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The superiority of the nanoparticle form of metformin over its conventional form on glucose levels and SIRT -1 gene expression in diabetic male rats

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ABSTRACT

Background: Insulin resistance, hyperinsulinemia, and hyperglycemia are hallmarks of type 2 diabetes (T2DM). T2DM's hyperglycemia is caused by an unusually high hepatic gluconeogenesis, which results in an increase in glucose production. Gluconeogenesis inhibition is the primary mechanism by which Metformin cures hyperglycemia. Gluconeogenic gene expression has been regulated by Sirtuin 1 (SIRT1). A new method for studying how metformin affects the levels and activity of SIRT1 in the liver and kidneys of diabetic rats is described in this research. SIRT1 will also be studied for its probable impact on diabetes-related metabolic problems, such as elevated blood glucose levels. This is a novel method of how metformin affected *SIRT-1* levels and activity in diabetic rat's liver and pancreas. Further, the possible role of *SIRT-1* on metabolic disorders associated with diabetes mellitus, including serum levels of glucose tests will be explored. Method:24 male albino rats were divided into control group (Gp I), DM diabetic group Gp II) were induced by alloxan 150 mg/kg (metformin+ DM) group (Gp III) and (nanoparticles+ DM) group (Gp IV) where each rat received 45 mg/kg metformin daily for 28 days after being induced with diabetes by alloxan. At the end of study: Serum glucose levels and *SIRT1*gene expression in the liver and kidneys were measured at 7 and 28 days. Results: Glucose levels were much lower for seven days and *SIRT-1*expression levels in the liver and kidneys were significantly upregulated according to our results. Conclusion: Metformin nanoparticles, rather than metformin, improved diabetes patients' SIRT1 levels.

1. INTRODUCTION

T2DM is due to excessively high hepatic gluconeogenesis (Nasri and Rafieian-Kopaei, 2014). Metformin improved insulin sensitivity (Madiraju et al., 2014), reduced hepatic glucose production and changes in cytosolic and mitochondrial redox states (mGPD) (MacDonald et al., 2021). Beta-cell death in T2DM is linked to an increase in beta-cell apoptosis (Du et al., 2021).

SIRT1 is primarily located in the nucleus (Parenti et al., 2015). Metformin has been shown to repair kidney lesions in diabetic rats and increase superoxide dismutase antioxidant activity. Use of metformin for three weeks at a level of 500 mg/day may have caused significant liver damage in one case (Meligi et al., 2021).

Nanoencapsulation of the drug is a viable alternative for patients who are concerned about the side effects from taking medication (Kumar et al., 2016). Carriers for medicines and other biomolecules in nanomers are known as nanoparticle drug delivery systems (Ab del-Hakeem et al., 2021).

A few nano-particle drug tablets are now available, which can help reduce the cost of diabetes management and improve adherence to the prescribed therapy (Cesur et al., 2021). Due to their method of action, metformin and metformin nanoparticles appear to be ideal for patient administration. Metformin polymeric nanocapsules encapsulated in chitosan can give prolonged release and increased efficacy at a lower dosage. Additionally, most side effects can be controlled by reducing the dosage (Kumar et al.,2017).

SIRT1 is a nicotinamide adenine dinucleotide-dependent deacetylase belonging to the class III histone deacetylases. It is abundantly expressed in the kidney, especially in the renal medulla. SIRT1 is closely involved in renal physiology and pathology (Guan and Hao, 2016). Sirt1 expression in T2DM monocytes and granulocytes may be linked to the state of glucose/lipolysis in these cells. SIRT1 activators

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increase insulin sensitivity and glucose balance throughout the body.

Rats with diabetes had significantly lower levels of SIRT1 and SIRT2 gene expression in their kidneys and livers than the blank control group (Arab Sadeghabadi et al.,2018). Metformin, the principal treatment for T2DM, corrects hyperglycemia and hyperinsulinemia (Shinu et al.,.2022). Metformin activates hepatic AMPK, which increases SIRT1 activity (Wang, 2021). At the time they investigated the potential impact of a 30 percent caloric restriction on insulin-stimulating receptor type 1 (SIRT1) in diabetic rats, both pre-and post-diabetes CR regimens helped to improve hyperglycemia while also reducing the drastic declines in liver and kidney levels of SIRT1 (Khowailed et al., 2015). The current study aim is to investigate the effect of metformin and metformin nanoparticles on SIRT1 expression levels and activity in diabetic rats' liver and kidneys. The potential role of SIRT1 in metabolic disorders associated with diabetes mellitus will also be investigated.

