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In Vivo Antidiabetic Potential Of Niosome Naringin Nanoconjugate In Streptozotocin Induced Diabetic Mice

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Abstract

Diabetes mellitus remains a significant global health concern, necessitating the exploration of novel therapeutic strategies. In this study, we investigated the *in vivo* antidiabetic potential of Niosome Naringin Nanoconjugate, a nanoformulation designed to enhance the bioavailability and therapeutic efficacy of naringin, a naturally occurring flavonoid with reported antidiabetic properties. Male Wistar rats were induced with diabetes mellitus using streptozotocin and treated with Niosome Naringin Nanoconjugate orally for a specified duration. The effects of the nanoconjugate on glucose tolerance, fasting blood glucose levels, insulin resistance, and lipid profile were evaluated. Our results demonstrate that Niosome Naringin Nanoconjugate significantly improved glucose tolerance, reduced fasting blood glucose levels, and ameliorated insulin resistance compared to diabetic control groups. Additionally, the nanoconjugate exhibited enhanced bioavailability and prolonged circulation time, suggesting potential for sustained therapeutic effects. These findings highlight the promising antidiabetic efficacy of Niosome Naringin Nanoconjugate and underscore its potential as a novel therapeutic agent for the management of diabetes mellitus. Further investigations, including long-term safety assessments and clinical trials, are warranted to fully elucidate its clinical applicability. Overall, this study contributes valuable insights into the development of innovative nanomedicine approaches for diabetes management, with Niosome Naringin Nanoconjugate emerging as a promising candidate for future therapeutic interventions.

Keywords: diabetes, polyphenols, nanotechnology, biochemical, toxicity, animal model

Introduction

Diabetes mellitus is a prevalent metabolic syndrome characterised by elevated levels of blood glucose. There has been a steady rise in the number of individuals diagnosed with diabetes, with projections indicating that the global count will surpass 600 million by 2045. Contemporary antidiabetic medications effectively address

hyperglycemia and the associated complications resulting from elevated blood glucose levels (Fouad et al., 2023). Nevertheless, the adverse reactions associated with these medications have sparked interest in exploring plant extracts and bioactive compounds that possess antidiabetic properties as potential alternative therapies for diabetes. It is widely acknowledged that natural products possess a range of advantageous qualities, such as biocompatibility, cost-effectiveness, and a reduced adverse effect, when compared to existing antidiabetic medications (Zolkepli et al., 2022).

Naringenin, a flavonoid categorized under the flavanones subclass, is widely present in various Citrus fruits, bergamot, tomatoes, and other fruits, often existing in its glycoside form, particularly as naringin. This phytochemical has demonstrated numerous biological activities, including antioxidant (Gerçek et al., 2021), antiviral (Tutunchi et al., 2020), antibacterial (Veiko et al., 2023), anti-inflammatory (Hassan et al., 2021), antiadipogenic (Dayarathne et al., 2021), and cardioprotective (Uryash et al., 2021) effects. Although the existing data are promising, there is a need for further research focusing on pharmacokinetic and pharmacodynamic aspects. This is crucial to refine production and delivery methods and to develop practical clinical formulations based on naringenin (Salehi et al., 2019) (Den Hartogh & Tsiani, 2019).

Nanotechnology, an innovative scientific field, focuses on the peculiarities of the nanoscale. Nanocarriers, characterized by a significant surface area to mass ratio, typically exhibit effective interaction with their surroundings. However, they can also function as confined carriers for their constituent molecules rather than engaging with these molecules in solution. Consequently, nanocarriers show promise as effective vehicles for targeted drug delivery in therapeutic applications. The potential of nanocarriers to act as "magic bullets," selectively targeting damaged organs and cells while sparing normal tissues, has sparked interest in utilizing nanotechnology for drug delivery. Enhancing the targeted delivery of drugs can be achieved through surface modifications of nanocarriers. Modifying the surface of nanocarriers, such as incorporating polyethylene glycol, proves beneficial in preventing opsonization (Jahangirian et al., 2017).

Vesicles known as niosomes have a bilayer membrane that surrounds a watery compartment, like liposomes. On the other hand, niosomes are formulated using non-ionic surfactants rather than phospholipids. Research has shown that niosomes have a higher drug encapsulation efficiency compared to liposomes. This is attributed to the lower levels of cholesterol present in niosomes. The stability of non-ionic surfactants used in niosome preparation exceeds that of lipids used in liposome production, both in terms of physical and chemical stability (Yeo et al., 2017).
By investigating the effects of Niosome Naringin Nanoconjugate on biochemical parameters, acute toxicity and histopathological analysis in diabetic animal models, this research provides valuable insights into the development of innovative nanomedicine approaches for diabetes management. The findings of this study hold promise for the development of Niosome Naringin Nanoconjugate as a novel therapeutic agent for diabetes mellitus.

