Resveratrol Improve the Adipokines levels in Induced Diabetes Mellitus Model

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Abstract

Background: The insulin-sensitizing ability of resveratrol has been studied mostly in experimental animals, with results showing a substantial improvement in insulin sensitivity and/or a reduction in circulating insulin concentration. The adipose tissue produces a class of bioactive chemicals known as adipokines, which function as both paracrine and endocrine hormones. There was a direct regulatory effect for insulin on adipokines release. Aim of study: The current study aimed to evaluate the effect of resveratrol on adipokines levels in relation to diabetes mellitus. Methods: Thirty six male rats were divided into 6 groups I: Negative control group, group II: Positive control diabetic induced by alloxan (120mg/kg), group III: diabetic rats treated with metformin 120 mg\kg\day after DM induction by alloxan (120mg/kg),, group IV: diabetic rats treated with resveratrol 5 mg/kg, Group V: diabetic rats treated with resveratrol 10 mg/kg,group VI: diabetic rats treated with resveratrol 20 mg/kg . Lipocaline2, Angiopoten like protein 2 LCN2, blood glucose, insulin, HBa1c were measured. HOMA and weight were also recorded. Results: This study showed an elevation in the lipocaline 2 level between negative and positive control groups. In diabetic group, lipocalin levels were increased from (8.63 ± 1.48 - 14.55 ± 1.1 ng/ml), significant 37% reduction (14.55 ± 1.1 to 9.2 ± 0.78 ng/ml) was found after treatment with 20 mg resveratrol compared with the positive control group at ($p \leq 0.01$), also showed significant raise between negative and positive groups, diabetes rises angiopoietin levels (from 370.63 ± 24.4 to 572 ± 72.4 ng/l), ($p < 0.001$), significant difference were found only between the use of 20 mg resveratrol and positive control groups ($p = 0.02$) i.e. the use of 20 mg of resveratrol lowers angiopoietin levels (from 572 ± 72.4 to 412.5 ± 81 ng/l). No difference was found between use of 20 mg resveratrol and negative control group ($p > 0.05$). Conclusions: Resveratrol improve the levels of lipocaline2, Angiopoten like protein 2 and glycemic status.

Keywords: Diabetes mellitus, Resveratrol, Adipokines

1. Introduction

Diabetus mellitus is a metabolic disease, characterize by high blood glucose despite of the cause and mechanism that led disease to develop. The most common mechanism that lead DM development either impaired in insulin secretion or resistance of insulin or both of them interchangeably [1].

Adipokines are functionally active molecules produced by adipose tissue which could be hormones, growth factors, angiogenic factors, and cytokines. They are include tumor necrosis factor-α (TNF-α), leptin, interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), adiponectin, resistin, and others which acts as paracrine and endocrine hormones, Adipokines are classified “proinflammatory adipokines” and “anti-inflammatory adipokines", imbalance between them which occur in inflammation will alter adipokine secretion toward a diabetogenic, proinflammatory and atherogenic pattern. DM developed when the insulin action impair in adipose tissue (AT), skeletal muscle, and the liver [2].

Resveratrol a phytoalexin, also known as (3,4′,5-trihydroxystilbene), is found in a wide range of plants, including grains, fruits, vegetables, dry beans, grapes, peanuts, and berries. It is created in reaction to fungus infections, UV radiation, and mechanical trauma [3]. Resveratrol is a SIRT1 activator, a mammalian version of the sirtuin family of proteins. Metabolism, stress resistance, cell survival, cellular senescence, inflammation-immune function, endothelial functions, and circadian rhythms are all affected by the SIRT1-regulated pathway [4]. Through the SIRT1 pathway, resveratrol has an anti-inflammatory effect on adipokine expression and secretion in human adipose tissue in vitro [5].

The current study aimed to evaluate the levels of adipokine in alloxan induced diabetes in animal model. In addition studying the effect of resveratrol on the level of adipokine and consequently their effects on diabetes diseases.

2. Materials and Methods

Animals and experimental design

Thirty-six albino Wister male weighing (230-250) g were purchased from Iraqi center for cancer research and kept in the animal house of the College of Pharmacy/ Mustansiriyah University, where this study had been conducted. Before being employed in tests, the rats were given a ten-day environmental adaptation period. Animal groups were as follows

Group I: Negative control group, rats have received normal saline (0.9%) by intraperitoneal (ip) route (10 mL/kg) for 21 consecutive days.