2. MATERIAL AND METHODS

2.1. Experimental Animals

24 male albino rats with a total weight of 200 grams each were fed a pelleted ration containing 20% protein, not to exceed 3% fiber, and 3% fat, all in accordance with Ain 97 NRC, as well as free access to water. Rats were kept at room temperature in sterile cages. Before administering the medication, the rats were fed a diet for 18–24 hours. Animals received human care in compliance with the ethical guidelines of the Animal Care and Use Committee Benha University by ethical number BUFVTM 21-03-22.

2.2. Chemicals

Alloxan monohydrate (from Sigma Aldrich, St. Louis) is used to induce hyperglycemia. El-NASR Co., Egypt, provided the pure powder Metformin (98 %) potency. Chitosan nanoparticles containing metformin were prepared by encapsulation method (Elkomy *et al.*, 2021).

2.3. Induction of diabetes

After fasting, rats were given 150 mg/kg b.w. in sterile saline intraperitoneally to test alloxan. Glucose levels were measured using Accuchek glucometer strips after 72 hours of fasting (Roche Diagnostics). Alloxan-treated rats were considered diabetic if elevation of fasting blood glucose above 250 mg/dl. Only diabetic rats were used in the investigation (Trinder, 1975).

2.4. Experimental design

Rats were divided into four groups of 6 rats each.
(All treatments were administered through an intragastric tube.):

Group 1(Normal control): Given 1 ml saline solution every day for 28 days.

Group 2(Diabetic control): Given 1 ml saline solution for 28 days after alloxan diabetic induction.

Group 3 (Metformin treated): Diabetic given metformin (45 mg/kg body weight) according to Nair and Jacob (2016) daily for 28 days, 1 ml saline solution.

Group 4 (metformin chitosan nanoparticles treated): Diabetic rats were given 45 mg/kg b.w. metformin chitosan nanoparticles orally daily,1 ml saline for 28 days.

2.5. Blood sampling:

Comparing the blood glucose levels between the four groups was determined by a different method (glucose oxidase method, O-toluidine method or glucose monitor with strips), after a specific fasting duration (for 12 h), a blood source (from orbital venus plexus) and different time for separating serum after sampling at 0, 30, 60, 90, 120, and 180 minutes. A mathematical model for the contribution of different components was evaluated and combined by parameters of half-width and range (Wang, *et al.*,2010) using an enzymatic colorimetric technique according to Trinder (1975).

2.6. Oral glucose tolerance test (OGTT)

OGTT for nondiabetic rats were performed according to the standard method (Du Vigneaud and Karr, 1925). In short, Group I to Group IV were selected for OGT test after deprivement of water for 12 hours. The test materials (metformin and metformin nanoparticles, 45 mg/kg b.w. each) were administered shortly after glucose oral administration (2 g/kg glucose One shot) into the rats. Blood sample was taken from orbital venus plexus (10 microliter) by using insulin syringe eat 0, 0.5, 1, 2, 4, 6, 8, and 10 hrs and estimated by using Accu-Check glucometer (Lima *et al.*, 2009). Normal overnight 12 hrs fasted rats were subjected to an oral glucose tolerance test. Metformin, metformin nanoparticles (45 mg/kg b.w. each), and standard reference drugs were administered shortly after glucose administration. Blood was withdrawn from each animals'orbital venus plexuses at 0, 0.5, 1, 2, 4, 6, 8, and 10 hrs. The O-toluidine method was used to calculate fasting blood glucose levels (Frings et al., 1970).

2.7. RNA isolation and reverse transcription

Total RNA was isolated from hepatic and kidney tissues using the QIAamp RNeasy Mini kit RNA extraction kit (Qiagen, Germany, Cat# 74104) according to the manufacturer's instructions. RNA concentration was determined spectrophotometrically at 260 nm using the NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific, Waltham, USA).

