

Antidiabetic Activity of Ayurvedic Niruryadi Gulika in Experimental Diabetes

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Diabetes mellitus is a chronic metabolic disorder associated with high rates of morbidity and mortality globally. Ayurvedic medicine offers promising approaches for managing diabetes, with Niruryadi Gulika (NG) emerging as a potential therapeutic agent. This study evaluated the effects of NG in comparison to metformin, a widely used antidiabetic drug, in streptozotocin (STZ)-induced diabetic rats. Experimental groups included control, diabetic control, metformin-treated and NG-treated groups, monitored over a 6-week period. Various parameters included circulating glucose levels, plasma insulin, total haemoglobin, glycosylated haemoglobin, lipid profile (cholesterol, triglyceride, HDL, LDL), antioxidant enzymes (catalase, GPx), and lipid peroxidation (TBARS) were assessed. Our results demonstrated that NG particularly at two doses, significantly reduced blood glucose levels, glycosylated haemoglobin, and lipid peroxidation, while enhancing plasma insulin levels, total haemoglobin, and antioxidant enzyme activity compared to diabetic control rats. NG also improved lipid profile markers, such as cholesterol and triglycerides, achieving effects comparable to metformin. Furthermore, NG treatment reduced elevated cytokine levels, including TNF- α and IL-6, in diabetic rats, reflecting the anti-inflammatory effects of metformin. These findings highlight NG's potential as an effective anti-diabetic agent, showing promising results comparable to metformin. Further research is warranted to better understand the mechanisms of NG and optimize its dosage regimen for managing diabetes mellitus.

Keywords: Antioxidants; Ayurvedic drugs; Diabetes; Niruryadi Gulika; Oxidative stress.

“Inducing diabetes in animal models has provided valuable insights into the physiological and biological disturbances connected with this metabolic disorder¹. Notably, hyperglycemic animals have been extensively studied, revealing significant alterations in lipid metabolism. Diabetes

Mellitus is a complex condition characterized by high blood sugar levels (hyperglycemia) due to issues with the production and use of insulin in the body. Diabetes is classified into two main categories: Type 1 Diabetes mellitus, which is caused by insufficient insulin production, and

Type 2 Diabetes Mellitus, which is caused by the occurrence of insulin resistance in the cells and eventual buildup of extraneous insulin in the body ². In diabetes, pathological changes are primarily related to redox imbalance and are closely associated with the onset of cardiovascular complications ³. Studies in diabetic rats have shown increased lipid peroxidation, particularly in association with hyperlipidemia ⁴. The liver, a crucial insulin-dependent organ responsible for maintaining glucose and lipid homeostasis, undergoes significant dysfunction during diabetes ⁵. The liver's functions, including the absorption, oxidation, and energy-based transformation of non-esterified fatty acids, the production of triglycerides, cholesterol, phospholipids are significantly impaired in diabetes. This disruption often results in marked alterations in lipid concentration and composition ⁶. Moreover, diabetes adversely affects glucose metabolism in the liver, leading to reduced glycolysis, impaired glycogenesis, and increased gluconeogenesis ⁷.

In recent years, the use of complementary medicine has seen significant growth, particularly in dietary interventions and traditional plant-based therapies derived from systems such as Ayurveda. Drawing upon centuries of accumulated knowledge and experience, Ayurveda emphasizes personalized treatment plans based on an individual's constitutions, or doshas, comprising Vata, Pitta, and Kapha. Ayurvedic therapies for diabetes encompass a holistic and multifaceted approach, including dietary modifications, herbal remedies, lifestyle changes, and specialized treatments like Panchakarma detoxification ⁸.

Key herbs commonly used in Ayurveda for diabetes management are, bitter melon (*Momordica charantia*) and fenugreek (*Trigonella foenum-graecum*), and Indian gooseberry (*Emblica officinalis*), all known for their potent blood sugar-regulating properties ⁹⁻¹¹. Ayurvedic principles underline the importance of maintaining balance in bodily functions while optimizing digestion, metabolism, and elimination to efficiently prevent and manage diabetes effectively. By addressing the root causes of diabetes and promoting overall well-being, Ayurvedic medicine offers a comprehensive and integrative approach to diabetes care. This holistic framework complements conventional

treatments, offering patients a pathway to achieve long-term health and vitality.