Materials and methods

Induction of diabetes mellitus

After an acclimatization on period (2 weeks), all rats were subjected to overnight fasting (16 h) except normal control animals. Thirty rats received an intraperitoneal (i.p) injection of STZ (65 mg/kg BW). STZ was diluted in citrate buffer to a concentration of 0.1 M and pH=4.5 just before use, 30 min after STZ administration. To avoid hypoglycemic shock and death, glucose solution (5%) was administered 30 min after STZ injection for the first 48 h. After 72 h, rats were fasted overnight (16 h) once more, and their blood glucose levels were assessed. Hyperglycemic rats (glycemia ≥150 mg/dL) were considered diabetic for treatment.

Experimental design

Thirty rats were divided into the following treatment groups:
Group 1: Normal control: Treated with distilled water
Group 2: Diabetic control: Streptozotocin (65 mg/kg) induced diabetic rats
Group 3: Streptozotocin-induced diabetic rats treated with Naringin (40 mg/kg)
Group 4: Streptozotocin-induced diabetic rats treated with Niosome Naringin Nanoconjugate (100 mg/kg)
Group 5: Streptozotocin-induced diabetic rats treated with standard Metformin (70 mg/kg)
All rats were given free access to water and food ad libitum during treatment.

Determination of body weight

Body weight determination was carried out for all groups of mice at the initiation of the study and on final day. The measurement of the experimental mice's body weight was conducted using a suitably calibrated electronic balance to ensure accurate and reliable results (Melesie Taye et al., 2020).

Serum biomarker analysis

Serum lipid profile indicators such as VLDL, Phospholipids, LDL, HDL, TC and TGs levels and hepatic functional markers such as ALP, ALT, AST, Total protein, Globulin and Albumin were analysed (Khan et al., 2022). Serum biochemical parameters such as insulin and glucagon Hb, HbA1C, and blood glucose in three-time interval and renal biomarkers were analysed for the experimental group.

Antioxidant profile

After 24 hours from the last administered dosage, all experimental animals were euthanized, and the pancreas, kidney and liver of each rat were dissected. Subsequently, the tissues underwent extraction, homogenization, and centrifugation for 30 minutes at 1500 rpm to isolate their supernatant. The concentrations of MDA, SOD, CAT, GPx, GSH, Vit C and Vit E in the supernatant were quantified (Khan et al., 2022).

Histological Analysis of Kidney, Liver, and Pancreas

An examination of the sample tissues was conducted through histological analysis to assess the impact of noisome narinjin nanoconjugate on their anatomy. Following the administration of anaesthesia, the liver, pancreas, and kidney tissues of mice were carefully isolated to prevent any degradation or post-mortem autolysis. The tissues were carefully preserved in a 10% formalin solution. The tissue samples were carefully sliced into thin sections measuring approximately 4 microns using a microtome. These sections were then dehydrated and stained using the H&E (Haematoxylin and Eosin) staining system. The slides were subsequently analysed using a light microscope (Wahab et al., 2022) .

Statistical analysis

The data is presented as the mean ± SEM. An analysis was conducted to compare the pharmacokinetic parameters and formulations, using analysis of variance. The statistical evaluation of the *in vivo* experiments was conducted using Duncan's multiple range test (DMRT). A comparison was made between group I (control) and groups II, III, IV, and V to determine any statistically significant variations. A significance level of less than 0.05 was considered to be statistically significant (Raafat & El-Zahaby, 2020).

Results

Effects of sample on Body weight and organ weight in normal and experimental diabetic rats

Internal organs were collected for weight study. Decreased weight of liver (8.29±0.29) g, pancreas (1.07±0.14) g, and kidney (1.67±0.06) g were observed in a diabetic group, but improvement was observed in naringin conjugate groups; the weight of liver (7.72±0.23) g, pancreas (0.88±0.04) g, and kidney (1.43±0.12) g were improved and presented in Table 1. Also, the body weight of the mice in different days have been represented in the same table.

