GN supplemented rats showed gradual and sharp decline in the glucose level as compared to the diabetic group. The results were shown in the Figure 1.

3.3 | Effect of GN in HbA1c level

The evaluation of glycated hemoglobin (HbA1c) provide a consistent degree of glycaemic index which is well related in developing complications during the chronic diabetic condition. Hence, it is considered as a main examination for assessing and managing of the diabetic condition. It also predicts the microvascular and macrovascular complications in diabetic patients. The HbA1c level was increased in the alloxan-induced rats when compared with normal rats. After treatment with GN, the level of HbA1c was decreased significantly (Figure 2).

3.4 | Effect of GN on specific liver marker enzymes

The liver function was analyzed by checking the serum levels of marker enzymes in each rat. Alloxan can induce damage to the liver cells thereby serum level of SGOT, SGPT, and ALP were elevated as compared to the other groups. By the administration of GN all these marker enzymes were revert back to normal level. These data demonstrate that liver function is also affected significantly during diabetic condition but it was counter balanced effectively by GN treatment (Figure 3).

3.5 | Effect of GN on pancreatic antioxidant enzyme

The inability of pancreatic cells to produce insulin in the diabetic animal was indirectly analyzed by the assay of antioxidant enzymes like CAT, SOD, and GPx. The results are shown in Figure 4, CAT, SOD, and GPx level in the alloxan-induced group were reduced than that in normal rats. These results indicate the occurrence of severe damage of cells in alloxan-induced rats. Treatment with GN significantly ($p < .05$) enhanced the level of CAT, SOD, and GPx. These results supported the fact that treatment with GN could boost up antioxidant status and provide a better host environment and prevent further diabetes-induced damages.

3.6 | Effect of GN on lipid peroxidation

Lipid peroxidation level was usually estimated in terms of its end product TBARS in pancreatic tissue. The result is shown in the

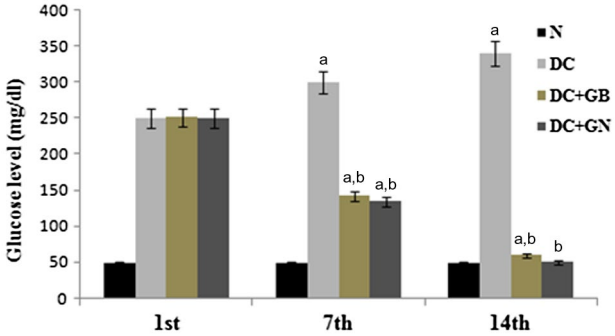

FIGURE 1 Effect of different groups on blood glucose (mg/dl) level. Values expressed as average of six samples ± *SD* in each group. (a) Statistical difference with control group at p < .05. (b) Statistical difference with diabetic-treated rats at p < .05

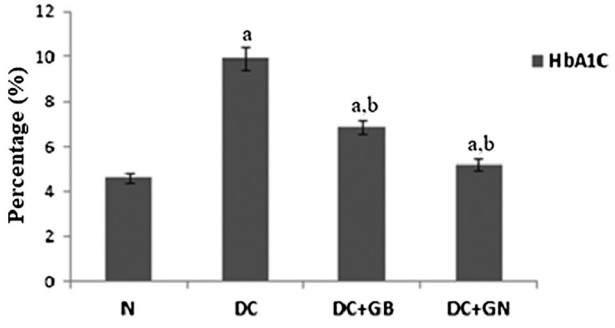

FIGURE 2 The level of HbA1c in different groups. (a) Statistical difference with control group at $p < .05$. (b) Statistical difference with diabetic-treated rats at $p < .05$

TABLE 1 Oral glucose tolerance test (mg/dl) level

Dose (2 g/kg glucose)	0 (min)	30 (min)	60 (min)	90 (min)	120 (min)
Normal control (*N*)	84.62 ± 1.5	103.14 ± 2.3	123.91 ± 2.6	92.17 ± 1.72	69.23 ± 1.0
Diabetic control (DC)	91.85 ± 2.8	134.72 ± 3.8	152.31 ± 3.2	121.42 ± 2.1	110.53 ± 1.5
Standard drug (DC + GB)	76.29 ± 1.3	118.28 ± 1.6	137.32 ± 2.3	109.64 ± 1.5	88.27 ± 1.1
Glothione (DC + GN)	73.72 ± 1.2*	109.48 ± 1.54*	128.37 ± 1.07*	102.98 ± 1.04*	72.53 ± 1.11*

Note: Value are expressed in mean ± *SD* (*N* = 6).

