Scientific Journal for Damietta Faculty of Science **15**(2) 2025, 1-11
Doi: 10.21608/sjdfs.2025.402875.1251
ISSN Print 2314-8594
ISSN Online 2314-8616

https://sjdfs.journals.ekb.eg/

The Role of Naringenin in Enhancing Metformin Efficacy in Diabetic Rats

Mayar Akram Shamkh[1], Abdel-Aziz Fatouh Abdel-Aziz[2] and Rasha Fekry Zahran[*1]

[1]Chemistry Department, Faculty of Science, Damietta University, 34517 Damietta, New-Damietta, Egypt.
[2]Chemistry Department, Faculty of Science, Mansoura University, Mansoura, Egypt.

Received: 11 July 2025 /Accepted: 25 July 2025

[*]Corresponding author's E-mail: zahran@du.edu.eg

Abstract

Background: Diabetes mellitus is linked to hepatic dysfunction, requiring effective therapeutic strategies. Metformin (MET), a standard antidiabetic agent, and naringenin (NAR), a citrus flavonoid, have shown complementary actions in enhancing insulin sensitivity and reducing oxidative damage through activation of AMP-activated protein kinase (AMPK). This study explores the dose-dependent effects of MET and NAR combinations on liver function and antioxidant status in diabetic rats.
Methods: Sixty male wistar rats were divided randomly into six groups: G1, normal control; G2 vehicle control (MET 50 mg/kg + NAR 50 mg/kg); G3, diabetic group (injected ip with nicotinamide (230 mg/kg) followed by streptozotocin (65 mg/kg), and three diabetic treated groups G4, G5 and G6 received MET (50 mg/kg) with NAR at 25, 50, or 100 mg/kg. respectively. Treatments were administered orally for five weeks. Body weight and fasting blood glucose (FBG) were recorded weekly, along with biochemical liver function parameters and total antioxidant capacity at the end of the experiment.
Results: Diabetic rats showed significant weight loss, elevated FBG, liver enzymes (ALT, AST), and bilirubin, alongside reduced total protein and total antioxidant capacity (TAC). Treatment with MET plus NAR significantly resulted in body weight increase, FBG, liver enzymes and TAC normalized compared to untreated diabetic rats.
Conclusion: These findings suggest that the combination of metformin and naringenin exerts synergistic antidiabetic and hepatoprotective effects. At the same time improve the antioxidant status in diabetic rats.

Keywords: antidiabetic, hepatoprotective, antioxidant, naringenin, streptozotocin

Introduction

Diabetes mellitus is a long-term metabolic disorder marked by persistently high blood glucose concentrations. This condition arises either due to insufficient insulin

production by the pancreas or due to impaired cellular responsiveness to insulin. Over time, such dysregulation in glucose homeostasis may result in significant health complications (Saeedi et al., 2019). Among its different types, type 2 diabetes is the most prevalent form.

Type 2 diabetes (T2D) represents the most prevalent form of diabetes, constituting more than 90% of cases worldwide (Rob et al., 2025)**.** It is a chronic metabolic condition marked by sustained hyperglycemia, which stems from both insulin resistance in peripheral tissues and an insufficient insulin secretion by pancreatic beta cells. This dysregulation disrupts normal metabolism of carbohydrates, fats, and proteins. The disease progression involves a decline in beta-cell function which disrupts the body's capacity to regulate blood glucose levels effectively, leading to elevated blood glucose levels and associated metabolic complications (Galicia-Garcia et al., 2020, Reed et al., 2021). According to previous studies which reported that diabetes mellitus induces a significant imbalance in the liver's oxidative status, characterized by elevated reactive oxygen species (ROS) production and diminished activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). This oxidative stress in the liver contributes to cellular damage, lipid peroxidation, and impaired hepatic function, which may underline the development of chronic liver disorders associated with diabetes, such as non-alcoholic fatty liver disease (NAFLD), steatohepatitis, and cirrhosis. The hyperglycemia and insulin resistance in diabetes disrupt normal metabolism of lipids, carbohydrates, and proteins, triggering both oxidative stress and an aberrant inflammatory response that activates pro-apoptotic pathways and damages hepatocytes. Pro-inflammatory mediators such as interleukin-1 beta (IL-1β), interleukin-6) (IL-6), and tumor necrosis factor alpha (TNF-α)exacerbate this oxidative damage, further promoting liver injury and progression to severe liver pathology (Lucchesi et al., 2013, Mohamed et al., 2016, Zahran et al., 2024).

As a first-line oral antidiabetic medication, metformin is extensively utilized in treating patients with type 2 diabetes mellitus due to its effective glucose-lowering properties and favorable safety profile (Pernicova and Korbonits, 2014, LaMoia and Shulman, 2021). Metformin primarily acts by inhibiting gluconeogenesis in the liver, leading to a reduction in hepatic glucose production, thereby lowering blood glucose levels (LaMoia and Shulman, 2021). Metformin is transported into hepatocytes through the organic cation transporter 1 (OCT1), where it inhibits complex I of the mitochondrial respiratory chain. This disruption alters the cellular energy balance, thereby stimulating AMP-activated protein kinase (AMPK), a central regulator of both glucose and lipid metabolism (Rena et al., 2017). This activation suppresses gluconeogenic gene expression and improves insulin sensitivity, contributing to better glycemic control (Pernicova and Korbonits, 2014).

Beyond its glucose-lowering effects, metformin exerts protective effects on the liver. It reduces hepatic lipid accumulation, inflammatory responses, and oxidative stress, which serve as major contributors to the development and progression of hepatic disorders, particularly nonalcoholic fatty liver disease (NAFLD) commonly associated with diabetes (Apostolova et al., 2020). By modulating AMPK activity, metformin improves lipid metabolism and inhibits pathways leading to liver fibrosis and injury, thus offering hepatoprotective benefits in diabetic patients (Rena et al., 2017).