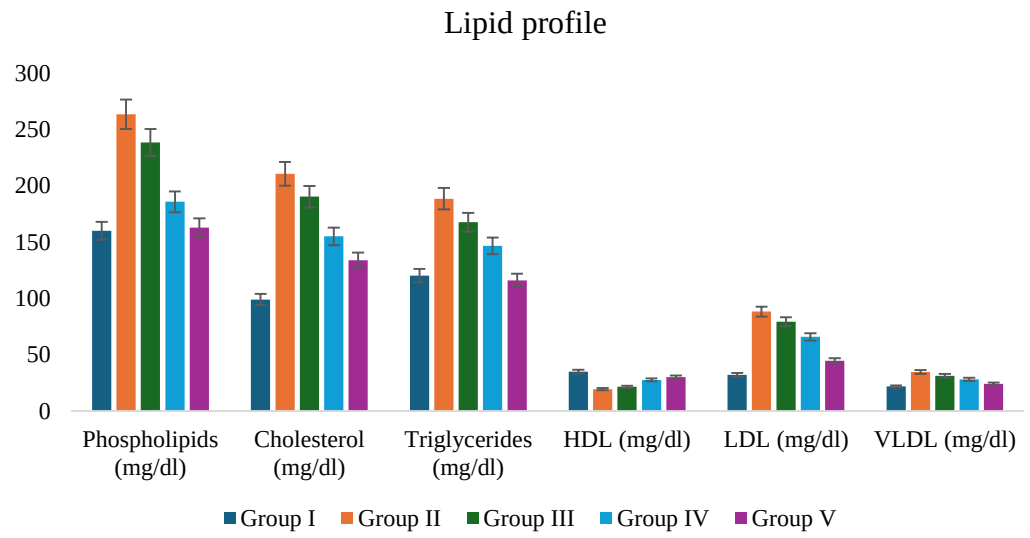
Table 1: Effects of sample on Body weight and organ weight in normal and experimental diabetic rats

Parameters	Group I	Group II	Group III	Group IV	Group V
Initial day (gm)	178.39±2.09	171.71±3.90^{a}	172.37±2.16^{a}	175.63±2.12^{a}	177.13±2.17^{a}
Final day (gm)	186.73±3.74	153.27±2.16^{b}	160.21±1.54^{b}	171.47±3.63^{a}	173.61±3.17^{a}
Liver weight (gm)	6.29±0.08	8.29±0.29^{b}	7.89±0.07^{a}	7.72±0.23^{c}	7.01±0.12^{a}
Kidney weight (gm)	1.15±0.08	1.67±0.06^{b}	1.57±0.19^{c}	1.43±0.12^{c}	1.29±0.08^{a}
Pancreases weight (gm)	0.75±0.15	1.07±0.14^{b}	0.95±0.03^{c}	0.88±0.04^{a}	0.80±0.04^{a}

Anti-hyperlipidemic potential

The mice of untreated diabetic control groups (DC) have shown an increase in cholesterol and triglycerides level as compared to the normal control group (NC). On the other hand, a noteworthy decrease in these parameters was observed in the mice of the treated group including the diabetic treated control group (TC) as compared to the untreated diabetic control group (DC). However, the best results have been observed in mice of the group treated with naringin nanoconjugate (Figure 1).

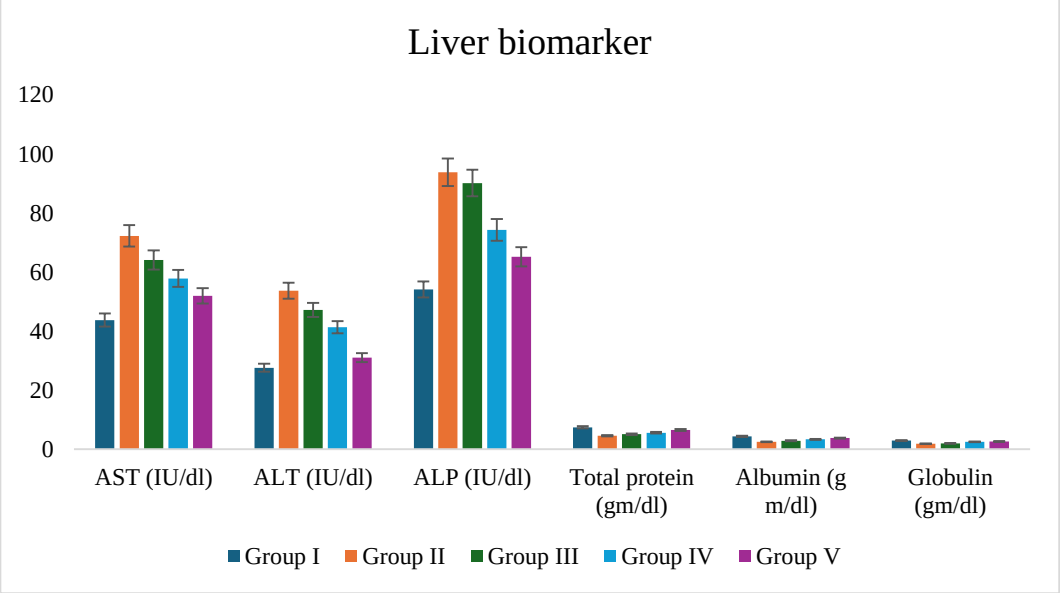
Figure 1: Effects of sample on lipid levels of control and experimental diabetic rats