Total RNA was reversed and transcribed to cDNA using the Go Script™ Reverse Transcription System Kit (Cat No. A5000, Promega). Real-time polymerase chain reaction (RT-PCR) was used to measure messenger RNA expression levels of *SIRT-1*gene in hepatic and kidney tissues qPCR kit (Cat No. A6001, Promega). Primer (10 pmol, Sigma-Aldrich, Egypt) sequences have been shown in Table (1). GAPDH gene was used as a reference gene. The relative expression fold changes using formula $2^{-\Delta\Delta CT}$which includes the control value using Equation:

$\Delta\Delta Ct = \Delta Ct_{target} - \Delta ct_{reference}$

Where ΔCt_{target} is for the target sample (experiment) and $\Delta Ct_{reference}$ is for the reference sample (control).

Table 1 Primer sequences GAPDH gene used as a reference gene

Gene	Accession number	Gene length (bp)	Forward primer (5' -3')	Reverse primer (5' -3')
Sirt1	XM_039098755.1	224	5′-CCAGATTTCAAGGCTGTTGGTTCC-3′	5′CCACAGGAACTAGAGGATAAGGCT-3
GAPDH	XM_039107008.1	138	5′-TGGAGTCTACTGGCGTCTT-3′	5′-TGTCATATTTCTCGTGGTTCA-3

2.8. Statistical analysis

IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 24, was used to analyze the data (SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine the normality of the variables. Because the variables were not normally distributed, comparisons between the four groups were made using the Kruskal-Wallis test, followed by a post-hoc Mann-Whitney U test. The Bonferroni corrections were used to adjust the p-values for inflation. A p-value of 0.05 or less was considered statistically significant. All of the tests were two-tailed.

3. RESULTS

3.1 Plasma glucose level (mg/dl):

After 28 days, administration of metformin and metformin nanoparticles significantly decreased serum glucose levels compared to diabetic control group. However, the glucose level was still higher than that of the normal control rats. The mean values were 98.80 ±8.35 and 93.00 ±5.61 for metformin and metformin nanoparticles; respectively, compared to diabetic control (190.20 ±5.54 mg/dl). (Table 2).

Table 2 The effect of metformin and metformin chitosan nanoparticles on serum glucose level in different study groups after oral administration (45mg/kg b.w) for four weeks. (n=6, Mean ± SD).

Group	Glucose level (mg/dl)
Diabetic	190.20 ±5.54 ^{b}
Metformin drug	98.80 ±8.35 ^{ab}
Metformin nanoparticles	93.00 ±5.61 ^{ab}
Normal rat	70.00 ±6.71 ^{a}
Overall p-value	0.001

3.2. Oral glucose tolerance test (mg/dl) for 7 days

After treatment with metformin and metformin nanoparticles (45 mg/kg b.wt.) daily for 7 days, blood glucose level started to decrease from 0.5 hr reaching the maximum decrease at 4 hrs in 1st and 2nd Days but in the 3rd day blood glucose levels reached the maximum at 6 hrs. Also, in 4th Day blood glucose levels reach maximum at 6 hrs. Finally, at 5, 6 and 7 days blood glucose levels reached maximum decrease at 6 hr also but it started to increase again at 10 hrs. There is a significant decrease in plasma glucose level in metformin and metformin nanoparticles treated groups than in diabetic groups.

The blood glucose level values are (184.83 ± 2.81 and 171.67 ± 3.43 mg/dl), respectively than in diabetic group (195.50 ± 3.64mg/dl) at 1st day (Table 3); (245.17 ± 3.70and 226.50 ± 3.92mg/dl) in treated groups, respectively than diabetic (265.67 ± 6.16 mg/dl) at 2nd Day (Table, 4); (286.50 ± 3.11 and 266.50 ± 7.08 mg/dl), respectively than diabetic (313.17 ± 9.52 mg/dl) at 3rd day (Table, 5);(295.17 ± 9.22and 252.22 ± 8.17mg/dl) in treated groups, respectively than diabetic (321.33 ± 9.87mg/dl) at 4th Day (Table, 6); (301.33±3.77 and 278.60±3.87 mg/dl) in treated groups, respectively than diabetic (323.17± 13.08mg/dl) at 5thDay (Table 7); (317.33± 3.50 and 303.33± 4.37 mg/dl) in treated groups, respectively than diabetic (332.5± 4.92mg/dl) at 6th Day (Table, 8) and (321.6±8.10 and 296.67±5.77 mg/dl) in treated groups, respectively than diabetic group (344.17± 10.46 mg/dl) at 8thDay (Table, 9). Moreover, metformin nanoparticles treatments resulted in a significant hypoglycemic effect than metformin in all treated groups allover treatment period (1-7 Days).