*$p < .05$. Significantly different from diabetic control.

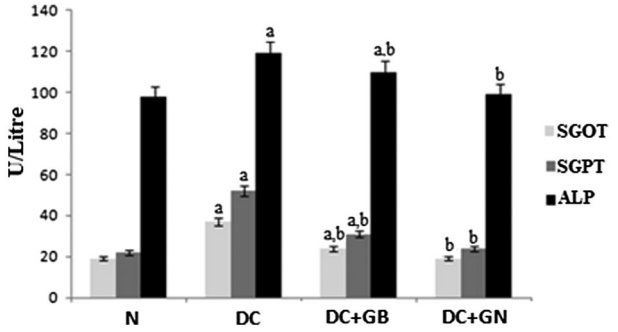

FIGURE 3 Effect of GN on liver enzymes. The values are expressed as mean ± *SD* of six rats in each group. U: SGOT—µmol of oxaloacetate liberated/min/mg protein. U: SGPT—µmol of pyruvate formed/min/mg protein. U: ALP—amount of enzyme to decompose 1 µmol of *p*-PNPP/min at 25°C. (a) Statistical difference with control group at $p < .05$. (b) Statistical difference with diabetic treated rats at $p < .05$

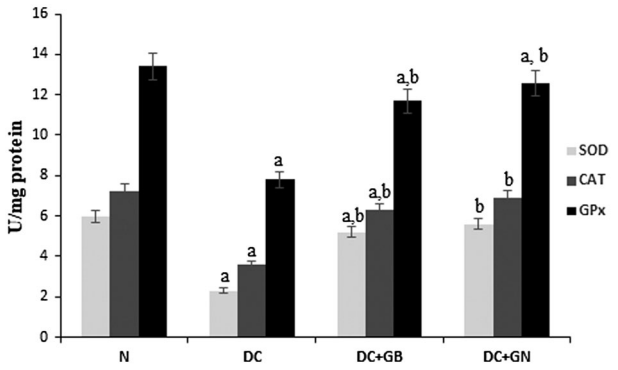

FIGURE 4 Effect of GN on the activities of SOD, CAT and GPx enzymes. The Values expressed as average of six samples ± *SD* in each group. (a) Statistical difference with normal group at $p \leq .05$. (b) Statistical difference with diabetic-treated rats at $p \leq .05$. SOD: U-enzyme concentration required to inhibit chromogen production by 50% in 1 min. Catalase: U-µmol H2O2 decomposed/min. GPx: U-µmol NADPH oxidized/min

Figure 5. TBARS level was significantly higher in diabetic rats in comparison with the normal control rats. GN administration showed that there was a significant ($p < .05$) decline in the level of TBARS. Therefore, antioxidant-enriched formulation GN can protect the cells from lipid peroxidation along with reducing the oxidative stress occur during the chronic state of diabetic condition.

3.7 | Effect of GN on glutathione level

Glutathione (GSH) helps to reduce the hydrogen peroxide radicals formed during stressful condition. Due to the overproduction of free radicals, GSH was decreased significantly in diabetic control rats. On the supplementation of GN, the amount of GSH markedly shot up when compared to diabetic control group. The result was shown in the Figure 6.