Liver profile study

The liver profile done during this study has shown strong hepatoprotective effects of the naringin nanoconjugate against diabetic changes. Liver enzymes of mice of the diabetic untreated diabetic control group (DC) were increased while a marked decrease was observed in the total protein and albumin contents of mice of this untreated control group as compared to the normal control group (NC). Naringin nanoconjugate exhibited strong hepatoprotective effects as a significant decrease was observed in the liver enzymes like AST, ALT and ALP along with improvement in total protein, globulin and serum albumin levels in the naringin nanoconjugate treated groups as compared to the diabetic untreated control group (DC) (Figure 2).

Figure 2: Effects of sample on biochemical markers in control and experimental diabetic rats



Reduction of blood glucose and glycosylated hemoglobin levels

A significant increase in glucose concentration was observed in mice of the untreated diabetic control group (DC) as compared to the normal control group (NC). After 21 days of administration of the samples (metformin, naringin and naringin nanoconjugate), an observable reduction was noted in the glucose level of almost all groups in comparison with the untreated control group (Table 2). Similar results were observed in glycosylated hemoglobin (HbA1C) level (Table 3) and the noticeable decrease was shown by all treated groups of mice as compared to the untreated diabetic control group (DC). Overall, maximum activity has been shown by naringin nanoconjugate and its glucose-lowering effects are comparable with the results of the group treated with standard drug (Figure 4).

Table 2: Effects of sample on plasma glucose in control and experimental diabetic rats

Parameters	Group I	Group II	Group III	Group IV	Group V
1st day	96.38±4.78	243.07±3.11^{b}	230.12±4.83^{c}	221.31±10.10^{c}	200.57±5.05^{a}
11th day	97.96±4.31	270.83±6.58^{b}	251.84±7.36^{c}	201.49±5.76^{c}	178.34±3.03^{a}
21st day	99.26±4.04	298.15±6.22^{b}	275.85±5.56^{c}	180.07±3.52^{a}	157.27±4.70^{a}

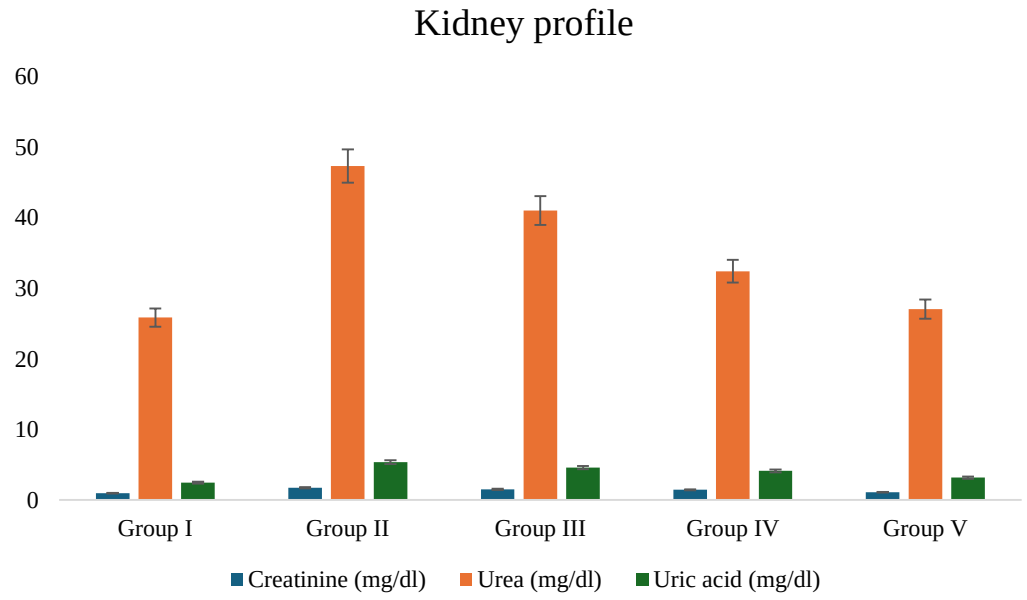
Table 3: Effects of sample on Haemoglobin and glycosylated haemoglobulin in control and experimental diabetic rats