Group II: Positive control diabetic group, received normal saline (0.9%) for seven days (day 1-7) followed by a single dose of alloxan (120mg/kg) in the eighth day.

Group III: Rats treated with metformin 120 mg\kg\day (dissolved in 0.9 % normal saline) and given orally for 21 days, and alloxan was injected ip on the 8th day.

Group IV: Rats treated with resveratrol which was dissolved in normal saline 0.9% and given orally at dose 5

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mg/kg for 21 days, and alloxan was injected ip on the 8th day.

Group V: Rats treated with resveratrol which was dissolved in normal saline 0.9% and given orally at dose 10 mg/kg for 21 days, and alloxan was injected ip on the 8th day.

Group VI: Rats treated with resveratrol which was dissolved in normal saline 0.9% and given orally at dose 20 mg/kg for 21 days, and alloxan was injected ip on the 8th day.

Serum sample collection

Blood was obtained from all animals at the end of the trail, and rats were anesthetized using the chloroform inhalation procedure. The blood samples were taken through heart puncture using a 10 ml syringe, then placed in a plain tube containing gel and allowed to clot for 15 minutes before being centrifuged at 3000x for 15 minutes to prepare the serum. For ELISA analysis, the serum was kept in eppindorf tubes at -40°C.

Measurements

Both lipocaline 2 and angiopoten like protein 2 levels were measured by using enzyme linked immune sorbent assay (Bioassay Co., China) Insulin levels were determined by ELISA (De mid tech, G (Blood glucose levels were calculated by spectrophotometer (Spain react Co., Morocco) [6] HOMA-IR is calculated by HOMA-CIGMA software (HOMA2 calculator program) [7].

3. Result

The effect of resveratrol and metformin on lipocalin Levels:

This study showed an elevation in the lipocaline 2 level between negative and positive control groups. In diabetic group, lipocalin levels were increased from (8.63 ± 1.48 - 14.55 ± 1.1 ng/ml), nearly 40% elevation at ($p < 0.001$). Significant 37% reduction (14.55 ± 1.1 to 9.2 ± 0.78 ng/ml) was found after treatment with 20 mg resveratrol compared with the positive control group at ($p \leq 0.01$). On the other hand, no difference was found between 20 mg Resveratrol treated group and negative control group as shown in table (3.3) & figure

Table 3.3: Lipocalin levels (ng/ml) according to groups

Groups	N	Lipocalin levels Mean ± SD	Range	
			Minimum	Maximum
Negative control	6	8.63 ± 1.48 a	6.89	10.52
Diabetic Positive control	6	14.55 ± 1.1 b	12.92	15.9
Diabetic with Metformin	6	14.1 ± 0.82 b	13.08	15.49
Diabetic with resveratrol 5 mg	6	13.42 ± 0.45 b	12.96	14.07
Diabetic with resveratrol 10 mg	6	13.13 ± 1.1 b	11.62	14.72
Diabetic with resveratrol 20 mg	6	9.2 ± 0.78 a	8.3	10.2

Relationship between different lower case letters represents significantly different statistics (P<0.05), however relationship between similar lower case letters showed no significance. (P > 0.05).

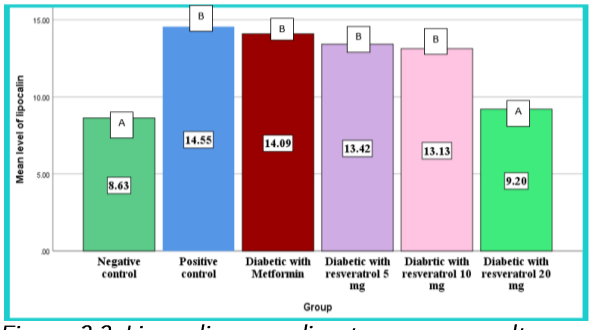

Figure 3.3: Lipocalin according to groups results were expressed as Mean±SD.