Table 3 Blood glucose level (mg/dl) on1st day in diabetic rats after oral administration of metformin and metformin nanoparticles (45 mg/kg b w) (Mean ± SE) (n=6).

Time	Control	Diabetic	D+ Metformin	D+Metformin NPS
0	95.33± 0.66^{b}	98.16± 0.94^{ab}	98.83± 1.24^{a}	98.90± 1.35^{a}
0.5	98.66± 0.71^{c}	259.67± 4.42^{a}	242.83± 430^{b}	235.83± 5.44^{b}
1	95.50± 0.67^{d}	229.83± 6.20^{a}	202.67± 3.65^{b}	175.00± 14.70^{c}
2	98.00± 1.01^{d}	209.67± 2.66^{a}	197.50± 3.67^{b}	181.67± 3.56^{c}
4	96.33± 2.43^{d}	195.50± 3.64^{a}	184.83± 2.81^{b}	171.67± 3.43^{c}
6	96.83± 0.65^{d}	217.83± 1.97^{a}	202.50± 4.44^{b}	189.67± 4.97^{c}
8	96.50± 1.02^{d}	237.67± 3.81^{a}	215.50± 3.45^{b}	203.83± 4.65^{c}
10	95.00± 0.93^{d}	256.83± 3.53^{a}	235.83± 6.42^{b}	216.83± 4.67^{c}

Different letters superscripts (a, b, ab) differ significantly ($p<0.05$), while similar letters differ insignificantly.

Table 4 Blood glucose level (mg/dl) on the 2nd day in alloxan-induced diabetic rats after oral administration of metformin and metformin nanoparticles (45mg/kg b w) (Mean ± SE) (n=6).

Time	Control	Diabetic	D+ Metformin	D+Metformin NPS
0	104.83± 1.22[d]	263.83± 4.57[a]	248.83± 5.90[b]	222.67± 3.44[c]
0.5	101.67± 1.25 [d]	286.67± 11.95 [a]	264.83± 6.23 [b]	242.17± 4.36 [c]
1	115.50± 2.61 [d]	315.83± 16.33 [a]	280.17± 5.33 [b]	244.67± 9.73 [c]
2	116.33± 3.27 [d]	312.33± 17.3 [a]	280.83± 6.05 [b]	247.83± 9.50 [c]
4	100.83± 2.67 [d]	265.67± 6.16 [a]	245.17± 3.70 [b]	226.50± 3.92 [c]
6	98.00± 1.73 [d]	286.67± 4.82 [a]	265.67± 6.03 [b]	242.67± .41 [c]
8	115.33± 4.66 [d]	294.83± 3.72 [a]	274.83± 5.89 [b]	257.50± 4.27 [c]
10	119.17± 5.62 [d]	317.83± 8.76 [a]	281.17± 5.53 [b]	252.50± 12.40 [c]

Different letters superscripts (a, b, ab) differ significantly (p<0.05), while similar letters differ insignificantly.

Table 5 Blood glucose level (mg/dl) on3rd day in diabetic rats after oral administration of metformin and metformin nanoparticles (45 mg/kg b w) (Mean ± SE) (n=6).

Time	Control	Diabetic	D+ Metformin	D+Metformin NPS
0	110.67± 3.94 [d]	311.83± 3.86 [a]	296.83± 2.95 [b]	281.50± 4.14 [c]
0.5	117.83± 4.02 [d]	333.50± 7.87 [a]	305.33± 2.38 [b]	297.83± 14.19 [c]
1	122.83± 3.99 [d]	338.00± 8.66 [a]	317.67± 2.43 [b]	295.17± 6.99 [c]
2	106.33± 1.17 [d]	330.50± 6.65 [a]	315.33± 3.67 [b]	296.17± 6.28[c]
4	105.67± 4.58 [d]	316.33± 10.54[a]	293.17± 3.88 [b]	270.00± 8.98[c]
6	110.50± 3.41 [d]	313.17± 9.52 [a]	286.50± 3.11 [b]	266.50± 7.08[c]
8	116.83± 2.85 [d]	324.33± 11.33[a]	295.67± 4.65 [b]	266.33± 8.04[c]
10	118.33± 2.55 [d]	319.17± 2.93 [a]	299.67± 6.33 [b]	281.83± 4.74 [c]

Different superscript letters in the same row indicate statistical significance at $P \leq 0.05$.