Parameters	Group I	Group II	Group III	Group IV	Group V
Hb (gm/dl)	14.85±0.11	8.28±0.26^{b}	9.37±0.28^{c}	11.39±0.34^{a}	12.51±0.58^{a}
HbA1c (% Hb)	6.38±0.09	13.68±0.59^{b}	12.71±0.97^{c}	11.10±0.15^{a}	8.70±0.07^{a}

Effects of the samples on kidney functions tests

It has been observed that the naringin nanoconjugate showed a positive effect on kidney functions of the mice groups. All parameters (urea, creatinine, uric acid) were observed to be high in the diabetic untreated control group and treatment of the mice with the naringin nanoconjugate decreased blood urea, creatinine and uric acid levels in all groups on comparison with the diabetic untreated control group (Figure 3).

Figure 3: Effects of sample on urea, creatinine and uric acid in control and experimental diabetic rats



Effect of samples on various glucose biomarker enzymes

Table 4 illustrates the impact of samples on the activities of glycogen, glucokinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, and glucose-6-phosphate dehydrogenase. In control rats there were no notable changes in the levels of these parameters. However, in diabetic rats, the activities of all these enzymes decreased significantly, while the activities of glucose-6-phosphatase increased significantly. Upon treatment with niosomes and metformin in diabetic rats, the altered enzyme activities were restored close to normal levels.

Table 4: Effects of sample on Glucose 6-phosphatase, Fructose 1, 6-bisphosphatase and glycogen in control and experimental diabetic rats

Parameters	Group I	Group II	Group III	Group IV	Group V
Glucose 6-phosphatase (µmoles of Pi liberated/h/mg protein)	1179.86±22.78	1817.93±22.25^{b}	1587.91±31.52^{c}	1392.54±31.63^{c}	1291.68±37.52^{a}
Fructose 1, 6-bisphosphatase (µmoles of Pi liberated/h/mg protein)	546.15±15.05	844.87±16.59^{b}	771.76±16.71^{c}	650.75±14.01^{a}	608.36±16.77^{a}
Glycogen (mg/100g tissue)	54.30±2.14	29.83±2.47^{b}	34.83±3.10^{c}	42.27±2.71^{a}	48.70±3.85^{a}
Insulin (µU/ml)	14.81±0.67	5.60±0.28^{b}	8.77±0.15^{c}	11.58±0.63^{a}	13.07±0.45^{a}
Glucagon (pg/ml)	132.45±12.5	180.21±0.21^{b}	170.58±2.13^{c}	161.69±1.20^{a}	140.23±2.31^{a}

Oxidative Stress Biomarkers

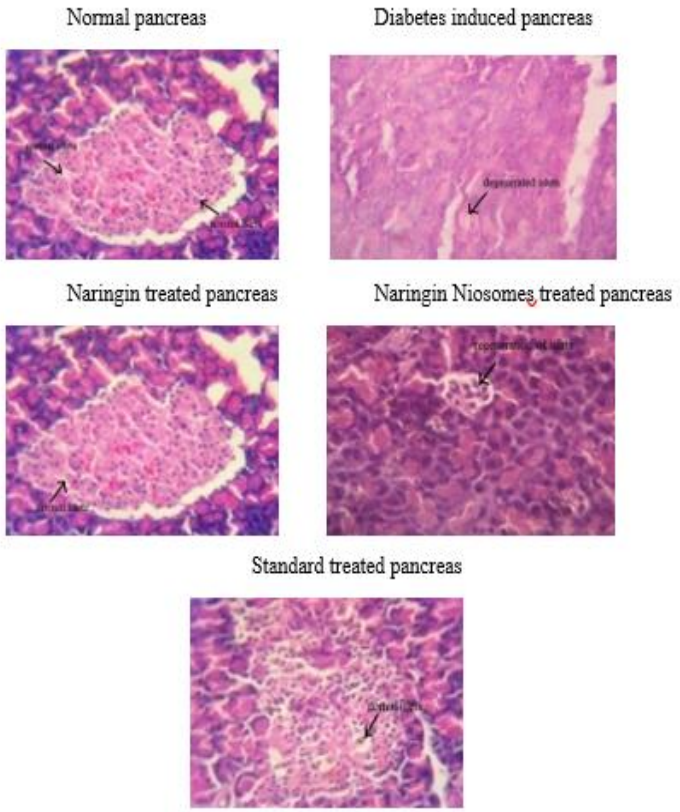
The synthesized noisome possesses antioxidant properties, which scavenged the oxidative stress. This effect was seen at different doses in serum, whole blood (hemolysate), liver, pancreatic, and kidney tissues samples of rats (Table 5). The present study indicated the elevation of MDA contents, a reliable marker of lipid peroxidation in the homogenate after diabetes induction with a significant ($p < 0.001$) in the noisome+metformin disease group, while treatment with noisome significantly ($p < 0.001$) reduced the MDA level to normalcy. The antioxidant parameters, including SOD, GPx, GSH, Vitamin C, Vitamin E, MDA, and CAT, were observed to be comparable between the normal group and the group treated with standard metformin in the diabetes-induced model. However, the serum antioxidant levels were noted to be diminished in the diabetes-induced group.