One such traditional Ayurvedic remedy, *Niruryadi Gulika* (NG), stands out for its potent therapeutic properties. Composed of a blend of medicinal herbs selected for their synergistic effects, NG has been used for generations to address various health concerns, including digestive, respiratory, and neurological issues ¹². NG is used for its anti-inflammatory and analgesic properties making it effective in managing conditions like joint pain & arthritis. Additionally, it promotes digestive health by alleviating symptoms of indigestion, bloating, and gas ¹³. Our research aims to investigate the properties of NG as a therapeutic agent in experimental diabetes. This investigation seeks to establish a foundation for integrating NG into modern therapeutic approaches for managing diabetes and its associated disorders.

MATERIALS AND METHODS

Composition and Preparation of extracts

NG, a traditional Ayurvedic herbal formulation, consists of a synergistic blend of medicinal herbs and natural ingredients meticulously selected for their therapeutic properties. This unique composition includes herbs such as Nirgundi (*Vitex negundo*) containing Vitamin C, carotene, vitexin and many anti-inflammatory flavonoids ¹⁴, Triphala which contains many essential tannins, gallic acid, flavanoids, anthraquinones ¹⁵, Haridra (*Curcuma longa* L.) which is rich in curcuminoids such as Curcumin, desmethoxycurcumin and bisdemethoxy and diarylheptanoids, contribute significantly to the antioxidant and anti-inflammatory properties of NG ¹⁶ and Daruharidra (*Berberis aristata*) which contains alkaloids such as xyberberine, palmatine, karachine, aromoline, taxilamine and oxyacanthine ¹⁷ each known for their specific health benefits including anti-inflammatory, immunomodulatory and antioxidant effects ¹⁸⁻²¹. Additionally, ingredients like Danti (*Baliospermum montanum* Muell) containing Baliospermin and montanin, Ativisha (*Aconitum heterophyllum*) containing many alkaloids, and Chitraka (*Plumbago zeylanica*) constituting of plumbagin, coumarins and chitranone contribute to the

formulation's digestive and carminative properties, aiding in the alleviation of gastrointestinal discomfort. Guggulu (oleo-gum) resin obtained from the bark of *Commiphora wightii* and Shilajit (*Asphaltum punjabianu*) containing more than 20 minerals essential for the body further enhance the formulation with their cholesterol-lowering, anti-inflammatory, and rejuvenating properties, promoting overall strength and well-being. The plant parts used in the study were collected, shade-dried and extracted using a Soxhlet extractor. The resulting compound was then purified, distilled, and evaporated under vacuum and the remaining extract was stored at 2-8°C for further studies.

Experimental animals

Male Wistar rats, weighing approximately 150-200g on average, were used as experimental animals. They were housed in an acclimatized environment, with temperatures maintained between 22°C to 25°C, while adhering to a natural 12-hour photoperiod. The rats had access to water and were fed commercial rat feed (Ratan Brothers, India) *ad libitum*. All study procedures were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee [Approval no IAEC/190/2022].

Induction of diabetes mellitus

To induce diabetes mellitus, the animals were initially divided into two groups. The first group (n = 6) was provided with a standard pelleted diet and served as the control, while the remaining rats (n = 40) were subjected to diabetes induction. Type 2 diabetes mellitus (T2DM) was induced in fasted rats by intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 40 mg/kg BW, dissolved in freshly prepared citrate buffer (pH 4.5)²². To prevent hypoglycemia from STZ administration, rats were given 5% glucose in their drinking water. After a three-day period, blood glucose levels of all experimental animals were measured using an Accu-Chek Instant Blood Glucose meter. Animals with blood glucose levels of ≥ 200 mg/dL were classified as diabetic and included in the study.

