In vivo study of polyphenol (3,5,4′ – trihydroxy stilbene) and its Derivative on Serum Urea Concentration on alloxan diabetic rabbits

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Abstract

The study has employed an *in vivo* evaluation of resveratrol and its derivative in female rabbits at concentrations (1mg/kg) given orally for 42 days after inducing diabetes mellitus type 2 by alloxan (100mg/1kg body weight). The serum was isolated from heart blood for the biochemical tests, including Serum urea concentration, total protein conc., albumin and serum globulin concentration. Statistical analysis showed a significant decrease in urea,Total protein,albumin, globulin concentration of serum blood levels on treated rabbits $p < 0.05$. We can concluded that the Resveratrol and their derivatives are safe and potent hypoglycemic and hypolipidemic agent which is capable of normalizing other biochemical and hematological abnormalities associated with diabetes mellitus type 2. Thus could be prescribed as adjunct to dietary therapy and main therapy for diabetes mellitus type 2.

KEYWORDS: Serum urea, serum globulin concentration, polyphenol compound,3,5,4′ – trihydroxy stilbene

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from the defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. The liver and kidney play a major role in the pathogenesis of type 2 diabetes.Scientists became interested in exploring potential health benefits of resveratrol in 1992 when its presence was first reported in red wine, leading to a speculation that resveratrol might help explain the " French Paradox" (Pussa*et al.*, 2006). The potential for resveratrol to treat diabetes mellitus type 2 the has continued to generate scientific interest (Howitz*et al.,* 2003).

Resveratrol(3,5,4′ – trihydroxy stilbene)), is a non flavonoid polyphenol and it has three phenolic hydroxyl groups and shown to have its biological effects (Tomas- Barberan*et al*,. 1997).

Phenolic and polyphenolic compounds, possess an aromatic ring bearing one or more hydroxyle substituents. Harborne (1984). These compounds are phytoalexins and have antidiabeteic properties.(Bruneton, 1999).The present study focuses on protein metabolic disorder management efficacy of resveratrol and their derivatives in alloxan-induced diabetic rabbits.

Materials and Methods

Preparation of Resveratrol Derivative

5-(4-(4-(2-(benzo [d] thiazol-2-yl) hydrazinyl) butanoyloxy)styryl)-1,3-phenylene bis(4-(2-(benzo[d]thiazol-2-yl) hydrazinyl)butanoate).

1.6gm (0.003 mole) of compound (2) was dissolved in 50ml of absolute ethanol then 1.58g 0.0096moles of 2- Mercptobenzothiazole then reflux for 8h. The solvent was removed and the precipitates was filtrated and dried (Stephen *et al* .,2006).

Induction of Diabetes

5,6-dioxyuracil,was used which marketing name (alloxan) and whose diabetogenic property is established with rabbits,.It is easier to handle and in which the diabetogenic property was also observed (Dubey *et al*., 1994). The alloxan injection depends on the weight of the animal: 70 mg of alloxan dissolved in 3 ml of distilled water for 1 kg of rabbit weight. The alloxan solution is injected through the marginal vein of the ear after fasted for 24 h before injection. This single dose of alloxan produced type 1 diabetes having fasting blood sugar level of 155±10.71 mg/dl after10 days of injection of alloxan and this diabetic state was maintained throughout the`1 duration of the experiment .Therefore, the health status of rabbits was closely monitored until the hyperglycemia appeared and was detected weekly by determination of blood sugar quantity(Dubey *et. al.*,1994).

Purified Resveratrol and its Derivative Dilutions

Pure resveratrol 100 mg was dissolved in 100ml PBS and 0.1ml DMSO as organic solvent for dissolving the substance. The stock was kept in a dark container at –20°C after sterilization with 0.22µm Millipore filter.

Resveratrol derivative 50mg were dissolved in 0.05ml DMSO and complete the volume to 50 ml with PBS. Then it was sterilized and kept in a dark container at –20°C.

Experimental Design:

Fifteen adult female rabbits were randomly divided into four groups and treated daily as follows for six weeks: The rabbits in first group received regular standard diet, tap water and served as control. After feeding them for about 1 week, their body weights and fasting blood sugar levels were taken. Other parameters which included levels were also taken and recorded.

Group 2 : Diabetic rabbits without treatment

At the expiration of 1 week, alloxan was subject into the control group and they formed group (2) rabbits. The rabbits were confirmed diabetic after estimation of their fasting blood sugar level, 2weeks after injection of alloxan. A rabbit was considered to be diabetic if it had a fasting blood sugar where level > 115 mg/dl. Other parameters were also taken and recorded.