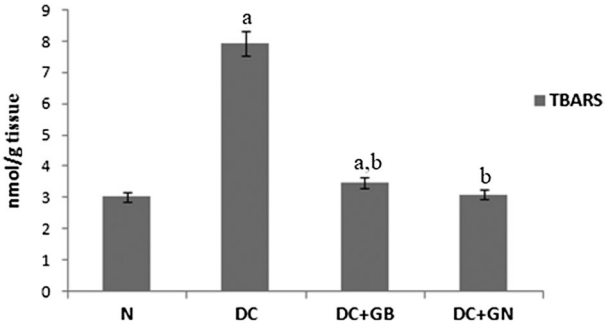

FIGURE 5 Effect of GN on lipid peroxidation level. Values expressed as average of six samples ± *SD* in each group. (a) Statistical difference with normal group at $p \leq .05$. (b) Statistical difference with diabetic-treated rats at $p \leq .05$

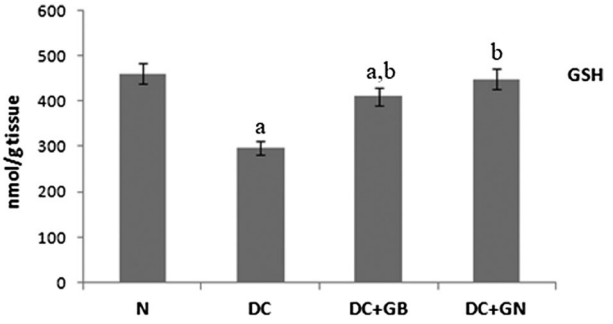

FIGURE 6 Effect of GN on GSH level. Values expressed as average of 6 samples ± *SD* in each group. (a) Statistical difference with normal group at $p \leq .05$. (b) Statistical difference with diabetic-treated rats at $p \leq .05$

3.8 | Effect of GN on inflammatory cytokines TNF-α & IL-6

As depicted in Figure 7, alloxan administration disturbed the normal equilibrium of metabolic activities and as a result, more and more cytokines like IL-6 and TNF-α was produced in alloxan induced control group when compared to normal control. The elevated pro-inflammatory cytokines were significantly downregulated by GN administration. These results indicate that GN can strongly control the inflammation associated with diabetes and hence has potent the anti-inflammatory activity.

3.9 | Histopathology of pancreas

Normal rat's pancreas (N) section shows exocrine pancreas (EP) made up of cuboidal cells having spherical nuclei located basally. Islets of Langerhans (IL) are embedded within the acini. IL are intact, its cortex (C) was rich with A, D cells and medulla (M) with B cells. B cells are larger and having eosinophilic granular cytoplasm. In diabetic rats, the size of pancreas is reduced and regular arrangement of β cells in the cord is lost. It loses its normal

architecture and becomes oedematous. Regular arrangement of B cells in the cords is lost; it also losses its granular cytoplasm and becomes degenerated (DB). EP was lined by atrophic, flat cells. In GN supplemented rats, the size of pancreas as well as number of cells in cord are similar to that of normal control. B cells appear healthy with eosinophilic granular cytoplasm. No fibrosis, inflammation or necrosis occurred in GN-treated group. The results are shown in the Figure 8.

3.10 | Histology of liver

Normal rat's liver (N) section demonstrated that lobules of liver contain normal liver cells and sinusoids (S) divided it separately. In the Sinusoidal walls, scattered phagocytic kupffer cells (KC) was observed. The liver hepatocytes (HE) contain nucleus and cytoplasm rich in glycogen. In alloxan-treated rats, hepatocytes show fatty changes and also sinusoids are prominent with minimal kupffer cells proliferation. Focal inflammation cell infiltration (IN) was also noted. In GN supplemented rats, hepatocytes are observed possessing a centrally placed nucleus with eosinophilic cytoplasm; sinusoids are dilated (DS). No degeneration, inflammation, necrosis, or hemorrhage was found. The results are shown in the Figure 9.

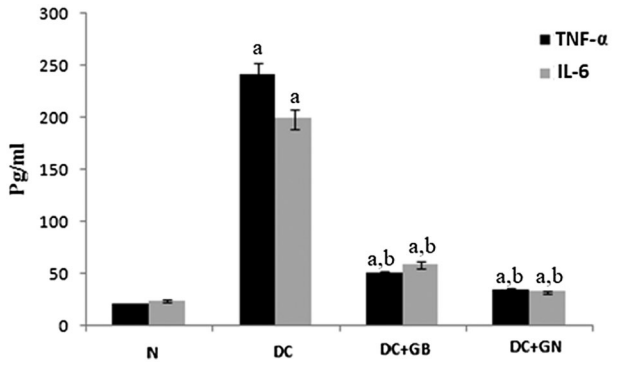

FIGURE 7 Effect of GN on inflammatory cytokines like IL-6, TNF-α. Values expressed as average of six samples ± *SD* in each group. (a) Statistical difference with normal group at $p \leq .05$. (b) Statistical difference with diabetic-treated rats at $p \leq .05$