The effect of resveratrol and metformin on angiopoietin like protein 2 levels:

This study showed significant raise between negative and positive groups, diabetes rises angiopoietin levels (from 370.63 ± 24.4 to 572 ± 72.4 ng/l), nearly 50% ($p < 0.001$), statistical difference were found only between the use of 20 mg resveratrol and positive control groups ($p = 0.02$) i.e. the use of 20 mg of resveratrol lowers angiopoietin levels (from 572 ± 72.4 to 412.5 ± 81 ng/l) about 28%. No difference was found between use of 20 mg resveratrol and negative control group ($p > 0.05$), table (3.4).

Table 3.4:Angiopoietin like protein 2 levels (ng/l) according to groups

Groups	N	Angiopoietin Mean ± SD	Range	
			Minimum	Maximum
Negative control	6	370.63 ± 24.4 a	342.29	406.86
Diabetic Positive control	6	572 ± 72.4 b	483.95	681.93
Diabetic with Metformin	6	537.2 ± 47 b	471.75	595.79
Diabetic with resveratrol 5 mg	6	494.63 ± 32.1 b	454.68	552.67
Diabetic with resveratrol 10 mg	6	494 ± 53.8 b	400.21	561.07
Diabetic with resveratrol 20 mg	6	412.5 ± 81 a	317.1	528.94

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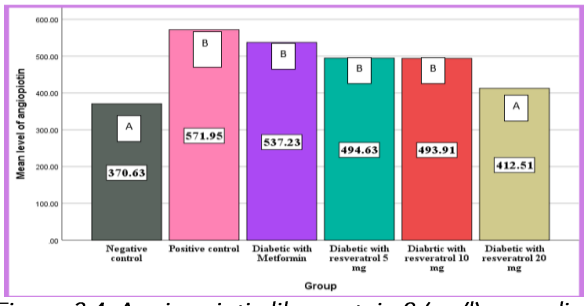

Figure 3.4: Angiopoietin like protein 2 (ng/l) according to groups Results were

The effect of resveratrol and metformin on level of insulin:

This study showed significant decrease between negative and positive control groups (20.1 ± 2.94 vs. 16.4 ± 1 µIU/mL,respectively), (p<0.05). There was no significant difference when compared among the remaining groups (p >0.05) as shown in table (3.6) and figure (3.6).

Table (3.6): Insulin levels according to groups

Groups	N	Insulin Mean ± SD	Range	
			Minimum	Maximum
Negative control	6	20.1 ± 2.94 a	17.54	24.16
Diabetic Positive control	6	16.4 ± 1 b	15.16	17.57
Diabetic with Metformin	6	17.8 ± 1.4 c	15.62	19.35
Diabetic with resveratrol 5 mg	6	17.6 ± 1.9 c	15.49	20.95
Diabetic with resveratrol 10 mg	6	17.7 ± 1.1 c	15.96	18.93
Diabetic with resveratrol 20 mg	6	18.3 ± 1.5 c	16.54	20.73
Relationship between different lower case letters represents significantly difference (P<0.05), however relationship between similar lower case letters showed no significance difference.				

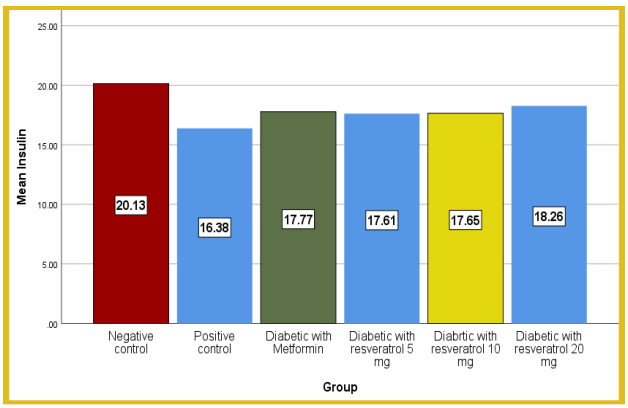

Figure 3.6: Insulin Levels according to groups Results were expressed as Mean±SD.

The effect of resveratrol and metformin on glucose levels:

This study resulted in significant elevation (p <0.001) between negative and positive control groups in regards to glucose levels (105 ± 8.6 vs. 369 ±37.3 mg/dl). Diabetic with metformin group showed significant reduction (p <0.05) in glucose levels compared to positive group (369 ± 37.3 mg/dl vs. 251 ± 51.6 mg/dl). The use of reseveratrol 20 mg lowered glucose levels significantly (p <0.05) when compared to positive control group (369 ±37.3 mg/dl to 206.8± 52 mg/dl). However, there were no difference between metformin group and resveratrol groups regarding glucose levels (p > 0.05), table (3.7) and figure (3.7).