Table 6 Blood glucose level (mg/dl) on 4th day in diabetic rats after oral administration of metformin and metformin nanoparticles (45 mg/kg b w) (Mean ± SE) (n=6).

Time	Control	Diabetic	D+ Metformin	D+Metformin NPS
0	99.16± 1.30 [d]	323.50± 6.92 [a]	303.17± 8.65[b]	281.67± 5.95 [c]
0.5	105.50± 0.99[d]	338.67± 9.96[a]	316.83± 4.86[b]	292.00± 7.07 [c]
1	106.33± 2.87[d]	360.83± 10.44[a]	323.67± 8.32 [b]	289.33± 10.54 [c]
2	110.67± 3.01 [d]	353.17± 6.71 [a]	329.83± 10.11[b]	303.50± 7.76 [c]
4	110.83± 2.44[d]	326.33± 4.93 [a]	309.50± 6.33[b]	293.50± 5.15 [c]
6	121.17± 2.12[d]	319.50± 5.66 [a]	301.83± 8.55[b]	284.67± 3.68 [c]
8	105.67± 2.49[d]	339.17± 9.20[a]	318.67± 8.48[b]	295.67± 4.48[c]
10	108.33± 3.87[d]	321.33± 9.87[a]	295.17± 9.22[b]	252.22± 8.17[c]

Different letters superscripts (a, b, ab) differ significantly (p<0.05), while similar letters differ insignificantly.

Table 7 Blood glucose level (mg/dl) on 5th day in diabetic rats after oral administration of metformin and metformin nanoparticles (45 mg/kg b w) (Mean ± SE) (n=6).

Time	Control	Diabetic	D+ Metformin	D+Metformin NPS
0	116.50± 3.55^{d}	342.67± 10.79^{a}	315.83± 5.41^{b}	288.00± 7.73 ^{c}
0.5	118.50± 1.47^{d}	334.66± 5.36^{a}	318.50± 6.88 ^{b}	302.00± 5.15 ^{c}
1	114.67± 2.20^{d}	346.6± 6.22^{a}	323.7± 6.60^{b}	302.50± 4.47 ^{c}
2	119.33± 2.89 ^{d}	370.33± 6.54^{a}	339.50± 9.74 ^{b}	315.83± 6.15 ^{c}
4	106.17± 4.78 ^{d}	339.83± 10.43 ^{a}	310.33± 3.30^{b}	285.17± 7.83^{c}
6	97.50± 1.17^{d}	323.17± 13.08^{a}	301.33± 3.77 ^{b}	278.60± 3.87 ^{c}
8	111.33± 4.59^{d}	350.50± 10.82^{a}	317.3± 6.33 ^{b}	290.17± 10.28^{c}
10	115.17± 3.36 ^{d}	328.17± 5.81^{a}	301.83± 4.43 ^{b}	283.00± 6.98^{c}

Different letters superscripts (a, b, ab) differ significantly ($p<0.05$), while similar letters differ insignificantly.

Table 8 Blood glucose level (mg/dl) on 6th day in diabetic rats after oral administration of metformin and metformin nanoparticles (45 mg/kg b w) (Mean ± SE) (n=6).

Time	Control	Diabetic	D+ Metformin	D+Metformin NPS
0	118.5± 2.64^{d}	331.17± 5.73^{a}	304.67± 4.39^{b}	286.5± 9.51^{c}
0.5	121.17± 2.72 ^{d}	346.5± 9.16^{a}	321.5± 5.46^{b}	293.00± 8.63 ^{c}
1	115.33± 3.08^{d}	367.17± 6.75^{a}	337.33± 7.97 ^{b}	312.67± 7.81 ^{c}
2	94.50± 0.92^{d}	373.5± 8.28 ^{a}	342.17± 7.23^{b}	317.67± 6.70^{c}
4	98.83± 1.86^{d}	353.5± 7.44^{a}	325.33± 9.70^{b}	302.67± 7.66 ^{c}
6	99.33± 2.45^{d}	332.5± 4.92^{a}	317.33± 3.50^{b}	303.33± 4.37^{c}
8	105.50± 2.40^{d}	356.5± 8.39^{a}	328.83± 10.01^{b}	301.17± 8.72^{c}
10	117.83± 3.41 ^{d}	357.5± 5.27^{a}	337.67± 15.49 ^{b}	311.5± 4.09^{c}

Different superscript letters in the same row indicate statistical significance at $P \leq 0.05$.