4 | DISCUSSION

Elevated glucose level in the blood (hyperglycaemia) is the main indication of DM. It also affects the normal functioning of vital organs such as liver, pancreas, kidney, eye etc. Many studies have confirmed that HbA_1C concentration is a good predictor of DM and also indicate both macrovascular and microvascular complications including coronary mortality and lower extremity amputations (Marica et al., 2005). During DM, the additional amount of glucose found in the blood will react with hemoglobin and form HbA_1C (Alagammal, Nishanthini, & Mohan, 2012). The quantity of HbA_1C in blood plasma of alloxan-induced control group was found that it was evidently elevated ($p < .05$). Treatment with GN significantly reverted the HbA_1C level close to normal control group.

Oral Glucose Tolerance Test is an another way to check the glucose tolerance capacity of the body, functioning of beta cells, and insulin resistance. By the supplementation of GN the concentration of glucose was significantly lowered at different intervals of time. Transition from normal to diabetic condition after the alloxan

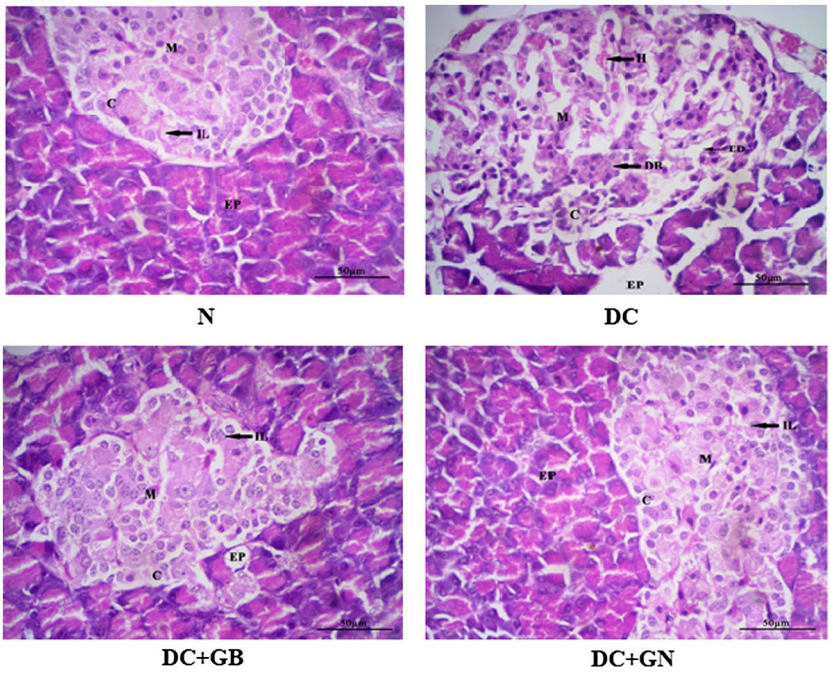

FIGURE 8 Histopathology of rat pancreas (H&E stain). N-Normal rats; DC-Alloxan-treated rats(DC); DC+GB– Alloxan+Standard drug; DC+GN– Alloxan + Glutathione-enriched formulation