Naringenin is a naturally occurring flavonoid predominantly found in citrus fruits, noted for its multifaceted biological activities, encompassing antioxidant, anti-inflammatory, antifibrogenic, and tumor-suppressive effects. It has attracted attention for its potential therapeutic role in various liver diseases caused by oxidative damage, inflammation, and tissue fibrosis (Hernández-Aquino and Muriel, 2018).

The mechanism of action of naringenin involves inhibition of oxidative stress and various signaling cascades, including transforming growth factor-beta (TGF-β) and the mitogen-activated protein kinase (MAPK) pathway, and toll-like receptor (TLR) pathways. This leads to reduced stimulation of hepatic stellate cells (HSCs) alongside a reduction in extracellular matrix (ECM) production, which are key processes in liver fibrosis (Naeini et al., 2021). Additionally, naringenin enhances the cellular antioxidant defense by upregulating enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thus

protecting liver cells from oxidative damage (Xu et al., 2024).

Naringenin's protective effects on the liver include prevention of lipid peroxidation, reduction of inflammation, and inhibition of fibrogenesis. It has demonstrated efficacy in experimental models of liver injury caused by toxins, alcohol, and metabolic disorders, improving liver function and histology. Furthermore, naringenin modulates lipid metabolism, which helps in managing nonalcoholic fatty liver disease (NAFLD) and prevents progression to more severe liver conditions (Mansour et al., 2023).

Streptozotocin (STZ) is a standard diabetogenic agent extensively utilized in preclinical models to simulate diabetes mellitus due to its selective cytotoxicity on pancreatic β-cells, leading to their necrosis and resulting in hyperglycemia similar to human diabetes (Ghasemi and Jeddi, 2023). At appropriate doses, STZ impairs insulin secretion by destroying β-cells and disrupting glucose metabolism, which can model both type 1 and type 2 diabetes depending on the dose and protocol(Rehman et al., 2023). To induce type 2 diabetes specifically, nicotinamide (NA) is administered prior to STZ. Nicotinamide partially protects β-cells from STZ-induced damage by inhibiting poly(ADP-ribose) polymerase-1 (PARP-1) activity, preventing NAD+ and ATP depletion, and thus allowing some β-cells to survive and respond to glucose stimulation(Yan, 2022) (Szkudelski, 2012). This combination produces a model of type 2 diabetes characterized by partial β-cell dysfunction and reduced insulin sensitivity, mimicking the pathophysiology of human type 2 diabetes more closely than STZ alone (Alenzi, 2009, Elamin et al., 2018).

The present study aims to evaluate the combined effects of metformin and differetnt concentrations of naringenin on liver function and metabolic parameters in a rat model of type 2 diabetes established by STZ and nicotinamide. This combination therapy is hypothesized to provide synergistic benefits by improving glycemic control and exerting hepatoprotective and antioxidant effects, potentially offering approach for managing diabetes-associated liver complications.

Methods

Chemicals

Metformin hydrochloride (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan)**,** naringenin were purchased from Glentham Life Sciences Ltd., England. Streptozotocin (Sigma Chemical Co., St Louis, MO, USA). Nicotinamide was purchased from Fluka AG, Switzerland. Sodium citrate dihydrate from Glentham Life Sciences Ltd., England. Portable glucometers and glucose test strips (perfecta Bianca, Italy) were purchased from a local pharmacy. All kits were procured from Biodiagnostic Company, Egypt.

Animals

Sixty adults male wister rats weighing (180–200 g) were obtained from the regional center for mycology and biotechnology, Al-Azhar university, Cairo, Egypt. The rats were housed in steel wire cages with mesh walls, which allowed for temperature control set at (22 ± 3) °C. The cages also had adjustable lighting to create a 12-hour cycle of darkness and light. The relative humidity in the cages was maintained at (80 ± 4) %. The rats were provided with free access to food and water. Prior to the experiments, the rats were placed in a standard laboratory setting for acclimation, two weeks in advance.

Experimental induction of diabetes mellitus

All rats were fasted for six hours prior to diabetes induction. To establish a non-obese type 2 diabetes model with residual β-cell function, nicotinamide was administered intraperitoneally at 230 mg/kg 15 minutes before injecting freshly prepared streptozotocin (STZ) at 65 mg/kg body weight dissolved in citrate buffer (50 mM, pH 4.5). This protocol partially protects pancreatic β-cells from STZ toxicity, allowing for a model of type 2 diabetes characterized by partial β-cell damage. Following diabetes induction, a combination of treatments was applied to the animals.

Estimation of blood glucose

Fasting blood glucose levels were measured 72 hours post-diabetes induction using a portable glucometer. Blood samples were collected by clipping the tip of the rat's tail to collect a drop of blood (Togashi et al., 2016).

Inclusion criteria required blood glucose values greater than 180 mg/dL to confirm diabetic status. Following confirmation of diabetes induction, combined treatment protocols were initiated.

Experimental design

The rats were randomly assigned into six groups each of ten rats. Following the experimental induction of diabetes, treatments with MET and NAR were administered daily via oral gavage for 5 weeks.