Group 3: Diabetic rabbits after treatment with glbcimaide

At the expiration of 2 weeks of induction of diabetes into the rabbits of group (2), they were force fed with glbcimaide 0.05mg orally for a period of 6 weeks and, thus they

formed the rabbits of group 3. At the end of 4 weeks their fasting blood glucose levels was estimated and recorded. Other parameters were also taken and recorded.

Group 4: Diabetic rabbits after treatment with Resveratrol

At the expiration of 2 weeks of induction of diabetes into the rabbits of group (2), they were force fed with resveratrol 1mg/ml orally for a period of 6 weeks and, thus they formed the rabbits of group 4. At the end of 4 weeks their fasting blood glucose levels were estimated and recorded. Other parameters were also taken and recorded.

Group 5: Diabetic rabbits after treatment with Resveratrol derivative

At the expiration of 2 weeks of induction of diabetes into the rabbits of group (2), they were force fed with resveratrol derivative 1mg/ml orally for a period of 6 weeks and they thus formed the rabbits of group (3). At the end of 4 weeks their fasting blood glucose levels was estimated and recorded. Other parameters were also taken and recorded.

Biochemical Estimation:

The serum cholesterol concentration was estimated according to (Young *et al*., 2000); Total protein (Peter*et al*., 1968); serum albumin (Drupt*et al*.,2000)]; Serum globulin was estimated by equation: Serum globulin conc. (g/dI)= Total protein-Serum albumin .

Statistical Analysis

Data were analyzed by using the SPSS package programmed. Multiple range test was used to detect the significant differences [13].The method which was used to measure the significances at level 0.05 or 0.01 was taken ([12].

Results and Discussion

Urea is the chief end product of protein metabolism in the body. The importance of the urea concentration in blood lies in its value as an indicator of kidney function. The clinical significance of the urea level in plasma is usually determined in conjugation with the plasma creatinine level.

Interestingly the levels of these electrolytes came to near normal levels by resveratrol and their compounds.

In diabetes, the obligatory renal water loss combined with the hyperosmolarity tends to deplete intracellular water, triggering the osmoreceptor of the thirst Centre of the brain and polydipsia, which leads to increase in water intake (UKPDS *et al.,* 1998).

Table (1) shows the effect of resveratrol and its derivative on urea level. The level of serum urea 36mg/dL± 1.0 is a sign of renal dysfunction in the diabetic rabbits when compared to control rabbits 20mg/dL±1.0. The results showed significant decrease in the level of serum urea of rabbits treated with resveratrol and its derivative significantly ($p<0.05$). Resveratrol derivative shows significant value after one week of treatment 20mg/dL± 1.0.

An experiment was conducted to study the efficacy of glimepiride (a new sulphonylurea) on the biochemical values and histomorphological features of streptozotocin-induced diabetic rabbits, by single intravenous administration of streptozotocin 2mg/kg. body weight daily for twenty-one days whereas The results indicated a significant decrease in

serum urea(P<0.001). Further, the histomorphological study showed an increase in the percentage of beta cells in pancreatic islets and recovery of renal tubules The findings indicate that glimepiride improves the biochemical values and ameliorates the histopathology of diabetic rabbits particularly restoring the morphology of beta cells of islets of Langerhan's (Sajad Hussain *et al*,.2008).

The effects of Oral administration of an aqueous extract of *A. squamosa*leaves (300 mg/kg body weight) of rats for 30 days were examined in the plasma, liver and kidney tissues of control and experimental groups. It shows significant decrease in diabetic rats on blood urea (p<0.05) (Kaleem*et al*,.2008).

Table (1): The treatment effect of resveratrol and its derivative on serum urea concentration mg/dL of rabbits' groups with weeks.