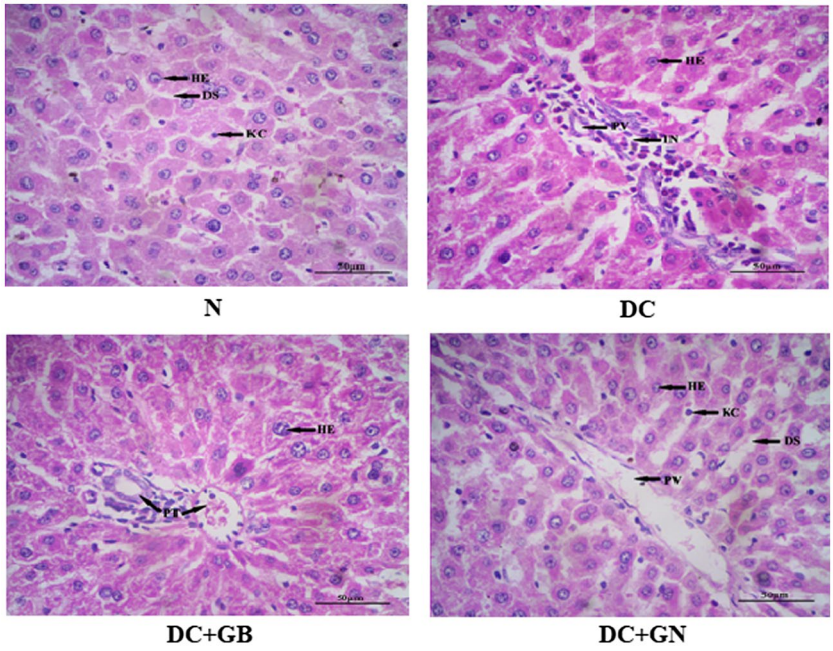

FIGURE 9 Histopathology of rat liver (H&E stain). N-Normal rats; DC-Alloxan-treated rats(DC); DC + GB− Alloxan+Standard drug; DC + GN− Alloxan + Glutathione-enriched formulation

induction was done by measuring the fasting blood glucose level and it was very high in the diabetic group as compared to the normal group. Treatment of diabetic rats with GN for 4 weeks significantly reduced their blood glucose levels when compared to those of diabetic group. Thus it is observed that GN has a potent hypoglycaemic property.

Alloxan will damage the beta cells of pancreas and result in the development of oxidative stress. Antioxidants will help to protect the biomolecules from extensive oxidative damage (Hisalkar, Patne, Fawade, & Karnik, 2012). There is a highly sophisticated antioxidant system in the body to protect all the cells and organs from reactive oxygen species (ROS) (Ojoye, Holy, Kemzi, Ferdinand, & Nsirim, 2016). Antioxidants also helps to restore the cells to their normal state from oxidative damage induced by free radicals (Krishnamurthy & Wadhwani, 2012).

Superoxide Dismutase is considered as the first line of defence against ROS. Reduced levels of the antioxidant SOD was found during DM in both liver and pancreatic tissues and blood (Giugliano et al.,1995; Thomas, Rao, Prasad, & Kumari, 2014). Another important antioxidant enzyme is Catalase (CAT) which has a major role in the elimination of H_2O_2––one of the major radical generated in the body (Chelikani, Fita, & Loewen, 2004). This enzyme helps in the degradation of H_2O_2 produced by peroxisomal oxidases to oxygen and water (Szaleczky, Prechl, Feher, & Somogyi, 1999). Deficiency of this enzyme can lead to the increased exposure of β cells to oxidative stress (Goth & Eaton, 2000). GPx also involves in the removal of H_2O_2 with the oxidation of glutathione along with its reduction (Ojoye et al., 2016). In diabetic patients, serum contains elevated level of free radicals that cause damage to proteins and lipids which leads to the development of many complications (Brownlee, 2001). These are the main antioxidant enzymes with powerful scavenging activity that regulate free radical activity (Holy & Ngoye, 2016). Most of the food items have powerful antioxidant property especially in Syzygium aromaticum, onion juice which help to improve the antioxidant status in the body (Majid et al., 2018; Maryam et al., 2017). From this study, we observed that the antioxidant status was significantly reduced in alloxan-treated rats. Upon supplementation with GN, the antioxidant status was significantly increased and thereby reduced the oxidative stress and ROS generation in diabetic condition.