Table (3.7): Glucose levels according to groups

Groups	N	Glucose Mean ± SD (mg/dl)	Range	
			Minimum	Maximum
Negative control	6	105 ± 8.6 a	93	119
Diabetic Positive control	6	369 ±37.3 c	315	413
Diabetic with Metformin	6	251 ± 51.6 b	189	321
Diabetic with resveratrol 5 mg	6	245 ± 64.3 b	185	348
Diabetic with resveratrol 10 mg	6	228.2 ± 65.3 b	143	301
Diabetic with resveratrol 20 mg	6	206.8 ± 52 b	137	258
Relationship between different lower-case letters showed significant difference (P<0.05), however relationship between similar lower case Letters showed no Significant difference (p>0.05).				

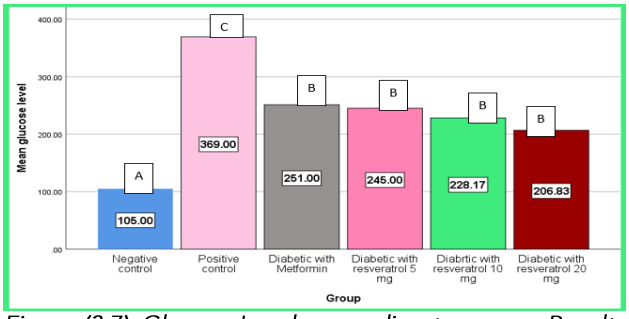

Figure (3.7): Glucose Levels according to groups Results were expressed as Mean±SD.

The effect of resveratrol and metformin on insulin resistance HOMA

This study resulted in significant increase between negative (5.2 ± 0.92) and positive control group (14.9 ± 0.77) in regards to HOMA levels (p <0.001)

There was significant lowering (p<0.05) between positive control group and metformin group (14.9 ± 0.77 to 11 ± 2.2). No significant change was found between metformin group and other resveratrol groups (p >0.005).

There were significant decline between 5, 10 and 20 mg resveratrol group and the positive group (p =0.006, 0.001 and

0.001 respectively). No significant differences were found among the resveratrol groups.

Table (3.9): HOMA IR levels according to groups				
Group	N	Insulin Resistance Mean ± SD	Range	
			Minimum	Maximum
Negative control	6	5.2 ± 0.92 a	4.4	6.9
Positive control	6	14.9 ± 0.77 c	13.6	15.6
Diabetic with Metformin	6	11 ± 2.2 b	7.7	13.5
Diabetic with resveratrol 5 mg	6	10.6 ± 2.5 b	7.3	14.6
Diabetic with resveratrol 10 mg	6	9.8 ± 2.4 b	6.2	12.6
Diabetic with resveratrol 20 mg	6	9.2 ± 1.8 b	6.3	10.8
# Relationship between different lower case letters showed Statistical significant (P<0.05),however relationship between similar lower case Letters showed no statistical Significance (p>0.05)				

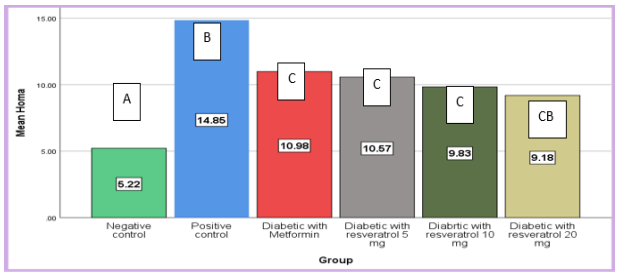

Figure 3-9: HOMA IR Levels according to groups.