Experimental design

The present study includes the following six groups:
Control group: untreated healthy rats.
MET + NAR group: healthy rats were administered with metformin (50 mg/kg) and naringenin (50 mg/kg).
Diabetic (NICO/STZ) group: diabetes was induced in rats using nicotinamide (230 mg/kg) and streptozotocin (65 mg/kg), without treatment.
MET_{50} + NAR_{25} group: diabetic rats were treated with metformin (50 mg/kg) and naringenin (25 mg/kg) daily for 5 weeks.
MET_{50} + NAR_{50} group: diabetic rats were treated with metformin (50 mg/kg) and naringenin (50 mg/kg) daily for 5 weeks.
MET_{50} + NAR_{100} group: diabetic rats were treated with metformin (50 mg/kg) and naringenin (100 mg/kg) daily for 5 weeks.
Weekly assessments of body weight and fasting glucose levels were recorded throughout the experiment. At the end of Met andNAR treatments, the rats were subjected to an overnight fast before being euthanized using CO2 for sample collection.

Blood sampling

At the end of the experiment, rats were euthanized using CO2, followed by careful opening of the peritoneal cavity to collect blood samples from the inferior vena cava. Blood was drawn into vacuum tubes to facilitate serum separation for subsequent biochemical analysis.

Estimation of liver function

Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed according to the method of (Reitman and Frankel, 1957)**.** The protein concentration was determined according to the method of (Kemp et al., 1961)**.** The total and direct bilirubin in the sample were determined according to the method of (Walter and Gerade, 1970) .

Estimation of total antioxidant capacity (TAC)

The serum antioxidant capacity is measured by adding a known amount of hydrogen peroxide (H_2O_2) to the sample, where antioxidants reduce its concentration. The remaining H_2O_2 is then quantified colorimetrically through an enzymatic reaction that produces a colored product from 3, 5-dichloro-2-hydroxybenzenesulfonate. The assay involves mixing the serum with diluted H_2O_2 substrate, incubating, adding a chromogen-enzyme mixture, further incubation, and measuring absorbance at 505 nm. TAC is calculated using the difference in absorbance between a blank and the sample, multiplied by a factor of 3.33. This method described by Koracevic et al (Koracevic et al., 2001)

Statistical analysis

All data are expressed as mean ± standard error (SE). Statistical evaluation was performed using SPSS version 25. Group comparisons were made through one-way ANOVA, and a p-value less than 0.05 was considered statistically significant.

Results

Effects of treatments on body weight

Normal rats treated with NAR +MET showed an insignificant change in body weight ($p = 0.068$) *versus* normal group. Additionally, diabetic rat …exhibited a significant reduction in body weight. (***p* < 0.001, Figure 1**) compared to normal group. In contrast, diabetic rats treated with (MET_{50} + NAR_{25}), (MET_{50}+NAR_{50}) and (MET_{50} + NAR_{100})

displayed significant increases in body weight relative to diabetic group (***p* < 0.001**).

Table 1: Mean Levels of ALT, AST, Total protein, Total Bilirubin and Direct bilirubin for all groups.

	Control	**MET +NAR**	**NICO/STZ**	**MET$_{50}$+ NAR$_{25}$**	**MET$_{50}$+ NAR$_{50}$**	**MET$_{50}$+ NAR$_{100}$**
ALT (U/mL)	49.2 ± 0.9	50.8 ± 3.2	148.7 ± 3.8^{a}	53.1 ± 3.5^{c}	65.7 ± 1.2^{c}	52.7± 2.5^{c}
AST (U/mL)	74.1 ± 1.1	86.8 ± 2.3^{a}	146.8 ± 3.3^{a}	77.0± 0.6^{c}	111.7 ± 0.5^{c}	113.1 ± 0.9^{c}
Total Protein(g/dL)	15.9 ± 0.4	15.6 ± 0.5	8.8 ± 0.5^{a}	15.5 ± 0.5^{c}	14.7 ± 0.5^{c}	15.5 ± 0.5^{c}
Total Bilirubin (mg/dL)	0.44±0.3	1.07±0.1^{a}	1.14±0.1^{a}	0.46±0.04^{c}	0.43±0.03^{c}	0.45±0.07^{c}
Direct bilirubin(mg/dL)	0.27±0.03	0.28±0.04	0.48±0.03^{a}	0.34±0.02^{c}	0.27±0.01^{c}	0.36±0.01^{c}
TAC(mM/L)	0.25 ± 0.01	0.25 ± 0.01	0.21± 0.01^{a}	0.26 ± 0.01^{c}	0.25±0.002^{c}	0.24±0.003^{c}

Data represented as mean ± SE, a =significant when compared with the control rat group, c =significant when compared with nicotinamide/streptozotocin injected rat. NICO: nicotinamide, STZ: streptozotocin, MET: metformin, NAR: naringenin, MET$_{50}$: metformin administered at 50 mg/kg, NAR$_{25}$: naringenin administered at 25 mg/kg, NAR$_{50}$: naringenin administered at 50 mg/kg and NAR$_{100}$: naringenin administered at 100 mg/kg. TAC: total antioxidant capacity.

Table 2. Pearson correlation between laboratory parameters in all studied groups

		body weight	**FBG**	**ALT**	**AST**	**T.ptn**	**T.BIL**	**D.BIL**	**TAC**
body weight	r		-.993**	-.952**	-.890**	.881**	-.586*	-.783 **	.714
	p		0.000	0.000	0.000	0.000	0.035	0.002	0.001**
FBG	r	-.993**		.993**	.881**	-.959**	0.472	.864**	-.819-
	p	0.000		0.000	0.001	0.000	0.168	0.001	0.002**
ALT	r	-.952**	.993**		.889**	-.951**	0.509	.829**	-.747
	p	0.000	0.000		0.000	0.000	0.076	0.000	0.000**
AST	r	-.890**	.881**	.889**		-.745*	0.294	.690*	-.953
	p	0.000	0.001	0.000		0.021	0.330	0.013	0.000**
T.ptn	r	.881**	-.959**	-.951**	-.745*		-0.504	-.813**	.697
	p	0.000	0.000	0.000	0.021		0.166	0.004	0.003**
T.BIL	r	-.586*	0.472	0.509	0.294	-0.504		0.437	-0.334
	p	0.035	0.168	0.076	0.330	0.166		0.080	0.379
D.BIL	r	-.783**	.864**	.829**	.690*	-.813**	0.437		-.809
	p	0.002	0.001	0.000	0.013	0.004	0.080		0.005**
TAC	r	.714**	-.819**	-.747**	-.953**	.697**	-0.334	-.809*	
	p	0.001	0.002	0.000	0.000	0.003	0.379	0.005	