Another important antioxidant enzyme is Glutathione (GSH) which is a tripeptide that is present in millimolar concentrations in all cells, which has a key role in intracellular radical scavenging and act as a substrate of many xenobiotic elimination reactions (Gregus, Fekete, Halaszi, & Klaassen, 1996; Lu et al., 1999). A marked reduction in the level of GSH in the plasma of diabetic patients was also reported. One of the factors that causes the oxidative DNA damage is decreased GSH level in the case of Type 2 DM (Dincer, Akcay, Alademir, & Ilkova, 2002). GN supplemented group showed significantly increased level of antioxidant GSH. These findings indicate the protective action of GN on pancreatic cells through its antioxidant.

Overproduction of free radicals cause lipid peroxidation. Malonaldehyde (MDA), as TBARS is frequently used to determine the pro-oxidant or antioxidant balance in type 2 diabetic patients because of its stability and easiness to measure the end product of lipid peroxidation (Ames, Cathcart, Schwiers, & Hochstein, 1981). Lipid peroxidation of fatty acids on the membrane react with free radicals to form a highly toxic end product, that is, TBARS. Increased TBARS concentration is correlated with poor glycaemic control (Shilpashree

& Tejaswi, 2016). Supplementation of GN significantly protects against the formation of lipid peroxides in the pancreas of diabetic rats which means, it inhibits the radical formation.

Measuring the enzymatic activities of SGOT, SGPT, and ALP have toxicological and clinical significance. It acts as an indicator of liver tissue damage due to the action of toxicants or any disease conditions. These enzymes are commonly used to evaluate the hepatic disorders. An increased level of these enzymes in the serum reflects the chronic liver damage which results in the leakage of enzymes into the blood stream from the cytosol of liver, which demonstrated the clear picture of hepatotoxic effect of alloxan (Lapshina et al., 2006). The level of SGPT, SGOT, and ALP in serum were increased than that of normal levels in diabetic animals and diabetic animals treated with GN revert the levels of these enzymes. Hence GN also functions to protect the liver.

Oxidative stress adversely affects the endogenous system, important signaling pathways and cellular mechanism, finally along with the free radicals, pro-inflammatory markers like TNF-α and IL-6 also elevated (Dominiczak, 2003). From the result, TNF-α and IL-6 level were significantly decreased with the GN treatment, this indicates its specific action on the cellular mediators involved during diabetes.

Histopathological analysis of liver and pancreas showed abnormality in the arrangement and structure of cells in diabetic rats. But treatment with our antioxidant rich GN helps to maintain the normal architecture of cells that is, GN can block the damage caused due to the oxidative stress generated in the tissues by its radical scavenging mechanism.

5 | CONCLUSION

The present study shows the efficacy and mechanism of antidiabetic effect of GN a new antioxidant-enriched formulation of glutathione along with essential nutrients. From the results, it clearly demonstrated the antioxidant and anti-inflammatory role of GN to protect against alloxan-induced hyperglycaemic condition by controlling the blood glucose level, HbA1c level, liver enzyme markers, inflammatory markers, and oxidative stress. GN also helps in the upregulation of endogenous antioxidants thereby the reduction of lipid peroxidation.

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CONFLICTS OF INTEREST

All the authors are disclosing the conflict of interest. Glothione is a patent pending product of Glowderma Pvt. Ltd. Mumbai.

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REFERENCES

Agergaard, N., & Jensen, P. T. (1982). Procedure for blood glutathione peroxidase determination in cattle and swine. *Acta Veterinaria Scandinavica*, *23*, 515–527.

Alagammal, M., Nishanthini, A., & Mohan, V. R. (2012). Anti-hyperglycemic and anti-hyperlipidaemic effect of Polygala rosmarinifolia Wright & Arn on alloxan induced diabetic rats. *Journal of Applied Pharmaceutical Science*, *2*, 143–148.

Ames, B. N., Cathcart, R., Schwiers, E., & Hochstein, P. (1981). Uric acid provides an antioxidant defense in humans against oxidant and radical caused aging and cancer: A hypothesis. *Proceeding of the National Academy of Science of the United States of America*, *78*, 6858–6862. https://doi.org/10.1073/pnas.78.11.6858

Ayala, A., Munoz, M. F., & Arguelles, S. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity*, *2014*, 1–31. https://doi.org/10.1155/2014/360438

Benke, G. M., & Murphy, S. D. (1974). Effect of TOTP pretreatment on paraoxon and methyl paraoxon detoxification in rats. *Research communications in chemical pathology and pharmacology*, *8*, 665–672.