Table 5: Oxidative Stress Biomarkers in treatment group

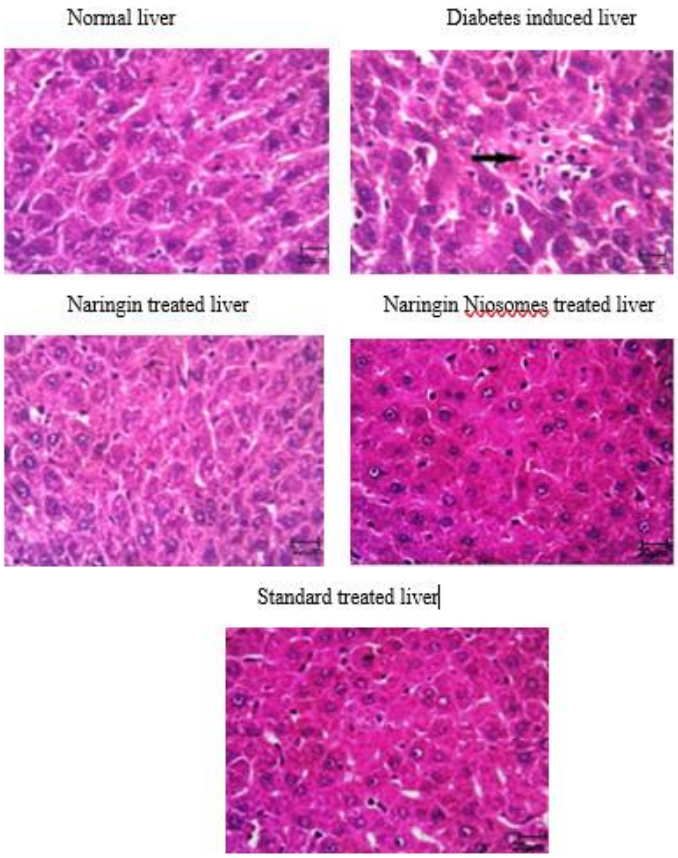
Parameters	Group I	Group II	Group III	Group IV	Group V
SOD	3.93±0.19	2.77±0.17[b]	2.39±0.19[c]	2.85±0.10[a]	3.32±0.01[a]
GPx	8.11±0.10	5.17±0.14[b]	5.63±0.11[c]	6.67±0.19[c]	7.09±0.10[a]
GSH	6.91±0.19	4.54±0.01[b]	4.79±0.12[c]	5.24±0.98[a]	6.24±0.11[a]
CAT	4.55±0.10	2.39±0.01[b]	2.17±0.10[c]	3.21±0.09[a]	3.85±0.01[a]
Vit C	5.28±0.14	3.41±0.17[b]	4.08±0.78[c]	4.55±0.10[a]	4.96±0.18[a]
Vit E	4.35±0.01	2.20±0.03[b]	2.61±0.02[c]	3.12±0.01[a]	3.73±0.01[a]
MDA (nmol of MDA formed/L)	7.08±0.56	16.09±1.71[b]	13.51±1.22[c]	12.04±0.68[a]	8.60±0.50[a]