4. Discussion

This study showed that induced diabetes raised Lipocalin levels,it was in accordance with a study done by Wu et al. [7] who reported increase in lipocaline 2 by exogenous recombinant LCN2 supplementation enhanced hepatocyte glucose production, indicating that LCN2 may play a causative role in insulin resistance and hyperglycemia [8]. This might point to a potential predisposition to consequences from LCN2, such as metabolic syndrome, insulin resistance, ischemic heart disease, and diabetic kidney disease [9]. LCN2, notably LCN2 expressed in macrophages, is crucial in vascular remodeling and plaque instability in atheroseclerosis as and a diabetic complication reference to diabetes consequences such atherosclerosis. Insulin sensitivity and glucose homeostasis are both significantly influenced by LCN2 [10].

The use of 20 mg resveratrol found to normalize the levels of lipocalin (p>0.005), this finding was in line of a Turkish study that showed an increase in liopcaline2 expression results from an increase in pro-inflammatory cytokines. With the use of resveratrol, the level of liopcaline 2 can return to normal, and the production of pro-inflammatory cytokines can be reduced [11]. In this study levels of angiopoietin like protein 2 (ANGPTL2) was high in diabetic group in regard to healthy negative group (p<0.05). similar finding was associated with a Japanese prospective study of a cohort of the general Japanese population study that revealed higher levels of ANGPTL2 with diabetic group [12].

By initiating a signaling cascade and releasing a number of inflammatory cytokines, toll-like receptors (TLRs) play a crucial part in the activation of both innate and adaptive immune responses. TLR4 was thought to be a possible ANGPTL2 receptor in endothelial cells and monocytes. It is well known that up-regulation of TLR4 is seen in people with diabetes [13].

In present study the levels of ANGPTL2 lowered near normality by the use of 20 mg resveratrol (p>0.05). however this was in line with a Chinese studies [14] showed that RSV may drastically reduce VEGF and Angiopoietin II production, and EX527 can reverse this effect, suggesting that RSV can enhance pancreatic microcirculation by fostering angiogenesis, maturation, remodeling, and stability [14]. Resveratrol has a significant pharmokinetic potential as an antidiabetic medication because it improves impaired insulin signaling, prevents pancreatic beta-cell apotopsis and malfunction, inhibits aberrant glucose uptake and storage, and reduces hyperlipidemia and dyslipidemia [15]. Resveratrol's ability to influence several pathways and its range of molecular targets, which include phospodiesterases, adenylyl cyclase, kinases, sirtuins, transcription factors, cytokines, and others, may be responsible for its complicated physiological effect as an anti-diabetic drug. The class of enzymes known as cyclic nucleotide phosphodiesterases (PDEs) hydrolyzes cAMP and cGMP's phosphodiester bonds to produce their physiologically inert 5′ derivatives. One important mediator in the control of metabolism is cyclic AMP,by inhibiting PDE, resveratrol raises cAMP levels, which boosts the insulin release caused by glucose [16].

Several meta-analysis Chinese study showed the same result, Consuming resveratrol dramatically decreased insulin resistance, glycated hemoglobin, fasting blood sugur, and insulin [17]. The preservation of glucose homeostasis depends on the control of glucose absorption and subsequent use. Insulin regulates blood sugar levels by promoting glucose absorption by moving the glucose transporter Glut-4 from the intracellular pool to the caveolar membrane system. Resveratrol stimulates the absorption of glucose via increasing the expression of the glucose transporter Glu-4 [18].

In both healthy and diseased skeletal muscle, the transcriptional coactivator PGC-1 has become a critical regulator of metabolic programming. Although PGC-1 has a variety of functions in many tissues, it drives the transcriptional pathway that leads to mitochondrial biogenesis in almost every situation. Insulin resistance in skeletal muscle is a result of PGC-1 malfunction, which leads to mitochondrial insufficiency. Resveratrol also has proven to enhance the PGC-1α–skeletal muscle protein levels. A modulator of the insulin-signaling pathway, Akt expression, is likewise activated by resveratrol. The phosphorylation of Akt causes it to become the primary effector of the IR-IRS-1-PI3K pathway. Treatment with resveratrol raises Akt's level of phosphorylation, especially at its Thr308 and Ser473 residues, which are necessary for both its minimal and complete activation.

Additionally, resveratrol may lessen insulin resistance by lowering the inflammatory response in diabetes.

In conclusions, resveratrol improved the levels of lipocaline2, Angiopoitin like protein 2 and glycemic status. When compare different doses of resveratrol mg/kg,the current study found the best improvement in adipokine and glycemic status at dose 20 mg/kg.

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