Brownlee, M. (2001). The pathobiology of diabetic complications: A unifying mechanism. *Diabetes*, *54*, 1615–1625. https://doi.org/10.2337/diabetes.54.6.1615

Chelikani, P., Fita, I., & Loewen, P. C. (2004). Diversity of structures and properties among catalases. *Cellular and Molecular Life Sciences*, *61*, 192–208. https://doi.org/10.1007/s00018-003-3206-5

Deepa, R., Subbulakshmi, P., & Krishnamoorthy, G. (2018). A review on role of antioxidants in diabetes. *Asian Journal of Pharmaceutical and Clinical Research*, *11*, 48–53.

Dincer, Y., Akcay, T., Alademir, Z., & Ilkova, H. (2002). Assessment of DNA base oxidation and glutathione level in patients with type2 diabetes. *Mutatation Research*, *505*, 75–81. https://doi.org/10.1016/S0027-5107(02)00143-4

Dominiczak, M. H. (2003). Obesity, glucose intolerance and diabetes and their links to cardiovascular disease. Implications for laboratory medicine. *Clinical Chemistry and Laboratory Medicine*, *41*, 1266–1278.

Engvall, E., & Perlman, P. (1971). Enzyme linked immunosorbant assay (ELISA). Quantitiative assay of immunoglobulin G. *Immunochemistry*, *8*, 871–874.

Fowler, M. J. (2008). Micro vascular and macro vascular complications of diabetes. *Clinical Diabetes*, *26*, 77–82. https://doi.org/10.2337/diaclin.26.2.77

Giugliano, D., Ceriello, A., & Paolisso, G. (1995). Diabetes mellitus, hypertension, and cardiovascular disease: Which role for oxidative stress? *Metabolism*, *44*, 363–368. https://doi.org/10.1016/0026-0495(95)90167-1

Goth, L., & Eaton, J. W. (2000). Hereditary catalase deficiencies and increased risk of diabetes. *The Lancet*, *356*, 1820–1821. https://doi.org/10.1016/S0140-6736(00)03238-4

Gregus, Z., Fekete, T., Halaszi, E., & Klaassen, C. D. (1996). Lipoic acid impairs glycine conjugation of benzoic acid and renal excretion of benzoylglycine. *Drug Metabolism and Disposition*, *24*, 682–688.

Hisalkar, P. J., Patne, A. B., Fawade, M. M., & Karnik, A. C. (2012). Evaluation of plasma superoxide dismutase and glutathione peroxidase in type 2 diabetic patients. *Biomedical Research and Clinical Practice*, *4*, 65–72.

Holy, B., & Ngoye, B. O. (2016). Clinical relevance of superoxide dismutase and glutathione peroxidase levels in management of diabetes Type 2. *International Journal of Contemporary Medical Research*, *3*, 2454–7379.

Kakkar, P., Das, B., & Viswanathan, P. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics*, *21*, 130–132.

Katz, D. L., Friedman, R. S. C., & Lucan, S. C. (2015). *Nutrition in clinical practice: A comprehensive, evidence-based manual for the practitioner* (3rd ed.). Philadelphia, PA: Wolters Kluwer.

Krishnamurthy, P., & Wadhwani, A. (2012). Antioxidant enzymes and human health. *Antioxidant Enzyme*, *1*, 3–18.

Lapshina, E. A., Sudnikovich, E. J., Maksimchik, J. Z., Zabrodskaya, S. V., Zavodnik, L. B., Kubyshin, V. L., … Zavodnik, I. B. (2006). Antioxidative enzyme and glutathione S-transferase activities in diabetic rats exposed to long-term ASA treatment. *Life Science Journal*, *79*, 1804–1811. https://doi.org/10.1016/j.lfs.2006.06.008

Lawrence, R. A., & Burk, R. F. (1976). Glutathione peroxidase activity in selenium-deficient rat liver. *Biochemical and Biophysical Research Communications*, *7*, 1952–1958.