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

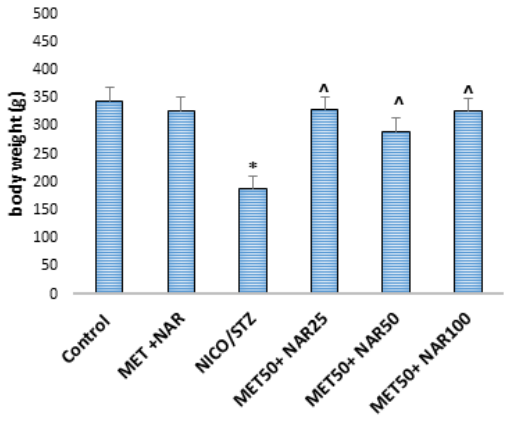

Figure 1. Effect of metformin and Naringenin on body weight.Values are expressed as mean ±SE.* significant when compared to control group,^ significant when compared to NICO/STZ group.

Effects of treatments on fasting blood glucose

Normal treated rats with MET + NAR exhibited insignificant changes in FBG compared to normal group (p =0.78, **Table 1** and **Figure 2**). Meanwhile, diabetic group exhibited a significant elevation in fasting blood glucose (FBG) levels relative to the normal group (***p* < 0.001**). In contrast, diabetic rats treated with (MET $_{50}$ + NAR $_{25}$), (MET $_{50}$ + NAR $_{50}$), and (MET $_{50}$ + NAR $_{100}$) had a significant reduction in FBG compared to diabetic group (***p* < 0.001**).

Effects of treatments on liver function tests

In **Table 1**, Normal treated rats with MET + NAR showed no significant change in ALT activity compared to normal group (p = 0.677). In contrast, ALT and AST activities in diabetic rats were significantly elevated compared to

normal group (***p* < 0.001**, **Figure 3a and Figure 3b**), whereas Diabetic rats treated with (MET $_{50}$ + NAR $_{25}$) , (MET $_{50}$ + NAR $_{50}$), and (MET $_{50}$ + NAR $_{100}$) demonstrated substantial reductions in these enzymes relative to diabetic group (***p* < 0.001**).

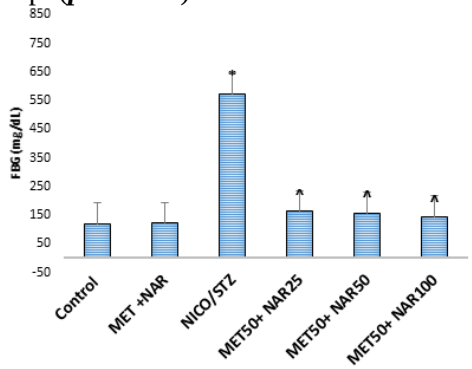

Figure 2. Effects of metformin and Naringenin on FBG. Values are expressed as mean ±SE.* significant when compared to control group, ^ significant when compared to NICO/STZ group.

Similarly, normal rats treated with MET + NAR experienced an insignificant decrease in total protein level compared to normal group (*p* = 0.642, **Table 1**). While total protein levels in diabetic group were lower than those in normal group (***p* < 0.001**), but whereas diabetic rats treated with (MET $_{50}$ + NAR $_{25}$), (MET $_{50}$ + NAR $_{50}$), and (MET $_{50}$ + NAR $_{100}$) exhibited significant increases in total protein level compared to diabetic group (***p* < 0.001, Figure 3C**). Regarding bilirubin levels, normal rats treated with MET + NAR experienced a significant increase in total bilirubin (***p* < 0.05**) with no change in direct bilirubin compared to normal group (p = 0.874). Additionally, diabetic rats had a significant rise in total and direct bilirubin compared to normal group (***p* < 0.01** and ***p* <0.001,** respectively). Meanwhile, diabetic rats treated with (MET $_{50}$ + NAR $_{25}$), (MET $_{50}$ + NAR $_{50}$) and (MET$_{50}$+NAR$_{100}$) showed a significant reduction in total bilirubin (***p* < 0.01, Figure 3d**). Notably, Diabetic rats treated with (MET $_{50}$ + NAR $_{25}$), (MET $_{50}$ + NAR $_{50}$), and (MET $_{50}$ + NAR $_{100}$) all displayed significant reductions in direct bilirubin compared to diabetic rats (***p* < 0.01**, ***p* < 0.001**, and ***p* < 0.05**, respectively) as shown in **Figure 3e**.