Experimental design

The animals were divided into six groups, each consisting of six rats, as follows: Group 1 - Untreated rats that received equivalent volume of the vehicle orally via gavage for 6 consecutive weeks; Group 2 - Diabetic control rats received

the same volume of the vehicle orally via gavage for 6 weeks; Group 3 - Diabetic rats treated with NG at 100 mg/kg /BW/day via oral gavage for 6 weeks; Group 4 - Diabetic rats treated with NG at 200 mg/kg/BW/day via oral gavage for 6 weeks; Group 5- Diabetic rats treated with NG at 300 mg/kg/BW/day via oral gavage for 6 weeks; Group 6 - Reference drug, metformin was administered at a dose of 500-mg/kg /BW/day via oral administration for 6 weeks. NG and the reference drug, metformin, were administered to the animals via oral gavage during the study. No observable discomfort or agitation was noted following drug administration and there were no significant adverse effects on the wellness or behavioural parameters of the experimental animals.

Blood glucose levels were monitored throughout the duration of 6 weeks. At the end of the study, the rats were starved for 6 hrs, and euthanized following CPCSEA guidelines using the CO₂ asphyxiation method. Serum was collected from whole blood by spinning at 2000 revolutions per minute for 5 minutes and was immediately stored in a deep freezer for subsequent analysis.

Measurement of glycated haemoglobin (HbA1C)

Haemoglobin A1c was estimated using a microcolumn (BioRad, CA, column size: 4.0 mm I.D \times 30 mm, Model no: D-10), and reported as a percentage of total haemoglobin²³. Additionally, HbA1C was measured using affinity chromatography with aminophenyl boronic acid gels (Millipore, USA) to minimize potential interference from malondialdehyde-modified hemoglobin (MDA-Hb) in the estimation.

Assessment of Antioxidant Enzymes Catalase (CAT) and Glutathione Peroxidase (GPx)

For CAT, a reaction mixture was prepared by combining phosphate buffer and H₂O₂ solution to achieve a final H₂O₂ concentration of 0.1-0.3%. The reaction mixture was pre-warmed to 37°C. Sample dilutions were prepared using phosphate buffer, and the reaction was initiated by adding an appropriate volume of the sample to the pre-warmed reaction mixture. The mixture was immediately transferred to a cuvette or microcentrifuge tube and placed in a spectrophotometer. The decrease in absorbance was measured at 240 nm to estimate CAT activity²⁴.

For GPx, enzyme activity was assessed by measuring the consumption of NADPH during the

reduction of oxidized glutathione (GSSG) catalyzed by glutathione reductase. One unit of GPx activity was defined as the amount of enzyme required to catalyze the formation of 1 micromole of reduced glutathione (GSH) per minute, corresponding to the oxidation of 0.5 micromole of NADPH per minute²⁵.

Estimation of TBARS

The standard solution of MDA was prepared by mixing 100 μ L of thiobarbituric acid reactive substances standard with 200 μ L of TBARS Acid Reagent, gently agitating the mixture

for 30 minutes to create a stock solution of 167 μ M. A 16.7 μ M solution act as the high standard, while deionized water was used as the blank. For the assay, 150 μ L of samples and standards were pipetted into the microplate wells, followed by the addition of 75 μ L of TBA reagent. The microplate was sealed with a cover strip and left undisturbed for 3 hrs at 45-50 $^{\circ}$ C and absorbance was estimated at 532 nm. The initial reading was subtracted from the final reading to calculate the unknown sample values²⁵.