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, *51*, 216–226. https://doi.org/10.1007/s00125-007-0886-7

Lu, W. P., Sun, Y., Bauer, M. D., Suzanne, P., Paula, M. K., & William, G. K. (1999). Penicillin-binding protein 2a from methicillin-resistant *Staphylococcus aureus*: Kinetic characterization of its interactions with beta-lactams using electrospray mass spectrometry. *Biochemistry*, *18*, 6537–6546.

Maehly, A. C., & Chance, B. (1954). The assay of catalases and peroxidases. *Methods of Biochemical Analysis*, *1*, 357–424.

Majid, S., Elahe, O. S. M., Arash, K., Hamed, S., Amir, A. K., Malihe, S., & Nava, A. (2018). Investigating the effects of onion juice on male fertility factors and pregnancy rate after testicular torsion/detorsion by intrauterine insemination method. *International Journal of Women's Health and Reproduction Sciences*, *6*, 499–505.

Marica, J. B., Veljko, B., Jandranka, B., Zeljko, M., Ivan, J., & Zeljko, R. (2005). Impact of glycemic control on antioxidant enzyme activity in patients with type 2 diabetes mellitus. *Diabetologia Croatica*, *33*, 131–135.

Maryam, M., Seyd-Hosein, A. E., Majid, S., Mostafa, A., & Malihe, S. (2017). Effect of Syzygium aromaticum (clove) extract on seminiferous tubules and oxidative stress after testicular torsion in adult rats. *Physiology and Pharmacology*, *21*, 343–350.

Ogurtsova, K., da Rocha Fernandes, J. D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N. H., … Makaroff, L. E. (2017). IDF diabetes atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Research and Clinical Practice*, *128*, 40–50. https://doi.org/10.1016/j.diabres.2017.03.024

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxidesin in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*, 351–358.

Ojoye, N. B., Holy, B., Kemzi, E., Ferdinand, E., & Nsirim, N. (2016). Superoxide dismutase and glutathione peroxidase levels in patients with long standing Type 2 diabetes in Port Harcourt, Rivers State. *International Journal of Science and Research*, *5*, 2319–7064.

Patel, D. K., Kumar, R., Prasad, S. K., Sairam, K., & Hemalatha, S. (2011). Antidiabetic and invitro antioxidant potential of Hybanthus enneaspermus (Linn) F. Muell in streptozotozine induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine*, *1*, 316–322.

Piero, M. N. (2006). Hyperglyceamic effect of some Kenyan plants traditionally used in management of diabetes mellitus in Eastern Province. *Journal of Diabetes and Metabolism*, *2*, 1–6.

Piero, M. N., Nazaro, G. M., & Njangi, J. M. (2014). Diabetes Mellitus—A devastating metabolic disorder. *Asian Journal of Biomedical and Pharmaceutical Science*, *4*, 1–7.

Prentice, A. M., & Jebb, S. A. (2003). Fast foods, energy density and obesity: A possible mechanistic link. *Obesity Reviews*, *4*, 187–194. https://doi.org/10.1046/j.1467-789X.2003.00117.x

Rambhade, S., Chakraborty, A. K., Patil, U. K., & Rambhade, A. (2010). Diabetes Mellitus—Its complications, factors influencing complications and prevention—An overview. *Journal of Chemical and Pharmaceutical Research*, *2*, 7–25.

Shilpashree, Y. D., & Tejaswi, H. L. (2016). Lipid peroxidation and superoxide dismutase activities in patients with type 2 diabetes complicated with peripheral neuropathy. *International Journal of Research in Medical Sciences*, *4*, 457–460.

Szaleczky, E., Prechl, J., Feher, J., & Somogyi, A. (1999). Alterations in enzymatic antioxidant defence in diabetes mellitus—A rational approach. *Postgraduate Medicine Journal*, *75*, 13–17. https://doi.org/10.1136/pgmj.75.879.13

Thomas, B., Rao, A., Prasad, B. R., & Kumari, S. (2014). Serum levels of anti-oxidants & SOD in periodontitis patients with diabetes type 2. *Journal of Indian Society of Periodontology*, *18*, 451–455.

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