Table 9 Blood glucose level (mg/dl) on 7th day in diabetic rats after oral administration of metformin and metformin nanoparticles (45 mg/kg b w) (Mean ± SE) (n=6).

Time	Control	Diabetic	D+ Metformin	D+Metformin NPS
0	119.17± 0.87^{d}	361.83± 5.15 ^{a}	332.33± 8.25^{b}	315.83± 3.64^{c}
0.5	118.5± 1.36 ^{d}	373.67± 7.12^{a}	352.67± 9.07^{b}	331.33± 6.94^{c}
1	109.67± 2.61^{d}	374.83± 6.94^{a}	342.5± 8.00 ^{b}	316.67± 6.21^{c}
2	108.17± 1.07^{d}	351.5± 3.31 ^{a}	331.33± 7.13^{b}	309.83± 3.62^{c}
4	98.33± 1.33^{d}	343.5± 6.44^{a}	325.67± 7.04^{b}	308.83± 4.36 ^{c}
6	109.17± 1.40^{d}	344.17± 10.46^{a}	321.6± 8.10 ^{b}	296.67± 5.77 ^{c}
8	110.33± 2.17^{d}	354.17± 5.31^{a}	332.83± 8.87 ^{b}	310.5± 7.40^{c}
10	117.17± 1.77 ^{d}	372.5± 6.97^{a}	334.17± 9.14^{b}	308.33± 6.32^{c}

Different superscript letters in the same row indicate statistical significance at $P \leq 0.05$.

3.3. Expression of SIRT-1 gene in kidney and liver in response to metformin and nano-metformin

Metformin and nano-metformin administration boosted SIRT-1 gene expression in liver and kidney tissues and inhibited gluconeogenesis. At 28 days, the fold of SIRT-1 expression in the kidney increased by 0.7, but the fold of gene expression increased by 1.5 in the case of nano-metformin. In the liver, there was a 0.6 and 2 fold increase in SIRT-1 expression in response to metformin therapy, as shown in Figure 1.

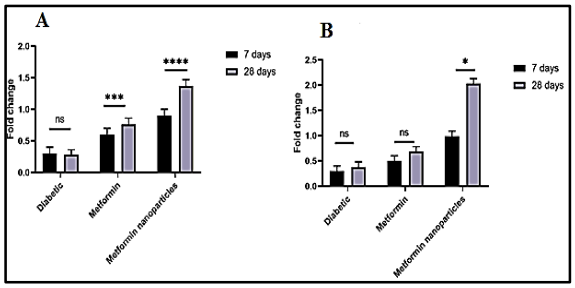

Fig (1) Effect of metformin and metformin nanoparticles on the expression levels of SIRT1 genes in kidney and liver tissues after 7 and 28 days of administration in diabetic induced rats. The qRT-PCR analysis results to measure the relative mRNA expression levels of SIRT1genes of kidney and liver tissues with metformin and metformin nanoparticles. T-test: *p < 0.05, ***p < 0.01. Bars represent mean values ± standard error.

Metformin and metformin nanoparticles induced expression of *SIRT-1* gene, the correlations of SIRT1 gene expression in liver and kidney after 7 and 28 days of metformin and metformin nanoparticles were investigated in figure (2). *SIRT-1*expression in both liver and kidney was positively correlated r = 0.979324 and 0.96028 respectively suggesting that metformin and metformin nanoparticles would promote the expression of *SIRT-1*gene.

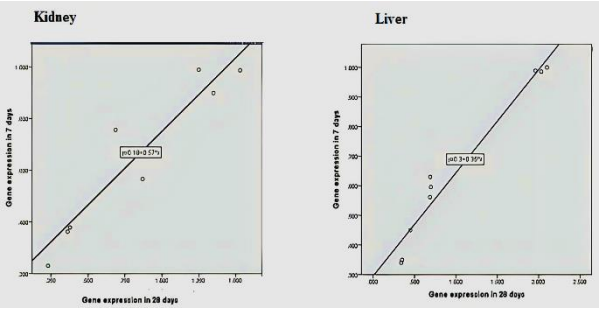

Fig (2) Spearman correlation analysis was used to examine the expression correlation of *SIRT-1*gene in kidney and liver after 7 and 28 days of metformin and metformin nanoparticles. Expression of *SIRT-1*gene had a highly positive correlation in both liver tissues (r = 0.979324) and kidney tissues (r = 0.96028).