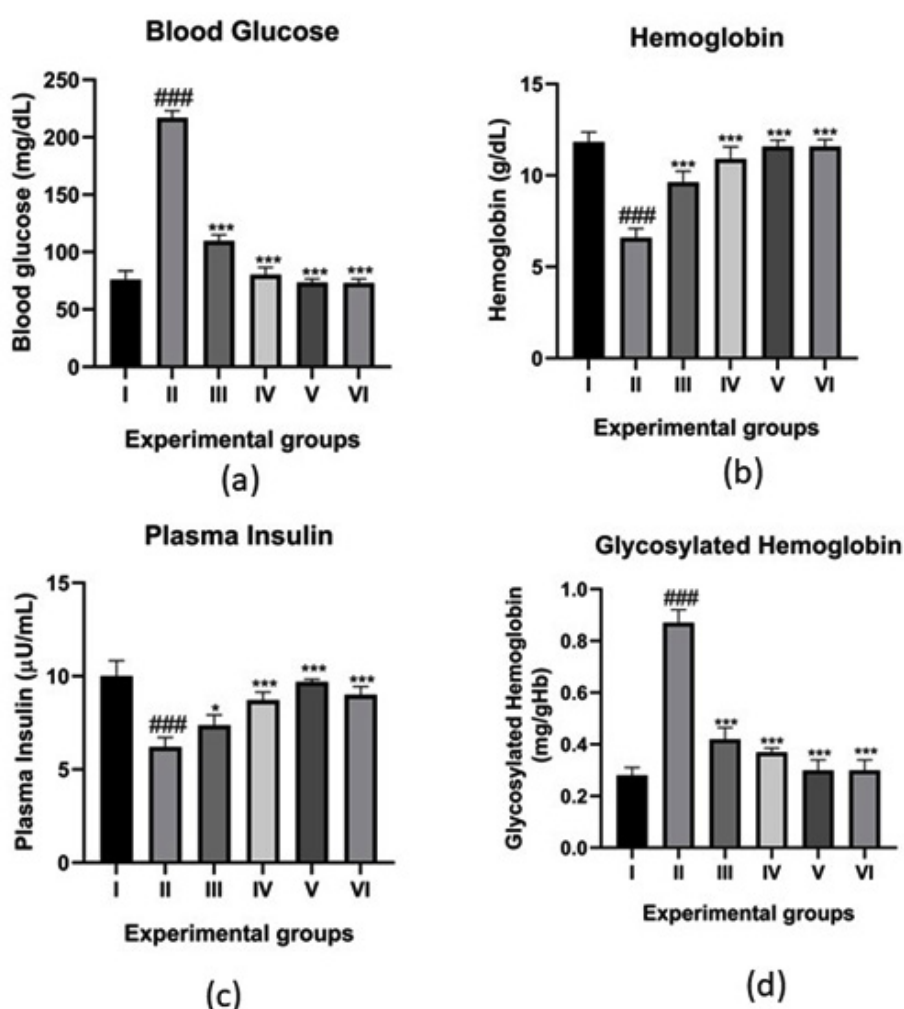


Fig. 1. Effect of NG on various biochemical parameters - (a) Blood glucose, (b) haemoglobin, (c) Plasma insulin, and (d) glycated haemoglobin levels in control and experimental diabetic rats. Data are expressed as Mean \pm SD from three separate experiments. Statistical analysis was conducted with One-Way ANOVA test. using GraphPad Prism 6.01 where *indicates comparison with diabetic control group and # indicates comparison with control group. (***) $p < 0.001$, (###) $p < 0.001$

Protocol for Lipid profile

The enzymatic method utilizing cholesterol esterase and cholesterol oxidase was adopted to estimate the serum levels of total cholesterol (TC) ^{26,27}. The HDL-c content in the supernatant was quantified in the supernatant after VLDL-c and LDL-c were precipitated using phosphotungstic acid and $MgCl_2$ ²⁸. LDL-c and VLDL-c were calculated using Fried Ewald's formula. Triglyceride levels were enzymatically estimated following hydrolysis by lipoprotein lipase, which generates glycerol-3-phosphate and dihydroxyacetone phosphate (DHAP) along with H_2O_2 , which was then converted to amino phenazone in the presence of peroxidase for measurement ²⁹.

Statistical analysis

All graphs and tables are presented as the mean \pm SD. Statistical analyses were conducted using GraphPad Prism (Version 5.01, GraphPad Software). Group comparisons were evaluated using Student's t-test, while multiple group analyses were performed with one-way ANOVA. Correlations between variables were assessed using

Pearson's correlation coefficient. A P-value of < 0.05 was considered statistically significant.

RESULTS

Figure 1 shows haemoglobin, plasma insulin, glycated haemoglobin, and glucose levels in control and diabetic rats. The diabetic group exhibited significantly elevated glucose levels (### $p < 0.001$) compared to controls. Treatment with NG at all doses significantly reduced blood glucose levels in a dose-dependent manner ($p < 0.001$), demonstrating comparable efficacy observed in the metformin-treated group ($p < 0.001$).