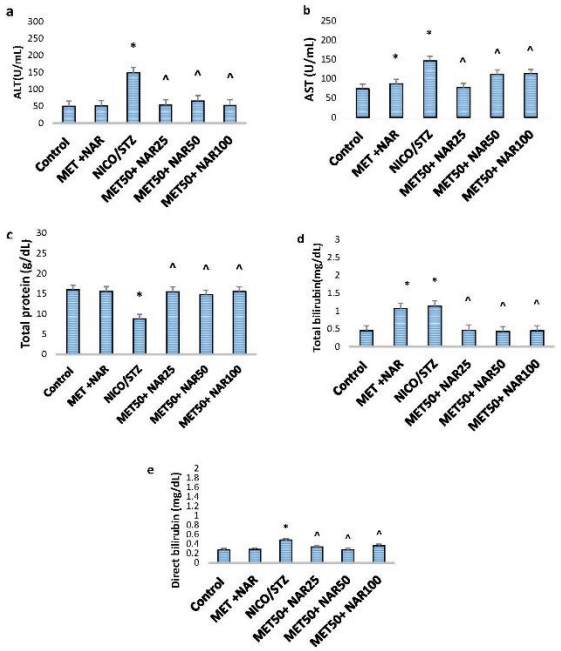

Figure 3. Effects of metformin and naringenin on a) ALT, b) AST, c) Total protein, d) Total bilirubin and e) Direct bilirubin. Values are expressed as mean ± SE. * significant when compared to control group.^ significant when compared to NICO/STZ group.

Effects of treatments on TAC

Normal rats treated with the combination of MET+NAR showed no significant change in total antioxidant capacity (TAC) compared to the normal group (*p* = 0.856, **Table 1** and **Figure 4**). In contrast, diabetic rats displayed a significant decrease in TAC (***p* < 0.01**) compared to normal rats. Diabetic rats were treated with (MET $_{50}$ + NAR $_{25}$), (MET $_{50}$ + NAR $_{50}$) and (MET $_{50}$ + NAR $_{100}$) significantly increased TAC (***p* < 0.001**, ***p* = 0.001**, and ***p* = 0.023**, respectively) compared to untreated diabetic rats.

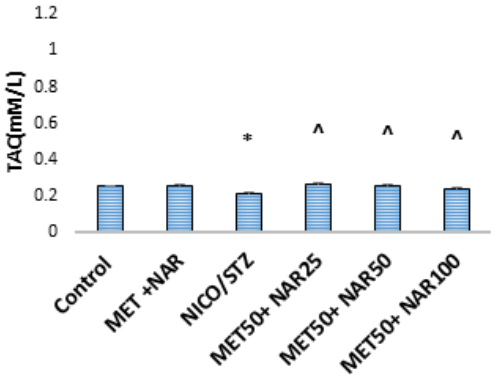

Figure 4. Effects of metformin and naringenin on TAC. Values are expressed as mean ± SE. * significant when compared to control group.^ significant when compared to NICO/STZ group

Discussion

Diabetes mellitus is a long-standing metabolic disease marked by elevated blood glucose levels resulting from inadequate insulin secretion, impaired insulin action, or both (Ehsan et al., 2025). Effective diabetes management frequently requires combination therapy, integrating two or more treatments to address the multiple pathways implicated in chronic hyperglycemia and its associated complications. (Nankar et al., 2017). This investigation focused on assessing the effect of naringenin (a citrus flavonoid) and metformin (a standard antidiabetic drug) on STZ-induced diabetic rats by comparing liver function. The findings highlight the therapeutic potential of these compounds, particularly in diabetic conditions, while also revealing dose-dependent variations in efficacy. The results showed a marked increase in serum levels of blood glucose, ALT, AST, total and direct bilirubin, with a concomitant decrease in total protein and body weight secondary to induction of diabetes in rats. These results align with other studies that have observed increased serum levels of blood glucose, ALT, AST, total and direct bilirubin (HJ, 1970, Zulkifle et al., 2024, Kakkar et al., 1998, Kumar et al., 2011).

In this study, the changes that occur in body weight may be a consequence of energy imbalance between intake and expenditure may lead to the utilization of alternative energy sources, primarily through increased lipid metabolism, reduced glucose utilization, and the breakdown of structural proteins (Ojo et al., 2017, Wilson and Islam, 2012). Additionally, an elevation in body weight was observed across all diabetic groups receiving treatment may be due to that naringenin enhances insulin sensitivity, promoting energy expenditure, and improving lipid metabolism thereby reducing weight loss (Coulter et al., 2023).

Notably, the reduction in fasting blood glucose levels in treated groups relative to the diabetic control supports the idea that naringenin improves glucose metabolism by modulating organic cation transporter 1 (OCT1) expression, enhancing metformin's hepatic uptake and subsequent glucose-lowering effects (Mato Mofo et al., 2020).

Diabetes mellitus is often associated with elevated levels of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which serve as biomarkers of hepatic injury and dysfunction (Targher, 2010). The persistent hyperglycemia characteristic of diabetes induces oxidative stress and inflammation in hepatic tissues, leading to hepatocellular damage and subsequent leakage of these enzymes into the bloodstream (Fang et al., 2019). In this study, all three treatment combinations significantly reduced ALT and AST levels compared to the untreated diabetic group, indicating improved liver function. Metformin's hepatoprotective effect is largely attributed to its activation of AMP-activated protein kinase (AMPK), which suppresses hepatic glucose synthesis and lipid accumulation, thereby mitigating oxidative stress and inflammation (Foretz et al., 2014). Conversely, naringenin exerts its protective role by scavenging free radicals and modulating inflammatory pathways, which helps restore liver integrity and enzyme levels (Assini et al., 2013).

Diabetes-induced reductions in total serum protein reflect underlying hepatic dysfunction caused by persistent hyperglycemia and oxidative injury, which impair the liver's ability to maintain normal protein synthesis (Dharmalingam and Yamasandhi, 2018). In this study, the three treatment combinations significantly increased total protein levels compared to the diabetic group, indicating improved liver function. Metformin acts primarily by activating AMPK, which reduces gluconeogenesis and improves insulin sensitivity, thereby decreasing metabolic burden on the liver and supporting protein synthesis (Nyane et al., 2017). Naringenin enhances hepatic function by promoting AMPK phosphorylation and providing antioxidant protection, which together restore hepatocyte metabolic balance and protein production (He et al., 2022).