4- DISCUSSION

Thyroid hormones (THs), T4 and T3, are released by the Incidence of type 2 diabetes necessitates the urgent development of new pharmaceutical therapy (Lv and Guo, 2020). Metformin polymeric nanoparticles may result in a significant improvement in bioavailability, lowering doses, delivery rates and side effects (Elkomy et al.,2021). Drugs are encapsulated in nanocarriers as part of a new technique of nano drug delivery (Zahin et al., 2020). Improved therapeutic efficacy at lower dosages and less adverse effects can be achieved by using this new technique (Kadian et al., 2018). Because of their low toxicity, mucoadhesion, and adaptable physical features, chitosan nanoparticles (CHNPs) are ideal for drug administration (Mohammed et al., 2017).

The glucose-lowering effect of acutely or long-term administered antidiabetic agents (metformin and metformin nanoparticles) in animal models. To quantify The OGTT method suggested the improvement for glycemic control in the single-dose treatment for 7 days. However, we applied two drugs used clinically for the treatment of diabetic disorders. Metformin is known to ameliorate diabetes mainly through activation of AMP kinase (AMPK) over time. A recent study demonstrated that acute administration of metformin was effective at lowering blood glucose levels and improving glycemic control in diabetic mice, but it did not significantly affect major insulin-sensitive tissues (Liu et al., 2022).

It indicated that the glucoregulatory influence of acute metformin treatment resulted from inhibition of intestinal glucose transport (AMPK) activation is thought to be the primary mechanism by which metformin improves diabetes. Acute metformin and metformin nanoparticles therapy has glucoregulatory effects because it inhibited intestinal glucose transfer in Diabetic mice (Bahne et al., 2018). SIRT1 has been identified as a regulator of the expression of gluconeogenic genes (Khowailed et al., 2018). Present work has been designed to investigate the possible link of the action of metformin on SIRT1 in diabetic rats and if metformin could be a line of treatment that is helpful in re-establishing the physiological relevant activity of SIRT1 known to be attenuated in diabetes. We also attempted to test if it has any preventive value through tempering of SIRT1 disorders associated with the development ofT2DM SIRT1 may regulate glucose-lipid metabolism through its deacetylase activity. SIRT1 also influences insulin secretion, adiponectin synthesis, inflammation, gluconeogenesis, oxidative stresses and developing insulin resistance. (Wang et al., 2021). Metformin and metformin nanoparticles may be used to restore SIRT1's physiologically appropriate activity in diabetic rats. Alloxan causes pancreatic b-cell partial destruction (Ossai et al., 2021). Gene expression results demonstrated that metformin and metformin nanoparticles cause overexpression in SIRT 1 gene in liver and kidney compared to control group. However, expression of SIRT1 gene was significantly overexpressed in metformin nanoparticles treated rats cause activation in AMPK (Nelson et al.,2011).

The present work has been designed to investigate the possible link of the action of metformin and metformin nanoparticles on SIRT1 in diabetic rats and if metformin nanoparticles can be a line of treatment that is helpful in re-establishing the physiological relevant activity of SIRT1 known to be attenuated with diabetes. We also want to see if it could serve as a preventative measure by reducing the severity of SIRT1 abnormalities linked to the onset of T2DM. SIRT1's deacetylase activity may help control glucose metabolism.

5. CONCLUSION

Our investigation showed that fasting serum glucose level was considerably higher than control, indicating hyperglycemia. Metabolic glucose levels were lowered after 28 days of metformin nanoparticles and metformin treatment. This is mostly due to a decrease in liver glucose synthesis and an increase in hepatocyte and adipocyte glucose absorption. From gene expression analysis metformin nanoparticles are considered a promising diabetic drug causing overexpression in *SIRT-1*gene that is

responsible for the regulation of blood glucose level throughout the body compared to metformin. We advise further studies concerning the treatment with metformin nanoparticles.

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