Figure 1(b) shows total haemoglobin levels across the experimental groups. The diabetic control group showed a significant reduction in haemoglobin levels compared to the control group ($p < 0.001$). However, rats treated with NG demonstrated a dose-dependent increase in haemoglobin levels ($p < 0.001$). Similarly, metformin-treated diabetic rats showed a significant increase in haemoglobin levels.

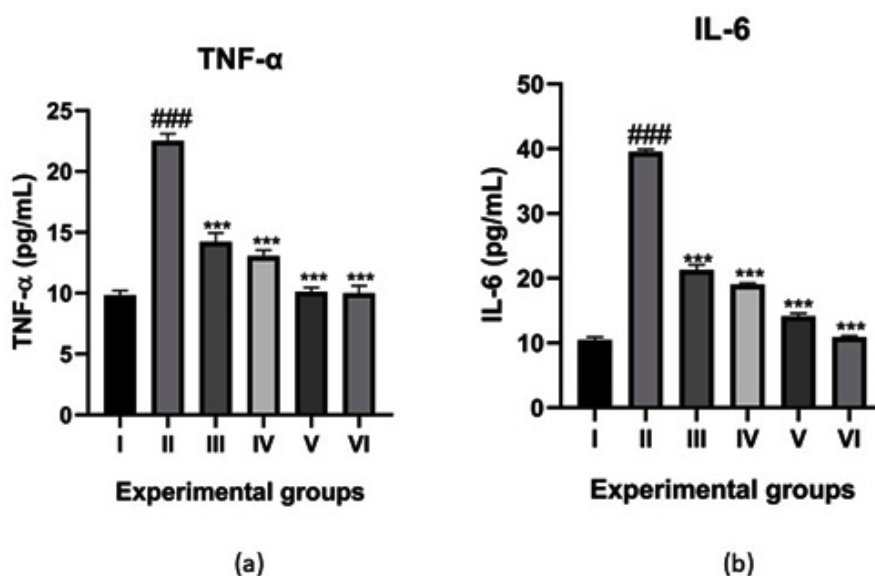


Fig. 2. Effect of NG on inflammatory cytokines – (a) TNF- α and (b) IL-6 levels control and experimental diabetic rats. Data are expressed as Mean \pm SD from three separate experiments. Statistical analysis was conducted with One-Way ANOVA test. One Way ANOVA test using GraphPad Prism 6.01 where * indicates comparison with diabetic control group and # indicates comparison with control group. (***) $p < 0.001$, (###) $p < 0.001$.

The diabetic control group exhibited significantly reduced plasma insulin levels compared to the control group $p < 0.001$) [Figure 1(c)]. In contrast, the diabetic rats treated with NG established a marked increase in insulin levels, demonstrating a similar trend as observed in the metformin-treated diabetic rats $p < 0.001$).

Figure 1(d) illustrates the HbA1c levels across the study groups. Diabetic control rats showed an approximately 0.7-fold increase in HbA1c levels compared to the control group ($p < 0.001$). In contrast, all NG treated diabetic rats exhibited a significant dose-dependent reduction in HbA1c levels ($p < 0.001$). Similarly, diabetic rats treated with metformin exhibited a marked decrease in HbA1c levels compared to the diabetic group.

Figure 2 depicts the effect of NG on the levels of inflammatory cytokine, TNF- α and IL-6, across the experimental groups. The cytokine levels were significantly elevated in the diabetic group compared to the control group. However, NG-treated diabetic rats exhibited a significant reduction in these cytokines, in a dose-dependent manner. Notably, the TNF- α levels in the 300 mg/kg NG-treated group were comparable to those observed in the metformin-treated rats, highlighting the potential anti-inflammatory properties of NG.