Diabetes is often associated with impaired liver function, reflected by increased levels of total and direct bilirubin, which serve as important biomarkers of hepatic injury and cholestasis. The elevated bilirubin levels in diabetic patients arise from oxidative stress and inflammation-induced hepatocyte damage, disrupting bilirubin metabolism and excretion pathways (Ramírez-Mejía et al., 2024). In our study, all three treatment combinations significantly reduced total and direct bilirubin compared to the diabetic group, indicating improved liver function. The hepatoprotective mechanism of metformin is largely dependent

on the upregulation of AMPK activity, which reduces hepatic glucose production and oxidative stress, thereby mitigating liver injury and normalizing bilirubin metabolism (Zhou et al., 2001). Meanwhile, naringenin acts as a potent antioxidant and anti-inflammatory agent, enhancing bilirubin conjugation and excretion by upregulating detoxifying enzymes such as UDP-glucuronosyltransferase (UGT1A1) and improving bile flow (Mulvihill et al., 2016).

In the present study, diabetic rats exhibited markedly lower TAC than the control group. Kawahito, et al, Inoguchi, et al and Nishikawa, et al., illustrated that, hyperglycaemia is known to induce oxidative stress and suppressing endogenous antioxidant defence systems (Kawahito et al., 2009, Inoguchi et al., 2000, Nishikawa et al., 2000). Nevertheless, diabetics treated with all combinations demonstrated substantial improvements in TAC levels compared to diabetic group, with the combination of (MET $_{50}$ + NAR $_{25}$) being of significant.

In summary, the co-administration of metformin and naringenin represents a promising therapeutic approach against diabetes-induced metabolic and hepatic dysfunction. This combination effectively reverses weight loss, normalizes fasting blood glucose, and significantly improves liver function markers in diabetic rats. These findings highlight that metformin plus naringenin holds great promise as an adjuvant treatment to mitigate diabetes-related hepatic complications, leveraging their complementary mechanisms to enhance glycemic control. These findings highlight the potential for tailored dosing to address specific diabetic complications.

Conclusion

This study underscores the efficacy of combining metformin with moderate doses of naringenin for managing diabetic liver dysfunction while providing insights into dose-dependent responses critical for optimizing treatment regimens.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval and consent to participate

All experimental procedures were carried out following the ethical standards and regulations set forth by the Research Ethics Committee of Damietta Faculty of Medicine, Al-Azhar University, Egypt (Permit No. IRB00012367). The study adhered strictly to the institutional guidelines and complied with internationally recognized protocols, including the ARRIVE guidelines, to ensure the humane treatment and welfare of the animals throughout the research.

References

Alenzi, F. Q. (2009). Effect of nicotinamide on experimental induced diabetes.

Apostolova, N., Iannantuoni, F., Gruevska, A., Muntane, J., Rocha, M. & Victor, V. M. (2020). Mechanisms of action of metformin in type 2 diabetes: Effects on mitochondria and leukocyte-endothelium interactions. Redox biology, 34, 101517.

Assini, J. M., Mulvihill, E. E. & Huff, M. W. (2013). Citrus flavonoids and lipid metabolism. Current opinion in lipidology, 24, 34-40.

Coulter, A. A., Greenway, F. L., Zhang, D., Ghosh, S., Coulter, C. R., James, S. L., He, Y., Cusimano, L. A. & Rebello, C. J. (2023). Naringenin and β-carotene convert human white adipocytes to a beige phenotype and elevate hormone-stimulated lipolysis. Frontiers in Endocrinology, 14, 1148954.

Dharmalingam, M. & Yamasandhi, P. G. (2018). Nonalcoholic fatty liver disease and type 2 diabetes mellitus. Indian journal of endocrinology and metabolism, 22, 421-428.

Ehsan, M., Ahmed, S., Majeed, W., Iftikhar, A., Iftikhar, M., Abbas, M. & Mehmood, T. (2025). Rhoifolin improves glycometabolic control in streptozotocin-induced diabetic rats by up-regulating the expression of insulin signaling proteins and down-regulating the mapk/jnk pathway. Pharmaceuticals, 18, 361.

Elamin, N., Fadlalla, I., Omer, S. & Ibrahim, H. (2018). Histopathological alteration in stz-nicotinamide diabetic rats, a complication of diabetes or a toxicity of stz. Int J Diabetes Clin Res, 5, 1-8.

Fang, S., Cai, Y., Li, P., Wu, C., Zou, S., Zhang, Y., Lin, X. & Guan, M. (2019). Exendin-4 alleviates oxidative stress and liver fibrosis by activating nrf2/ho-1 in streptozotocin-induced diabetic mice. Nan fang yi ke da xue xue bao= Journal of Southern Medical University, 39, 464-470.

Foretz, M., Guigas, B., Bertrand, L., Pollak, M. & Viollet, B. (2014). Metformin: From mechanisms of action to therapies. Cell metabolism, 20, 953-966.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H. & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. International journal of molecular sciences, 21, 6275.

Ghasemi, A. & Jeddi, S. (2023). Streptozotocin as a tool for induction of rat models of diabetes: A practical guide. EXCLI journal, 22, 274.

He, W., Wang, Y., Yang, R., Ma, H., Qin, X., Yan, M., Rong, Y., Xie, Y., Li, L. & Si, J. (2022). Molecular mechanism of naringenin against high-glucose-induced vascular smooth muscle cells proliferation and migration based on network pharmacology and transcriptomic analyses. Frontiers in Pharmacology, 13, 862709.

Hernández-Aquino, E. & Muriel, P. (2018). Beneficial effects of naringenin in liver diseases: Molecular mechanisms. World journal of gastroenterology, 24, 1679.