Histological study

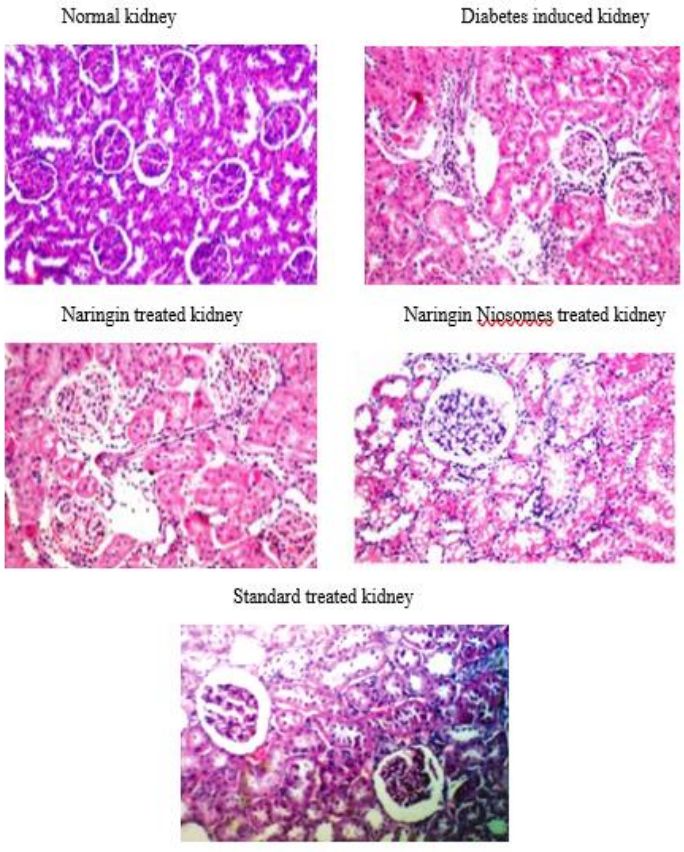
The Figure 4a, 4b and 4c represents the histopathological studies on pancreas, liver and kidney. The results suggest that treatment with metformin and naoconjugate was shown to be effective compared to the treatment with noisome and naringin alone, this could be due to the synergistic effect of the compounds in diabetes treatment. The cell was normalised in diabetic rat in the group V compared to the control.



4a


Normal liver
Diabetes induced liver
Naringin treated liver
Naringin Niosomes treated liver
Standard treated liver

4b


Normal kidney
Diabetes induced kidney
Naringin treated kidney
Naringin Niosomes treated kidney
Standard treated kidney

4c

Discussion

The rise in popularity of herbal remedies suggests they could serve as viable alternatives to conventional allopathic medication. Yet, ensuring the safety of plant-based treatments requires thorough examination of historical uses, effects on humans and animals, and results from standard toxicity tests. Various established screening methods are employed to evaluate the effectiveness, safety, and active components of herbal medicines (van Wyk & Prinsloo, 2020). Recent investigations suggest that *S. decora* has a reminiscent mechanism as metformin. Metformin, an oral hypoglycemic drug belonging to the dimethylbiguanide class, is derived from guanidine, an active hypoglycemic compound extracted from *Galega officinalis*, a medicinal plant with a centuries-old history of use in managing diabetes (Hawley et al., 2002). Widely utilized globally for individuals diagnosed with Type 2 diabetes, metformin is proficient in hindering hepatic glucose production and functions as an insulin sensitizer in isolated skeletal muscle derived from humans exhibiting insulin resistance (Musi et al., 2002).

In the present study, upon treatment with naringin nanoconjugate and metformin significantly lowered the level of blood glucose and improved the insulin levels in high fat diet and streptozotocin – induced diabetic rats. This glucose lowering and elevated insulin levels are brought by the antioxidant potential of naringin (Rangkadilok et al., 2007). The consumption of natural phytochemicals containing antioxidant effect were reported to have potential health benefits and help to regenerate β cells and protect pancreatic islets against cytotoxic effects of diabetes induced drug (Fernández-Alvarez et al., 2004). The predominant intracellular storable form of glucose is glycogen, which is directly regulated by insulin by increasing glycogen synthase and inhibiting glycogen phosphorylase in liver and skeletal muscles. Streptozotocin destroys β cells in the islets of Langerhans, lowering insulin levels and reducing glycogen stores in liver and muscle tissues. This process inhibits glucose invasion in the liver without insulin (Vats et al., 2004). We found that supplementing diabetic rats with naringin niosome and metformin enhanced glycogen content in both hepatic and skeletal muscles by increasing glycogen synthase and blocking glycogen phosphorylase, possibly due to elevated insulin levels.