Figure 3 shows the effect of NG on lipid profile markers, indicating that triglycerides were significantly elevated in diabetic animals (### $p < 0.001$), while NG treatment resulted in a dose-dependent decrease. Total cholesterol levels also elevated in the diabetic group, were markedly

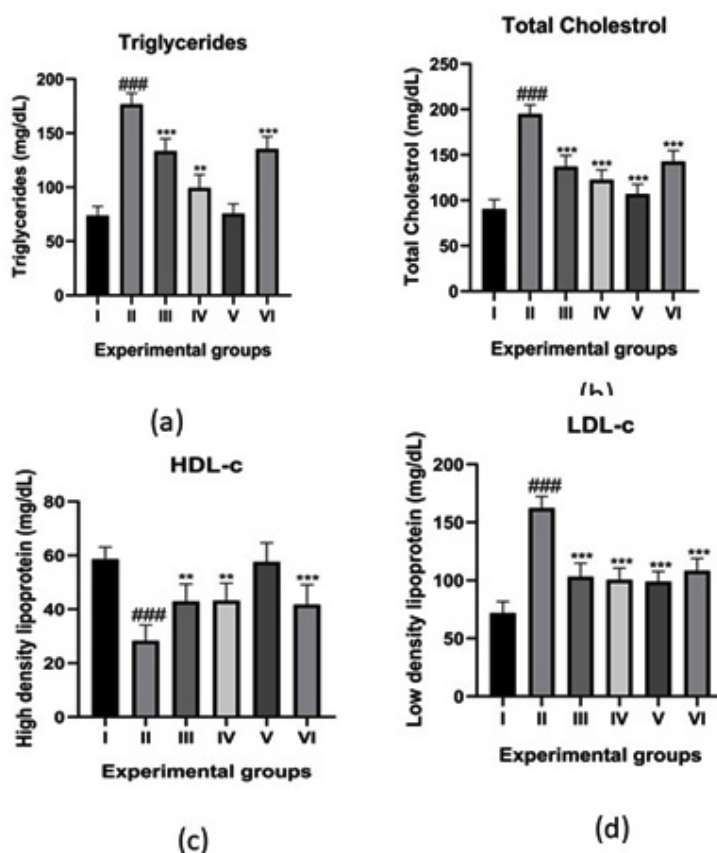


Fig. 3. Effect of NG on lipid profile markers (a) Triglycerides, (b) Total Cholesterol, (c) HDL-c and (d) LDL-c in the control and experimental diabetic rats. Data are expressed as Mean \pm SD from three separate experiments.

Statistical analysis was conducted with One-Way ANOVA test using GraphPad Prism 6.01 where * indicates comparison with diabetic control group and # indicates comparison with control group. (** $p < 0.01$, *** $p < 0.001$, ### $p < 0.001$)

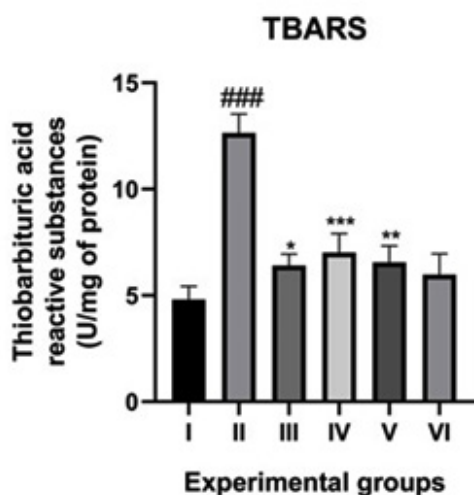


Fig. 4. Effect of NG on TBARS levels in the control and experimental diabetic rats. Data are expressed as Mean \pm SD from three separate experiments. Statistical analysis was conducted with One-Way ANOVA test using GraphPad Prism 6.01 where *indicates comparison with diabetic control group and # indicates comparison with control group. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ### $p < 0.001$).

reduced in the NG-treated groups ($p < 0.001$), with reductions comparable to those observed in the metformin treatment. HDL-c levels were significantly reduced in the diabetic control group ($p < 0.001$) but increased with NG treatment and metformin. LDL-c levels were higher in the diabetic control group and reduced in all NG-treated groups, showing similar reductions to the metformin-treated rats ($p < 0.001$).

Figure 4 depicts the levels of TBARS, a marker of lipid peroxidation, which was significantly elevated in the diabetic group ($p < 0.001$). Treatment with NG resulted in a dose-dependent reduction in TBARS levels, with similar effects observed in the metformin-treated rats.