Hj, Z. (1970). Enzymes in hepatic disease. Diagnostic enzymology, 24-26.

Inoguchi, T., Li, P., Umeda, F., Yu, H. Y., Kakimoto, M., Imamura, M., Aoki, T., Etoh, T., Hashimoto, T. & Naruse, M. (2000). High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase c--dependent activation of nad (p) h oxidase in cultured vascular cells. Diabetes, 49, 1939-1945.

Kakkar, R., Mantha, S. V., Radhi, J., Prasad, K. & Kalra, J. (1998). Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. Clinical science, 94, 623-632.

Kawahito, S., Kitahata, H. & Oshita, S. (2009). Problems associated with glucose toxicity: Role of hyperglycemia-induced oxidative stress. World journal of gastroenterology: WJG, 15, 4137.

Kemp, P., Hübscher, G. & Hawthorne, J. (1961). Phosphoinositides. 3. Enzymic hydrolysis of inositol-containing phospholipids. Biochemical Journal, 79, 193.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S. & Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. Journal of clinical pathology, 54, 356-361.

Kumar, D., Kumar, S., Kohli, S., Arya, R. & Gupta, J. (2011). Antidiabetic activity of methanolic bark extract of albizia odoratissima benth. In alloxan induced diabetic albino mice. Asian Pacific Journal of Tropical Medicine, 4, 900-903.

Lamoia, T. E. & Shulman, G. I. (2021). Cellular and molecular mechanisms of metformin action. Endocrine reviews, 42, 77-96.

Lucchesi, A. N., Freitas, N. T. d., Cassettari, L. L., Marques, S. F. G. & Spadella, C. T. (2013). Diabetes mellitus triggers oxidative stress in the liver of alloxan-treated rats: A mechanism for diabetic chronic liver disease. Acta Cirurgica Brasileira, 28, 502-508.

Mansour, L. A., Elshopakey, G. E., Abdelhamid, F. M., Albukhari, T. A., Almehmadi, S. J., Refaat, B., El-Boshy, M. & Risha, E. F. (2023). Hepatoprotective and neuroprotective effects of naringenin against lead-induced oxidative stress, inflammation, and apoptosis in rats. Biomedicines, 11, 1080.

Mato Mofo, E. P., Essop, M. F. & Owira, P. M. (2020). Citrus fruit-derived flavonoid naringenin and the expression of hepatic organic cation transporter 1 protein in diabetic rats treated with metformin. Basic & clinical pharmacology & toxicology, 127, 211-220.

Mohamed, J., Nafizah, A. N., Zariyantey, A. & Budin, S. (2016). Mechanisms of diabetes-induced liver damage: The role of oxidative stress and inflammation. Sultan qaboos university medical journal, 16, e132.

Mulvihill, E. E., Burke, A. C. & Huff, M. W. (2016). Citrus flavonoids as regulators of lipoprotein metabolism and atherosclerosis. Annual review of nutrition, 36, 275-299.

Naeini, F., Namkhah, Z., Ostadrahimi, A., Tutunchi, H. & Hosseinzadeh-Attar, M. J. (2021). A comprehensive systematic review of the effects of naringenin, a citrus-derived flavonoid, on risk factors for nonalcoholic fatty liver disease. Advances in nutrition, 12, 413-428.

Nankar, R., Prabhakar, P. & Doble, M. (2017). Hybrid drug combination: Combination of ferulic acid and metformin as anti-diabetic therapy. Phytomedicine, 37, 10-13.

Nishikawa, T., Edelstein, D., Du, X. L., Yamagishi, S.-i., Matsumura, T., Kaneda, Y., Yorek, M. A., Beebe, D., Oates, P. J. & Hammes, H.-P. (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature, 404, 787-790.

Nyane, N. A., Tlaila, T. B., Malefane, T. G., Ndwandwe, D. E. & Owira, P. M. O. (2017). Metformin-like antidiabetic, cardio-protective and non-glycemic effects of naringenin: Molecular and pharmacological insights. European Journal of Pharmacology, 803, 103-111.

Ojo, O. A., Ajiboye, B. O., Ojo, A. B., Oyinloye, B. E., Imiere, O. D. & Adeyonu, O. (2017). Ameliorative potential of blighia sapida kd

koenig bark against pancreatic β-cell dysfunction in alloxan-induced diabetic rats. Journal of complementary and Integrative medicine, 14, 20160145.

Pernicova, I. & Korbonits, M. (2014). Metformin—mode of action and clinical implications for diabetes and cancer. Nature Reviews Endocrinology, 10, 143-156.

Ramírez-Mejía, M. M., Castillo-Castañeda, S. M., Pal, S. C., Qi, X. & Méndez-Sánchez, N. (2024). The multifaceted role of bilirubin in liver disease: A literature review. Journal of Clinical and Translational Hepatology, 12, 939.

Reed, J., Bain, S. & Kanamarlapudi, V. (2021). A review of current trends with type 2 diabetes epidemiology, aetiology, pathogenesis, treatments and future perspectives. Diabetes, Metabolic Syndrome and Obesity, 3567-3602.

Rehman, H. u., Ullah, K., Rasool, A., Manzoor, R., Yuan, Y., Tareen, A. M., Kaleem, I., Riaz, N., Hameed, S. & Bashir, S. (2023). Comparative impact of streptozotocin on altering normal glucose homeostasis in diabetic rats compared to normoglycemic rats. Scientific reports, 13, 7921.

Reitman, S. & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology, 28, 56-63.

Rena, G., Hardie, D. G. & Pearson, E. R. (2017). The mechanisms of action of metformin. Diabetologia, 60, 1577-1585.