Gluconeogenic enzymes like glucose-6-phosphatase and fructose-1, 6-bisphosphatase regulate blood glucose homeostatically in the liver and kidney and supply glucose to other organs during diabetes, prolonged fasting, and starvation (Bouché et al., 2004). Increased glucose-6-phosphatase and fructose-1, 6-diphosphatase in liver and kidney of high-fat diet and streptozotocin-induced diabetic rats may be attributed to insulin deficiency. Naringin niosome and metformin administration in diabetic rats dramatically reduced glucose-6-phosphatase and fructose-1, 6-diphosphatase activity. Improved insulin secretion may suppress gluconeogenic core enzymes.

Hyperglycemia generates free radicals that exhaust antioxidant defenses, disrupting cellular activities, damaging membranes, and increasing lipid peroxidation risk. Lipid hydroperoxides result from high lipid peroxidation. In diabetic rats' hepatic and renal tissues, lipid peroxides and hydroperoxides increased while enzymatic antioxidants (SOD, CAT, GPx) and non-enzymatic antioxidants (Vitamin C, Vitamin E, and GSH) decreased (Parveen et al., 2010). The antioxidant and antiperoxidative activities of naringin niosome oral treatment reduced lipid peroxidation and hydroperoxides in liver and kidney tissues of diabetic rats.

Elevated levels of AST, ALT, and ALP enzyme activity serve as indicators of liver function in individuals with diabetes, indicating the harmful impact of streptozotocin on the liver. These enzymes are accountable for the transformation of amino acids into keto acids, and their concentrations may be increased primarily due to liver injury, resulting in their release into the bloodstream (Mahendran et al., 2014; Veiko et al., 2023). The therapy of naringin niosomes has demonstrated a protective effect by decreasing the activity of these enzymes, indicating their potential for hepatoprotection.

The lipid profile parameters of diabetic and treated mice models reveal insights into metabolic alterations. Elevated levels of lipid biomarkers often accompany diabetes, while treatment may mitigate these effects, potentially reducing cardiovascular risk. As observed in the present study treatment with nanoconjugate and metformin group of diabetes induced model there was significant reduction in lipid parameters as in control group. Elevated levels of lipid biomarkers were observed in diabetes induced group. In a similar study, antiatherogenic properties of naringin in type 2 diabetes (T2DM) using a high-fat, low-streptozocin rat model was studied, the results indicated that naringin treatment for 21 days resulted in dose-dependent reductions in lipid parameters also gene expression analysis suggests naringin enhances reverse cholesterol transport and paraoxonase activity, implicating its potential in managing T2DM complications (Rotimi et al., 2018). Understanding lipid changes in these models aids in evaluating therapeutic efficacy and disease progression.

Insulin, the primary hormone responsible for regulating blood sugar levels in our bodies, in a similar study a mean level of 4.16 ± 0.75 ng/mL was observed in healthy animals, which decreased to 0.83 ± 0.75 ng/mL in diabetic groups. However, in groups treated with naringin, there was a dose-dependent increase in insulin levels. Similarly,

the mean glycated hemoglobin level in diabetic animals was 7.66 ± 0.51, while normal animals and naringin-treated groups exhibited lower mean values of 3.5 ± 0.54. As the naringin dose increased, there was a notable reduction in HbA1c%. Specifically, the mean HbA1c% for NR-1, NR-2, and NR-3 was 6.66 ± 0.51, 5.66 ± 0.51, and 4.66 ± 0.51, respectively (Ahmad et al., 2022). This result was in par with our where the level of insulin was found to be 5.60±0.28 µU/ml in diabetic animals and in group V the level was 13.07±0.45 µU/ml. Similarly, the Hb levels were found to be 8.28±0.26 mg/dl in diabetic animals and the levels was found to be equal to normal in group V-12.51±0.58 mg/dl.

Conclusion

In conclusion, this study demonstrates the promising potential of Niosome Naringin Nanoconjugate as an effective agent in the management of diabetes mellitus. Through *in vivo* experimentation, the nanoconjugate exhibited significant antidiabetic activity, as evidenced by its ability to improve glucose tolerance, reduce fasting blood glucose levels, and ameliorate insulin resistance in experimental animal models. Moreover, the nanoconjugate demonstrated enhanced bioavailability and prolonged circulation time, suggesting its potential for sustained therapeutic effects. These findings underscore the importance of further exploration and optimization of Niosome Naringin Nanoconjugate as a novel therapeutic strategy for the treatment of diabetes mellitus. Further studies, including long-term safety assessments and clinical trials, are required to fully elucidate its efficacy and safety profile for clinical translation. Overall, the results of this study contribute valuable insights towards the development of innovative nanomedicine approaches for the management of diabetes and underscore the potential of Niosome Naringin Nanoconjugate as a promising candidate for future therapeutic interventions.

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