Figure 5 highlights the significant decrease in antioxidant enzyme activity, specifically catalase and glutathione peroxidase (GPx), in the diabetic control group compared to the control group (### $p < 0.001$). NG treatment led to a dose-dependent increase in these antioxidant enzyme levels, with the 300 mg/kg NG-treated group showing the maximum improvement in catalase activity ($p < 0.01$). Similarly, both catalase and GPx levels were significantly elevated in the metformin-treated rats, reflecting enhanced antioxidant defense.

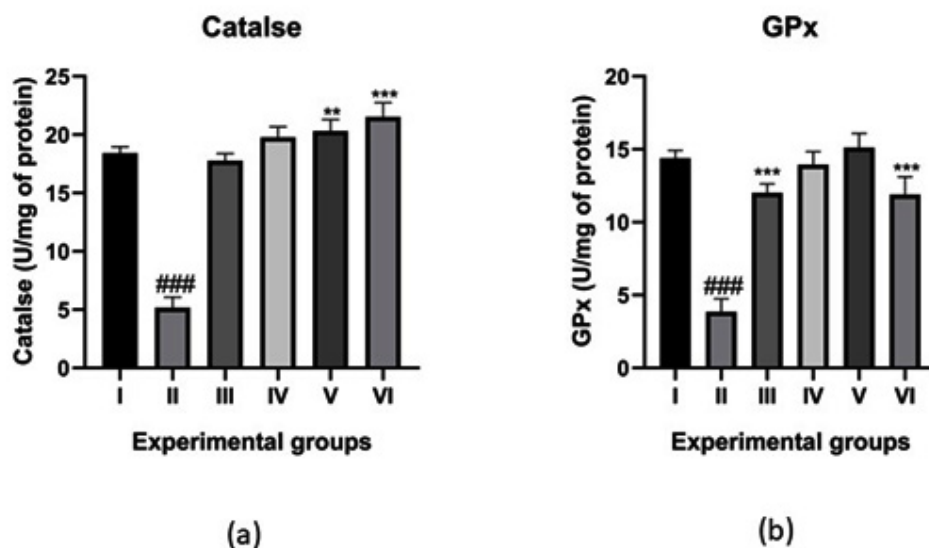


Fig. 5. Effect of NG on antioxidant enzymes (a) Catalase and (b) GPx levels in the control and experimental diabetic rats. Data are expressed as Mean \pm SD from three separate experiments. Statistical analysis was conducted with One-Way ANOVA test using GraphPad Prism 6.01 where *indicates comparison with diabetic control group and # indicates comparison with control group. (** $p < 0.01$, *** $p < 0.001$, ### $p < 0.001$)

DISCUSSION

Diabetes mellitus is a global health issue with a substantial impact, affecting approximately 500 million adults worldwide, with projections estimating an increase to over 750 million by 2045³⁰. This alarming rise underscores the urgent need for effective management and treatment strategies. Conventional diabetes treatments often have adverse effects, prompting researchers to explore alternative therapies with improved safety profiles. Herbal medicines have garnered increasing attention due to their minimal side effects and enhanced therapeutic potential^{31,32}. This study investigates the anti-diabetic and antioxidative role of NG in streptozotocin (STZ)-induced diabetic rats.

STZ induces diabetes by selectively destroying pancreatic beta cells, leading to decreased plasma insulin levels and elevated blood glucose^{22,33}. It damages DNA through alkylation in β cells by entering via the GLUT2 transporter, generating ROS, and releasing nitric oxide, which causes oxidative stress³⁴. This oxidative stress overwhelms the cell's antioxidant defences, resulting in cellular damage and triggering apoptosis and necrosis³⁵. Additionally, STZ induces inflammation, releasing pro-inflammatory cytokines, which are major contributors for beta cell death^{36,37}.

The combination of DNA damage, redox imbalance, and mitochondrial dysfunction results in significant β -cell loss, leading to a dramatic reduction in insulin secretion. This, in turn, results in hyperglycemia, mimicking the pathophysiological characteristics of diabetes, and thus provides a valuable model for studying the disease and potential treatments²². STZ also adversely affects lipid metabolism, increasing TG, LDL, and total lipid levels while decreasing HDL levels^{38,39}. Moreover, STZ disrupts the redox balance, contributing to oxidative stress and further beta cell damage^{40,41}. Our findings align with these previous studies, as the diabetic control group showed elevated blood glucose and haemoglobin levels, decreased plasma insulin and total haemoglobin levels, increased TNF- α and IL-6 levels, abnormal lipid profiles, and heightened lipid peroxidation. Treatment with NG

significantly ameliorated these adverse effects in a dose-dependent manner.