Rob, A., Hoque, A., Asaduzzaman, M. M., Khatoon, M. A. A., Khalil, R., Thomas, D., Ilyas, M., Mahmood, H. & Chowdhury, T. A. (2025). The global challenges of type 2 diabetes. Bangladesh Journal of Medicine, 36, 92-98.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A. & Ogurtsova, K. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes atlas. Diabetes research and clinical practice, 157, 107843.

Szkudelski, T. (2012). Streptozotocin–nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Experimental biology and medicine, 237, 481-490.

Targher, G. (2010). Elevated serum γ-glutamyltransferase activity is associated with increased risk of mortality, incident type 2 diabetes, cardiovascular events, chronic kidney disease and cancer–a narrative review. Clinical Chemistry and Laboratory Medicine, 48, 147-157.

Togashi, Y., Shirakawa, J., Okuyama, T., Yamazaki, S., Kyohara, M., Miyazawa, A., Suzuki, T., Hamada, M. & Terauchi, Y. (2016). Evaluation of the appropriateness of using glucometers for measuring the blood glucose levels in mice. Scientific reports, 6, 25465.

Walter, M. & Gerade, R. (1970). Bilirubin direct/total. Microchem J, 15, 231-233.

Wilson, R. D. & Islam, M. S. (2012). Fructose-fed streptozotocin-injected rat: An alternative model for type 2 diabetes. Pharmacological reports, 64, 129-139.

Xu, J., Chen, J., Deng, J., Chen, X., Du, R., Yu, Z., Gao, S., Chen, B., Wang, Y. & Cai, X. (2024). Naringenin inhibits apap-induced acute liver injury through activating ppara-dependent signaling pathway. Experimental Cell Research, 437, 114028.

Yan, L.-J. (2022). The nicotinamide/streptozotocin rodent model of type 2 diabetes: Renal pathophysiology and redox imbalance features. Biomolecules, 12, 1225.

Zahran, R. F., Rady, G. M. & El-Sonbati, A. Z. (2024). Evaluation of antioxidant and anti-inflammatory activities of 4-((quinolin-2-yl) methyleneamino)-1, 2-dihydro-2, 3-dimethyl-1-phenylpyrazol-5-one on ehrlich ascites carcinoma bearing mice. Scientific Journal for Damietta Faculty of Science, 14, 1-12.

Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T. & Fujii, N. (2001). Role of amp-activated protein kinase in mechanism of metformin action. The Journal of clinical investigation, 108, 1167-1174.

Zulkifle, N., Shaari, M., Omar, N. & Tajuddin, S. (2024). The effects of aquilaria malaccensis leaf extracts on blood glucose level, kidney profile and liver function in streptozotocin induced diabetic rats. J. Trop. Agric. and Fd. Sc, 52, 105-111.

الملخص العربي

عنوان البحث: دور النارنجينين في تعزيز فعالية الميتفورمين لدى الفئران المصابة بداء السكري.

ميار أكرم شامخ[١]، عبد العزيز فتوح عبد العزيز[٢]، رشا فكري زهران*[١]

[١]قسم الكيمياء، كلية العلوم، جامعة دمياط، دمياط، مصر
[٢]قسم الكينياء، كلية العلوم، جامعة المنصورة، المنصورة، مصر

يرتبط داء السكري بحدوث خلل في وظائف الكبد وزيادة الإجهاد التأكسدي، مما يستدعي البحث عن استراتيجيات علاجية فعّالة للحد من هذه التأثيرات. تهدف هذه الدراسة إلى تقييم التأثير المشترك لكل من الميتفورمين (دواء خافض لسكر الدم من الخط الأول) والنارينجينين (مركب فلافونويدي موجود في الحمضيات) على مؤشرات وظائف الكبد والحالة المضادة للاكسدة وذلك من خلال آلية تنشيط إنزيم.AMPK

تم إجراء التجربة على ٦٠ جرذا من نوع ويستر، قُسِّموا عشوائيا إلى ست مجموعات تشمل مجموعة تحكم سلبية، وتحكم مركبة، ومجموعة سكري غير معالجة، وثلاث مجموعات سكري عولجت بالميتفورمين (٥٠ ملغم/كغ) مدموجًا بجرعات مختلفة من النارينجينين (٢٥، ٥٠، ١٠٠ ملغم/كغ) علي التوالي. تم تحفيز السكري باستخدام مادة النيكوتيناميد و الستربتوزوتوسين، وأُعطي العلاج يوميًا عبر التناول الفموي لمدة خمسة أسابيع. شملت التقييمات قياس مستوى الجلوكوز الصائم والوزن أسبوعيًا، بالإضافة إلى تحاليل كيميائية لوظائف الكبد وقياس السعة الكلية المضادة للأكسدة.

أظهرت النتائج أن الجرذان المصابة بالسكري عانت من انخفاض في الوزن وارتفاع في الجلوكوز الصائم، وإنزيمات الكبدALT) ، (AST، والبيليروبين، مع انخفاض في البروتين الكلي والسعة الكلية المضادة للأكسدة. بينما أدى العلاج المشترك بالميتفورمين والنارينجينين إلى تحسُّن واضح في هذه المؤشرات، حيث سُجّل تحسُّن في الوزن، وانخفاض في الجلوكوز، واستعادة وظائف الكبد والسعة الكلية المضادة للأكسدة.

تشير هذه النتائج إلى أن الدمج بين الميتفورمين والنارينجينين يُحدث تأثيرًا تآزريًا مضادًا للسكري ومحسّنًا لوظائف الكبد، والسعة الكلية المضادة للأكسدة مما يساهم في تحسين التحكم في سكر الدم والحد من الضرر التاكسدي ويدعم دور النارينجينين في تعزيز فعالية الميتفورمين من خلال تعديل تعبير ناقلات الكبد.