NG led to a marked reduction in glucose and haemoglobin levels, demonstrating its potent anti-diabetic properties. The most substantial reduction was observed in the rats administered 300 mg/kg NG. This group also displayed a significant increase in plasma insulin levels, indicating improved beta-cell function or enhanced insulin sensitivity. The dose-dependent response suggests that higher doses of NG might have a more pronounced therapeutic effect, although future findings are essential to confirm this.

Inflammatory cytokines play critical roles in the development of diabetes and its complications by contributing to beta cell destruction^{42,43}. Our study revealed that NG treatment significantly downregulated these cytokines. Notably, the 300 mg/kg NG-treated rats exhibited cytokine levels comparable to those in metformin-treated rats. This anti-inflammatory effect of NG may occur through the suppression of the NF-KB pathway, a known mechanism underlying metformin's anti-inflammatory action^{44,45}.

Diabetes is often associated with dyslipidaemia, and an elevated lipid profile^{46,47}. In our study, NG treatment normalized these lipid abnormalities, with the 300 mg/kg dose showing the most significant improvements. These findings suggest that NG may help mitigate the cardiovascular risks associated with diabetes by improving lipid metabolism.

Lipid peroxidation, indicated by elevated TBARS levels, is a common consequence of diabetes-induced oxidative stress^{48,49}. NG treatment significantly reduced TBARS levels in diabetic rats, indicating its potential to inhibit lipid peroxidation. This effect is crucial as it helps in protecting cellular membranes from oxidative damage, thereby preserving cell integrity and function.

Antioxidant genes such as catalase and GPx play vital roles in combating oxidative stress. Our study found that NG treatment significantly elevated these enzymes in diabetic rats. The 300 mg/kg NG-treated group exhibited particularly high levels of catalase, suggesting a potent antioxidative response. This enhancement of antioxidant defences aligns with previous studies

highlighting the role of dietary antioxidants in improving insulin responses and overall diabetic status^{50,51}.

The exact mechanisms through which NG exerts its anti-diabetic and antioxidative effects remain to be fully elucidated. However, it is plausible that NG enhances insulin secretion or sensitivity, modulates inflammatory pathways, improves lipid metabolism, inhibits lipid peroxidation, and boosts antioxidant defense mechanism. Further research, including molecular studies, is needed to uncover the precise pathways involved.

The findings of this study have significant clinical implications. NG, a natural compound, shows promise as a potential treatment for diabetes management, offering a safer alternative to conventional drugs with fewer side effects. Future studies should focus on clinical trials to validate these findings in humans. Additionally, exploring the synergistic effects of NG with other anti-diabetic agents could provide valuable insights into more effective combination therapies.

CONCLUSION

In conclusion, our research supports the role of NG as a potent anti-diabetic formulation, with beneficial effects on cellular antioxidant response and lipid peroxidation. NG treatment moderated plasma insulin levels, reduced high blood glucose, restored lipid profiles, lowered TBARS levels, and enhanced antioxidant enzyme activity in STZ-induced diabetic rats. These findings highlight the potential of NG in diabetes management and emphasize the need for further investigation into its mechanisms of action and clinical applications.

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Conflict of interest

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Data availability Statement

This statement does not apply to this article.

Ethical Approval

All study procedures were accomplished in agreement with Institutional Animal Ethics Committee guidelines [Approval no IAEC/190/2022]. This study did not involve human participants, so informed consent was not required.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author Contributions

Conceptualization, R.C.S, C.D., K.M.R.; Methodology and analysis, R.C.S, M.B.; Investigation, R.C.S, M.B.; Writing—original draft preparation, R.C.S, M.B.; Writing-review and editing, K.M.R., C.D.; Funding - R.C.S, C.D.; Supervision, C.D.

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