Groups	Treatment periods							
	WK0	**PT**	**Wk1**	**Wk2**	**Wk3**	**Wk4**	**Wk5**	**Wk6**
C	20± 1.05^{a} A	20± 1.05^{b} A	22± 1.1^{e} A	22± 1.1^{d} A	21± 1.04^{d} A	20± 1.05^{d} A	20± 1.05^{d} A	20± 0.5^{c} A
D	20± 1.05^{a} B	36± 1.05^{a} A	36± 1.5^{a} A	36± 1.5^{a} A	35± 1.55^{a} A	36± 1.5^{a} A	35± 1.55^{d} A	36 ± 1.5^{a} A
DI	20 ± 1. 05^{a} C	35± 1.55^{a} A	30± 1.5^{bc} B	30± 1.6^{b} B	31± 1.62^{b} B	29± 1.04^{b} B	30± 1.6^{b} B	30 ± 1.6^{b} B
DR	20± 1. 05^{a} D	35± 1.55^{a} A	33± 1.57^{b} A	32± 1.63^{b} AB	30± 1.6^{bc} B	29± 1.04^{b} B	25± 1.2^{c} C	22 ± 1.6^{b} D
DRD	21 ± 1.07^{a} B	35± 1.55^{a} A	22± 1.6^{e} B	21± 1.08^{d} B	20± 1.04^{d} B	20± 1.05^{d} B	20± 1.05^{d} B	20 ± 1.05^{c} B

Each value represent mean ± SD

Values with non-identical superscripted (a, b, c, d, e& f) are considered as significantly different (p<0.05) among the same group of rabbits, Values with non-identical superscripted (A, B, C, D, E& F) are considered as significantly different (p<0.05) among the different groups of rabbits, N(number of animals)=3.

C=Control ;D=Diabetic; I=Diabetic after treated with glbcimide;DR= Diabetic after treated with resveratrol; DRD= Diabetic after treated with derivative.

The results show significant increase in the level of Serum total protein which is a sign of renal dysfunction in the diabetic rabbits when compared to control rabbits. The diabetic rabbits treated with the resveratrol and its derivativeshowed significant decrease in levels of Serum Total protein (p<0.05) . Resveratrol derivative and resvertrol show significant value after one week of treatment 6.9±0.03and6.6±0.03respectively as shown in table(2). In diabetes, protein catabolism increases due to deficiency of carbohydrate-derived

energy in connection with low-serum insulin. The present study focuses on protein metabolic disorder management efficacy of resveratrol and their derivatives in alloxan-induced diabetic rabbits.Our study agreed with the effects of Z. *multiflora* Essential Oil (EO) were investigated for anti-diabetic properties in the STZ-induced diabetic rats. The main components of this EO were carvacrol (53%), p-cymene (17%), thymol (11%), myercene (2.3%), α-pinene (2%), carvacrol methyl ether (2%), α-terpiene (1.3%), β-caryophyllene (1.2%), and α- thujene (1.1%).The concentration of total protein in the plasma of nondiabetic control rat was 7.75 ± 0.28 mg/dl. In the nondiabetic rats administered EO, this value was 7.67 ± 0.69 mg/dl, but in the diabetic control rats, it was 4.47 ± 0.41mg/dl. In the diabetic rats administered Essential Oil (EO) this value was 6.25 ± 0.64 mg/dl. Thus, *Z. multiflora* Essential Oil (EO) had no effects on the total protein level in the healthy rats but in the diabetic rat the level of total protein increased to the nondiabetic control level (p = 4 × 10-6, n=8) (Brownlee *et al*,. 2005). Results indicated a reduction in the total protein in the diabetic rats. Oral administration of EO restored protein concentration to the control level. Probably oxidative stress and at the same time production of nitric oxide can produce peroxynitrite, which led to tyrosine nitrosationincluding ribosomal protein) and consequently reduced protein production (Brownlee *et al*,. 2005).

Table (2): The effect of resveratrol and its derivative on serum total protein concentration
mg/dL of rabbits' groups with weeks.

Groups	Treatment periods							
	WK0	**PT**	**Wk1**	**Wk2**	**Wk3**	**Wk4**	**Wk5**	**Wk6**
C	6.9± 0.03 [a] A	7.7± 0.07 [b] A	7.1± 0.02 [a]A	7.1± 0.02 [a]A	7.1±0. 02 [a]A	6.9± 0.03 [a]A	7± 0.05 [b] A	7.1± 0.02 [b] A
D	7.1± 0.03 [a] B	5.1± 0.05[e] E	5.2± 0.03 [f] E	5± 0.05 [e] E	5± 0.05[e] E	5± 0.05 [e] E	5.3± 0.01 [f] E	5 ± 0.05 [e] E
DI	7.0± 0. 02 [a]A	5.3± 0.08 [e] F	7.1± 0.02[fdc] A	7.2± 0.06 [b] A	7.2± 0.06[b] A	7.1± 0.02 [b] B	6.9± 0.03 [a] D	7 ± 0.023 [b] A
DR	7.1± 0. 02[a] B	5.3± 0.05[e] F	6.6± 0.03 [dc] CB	6.8± 0.10 [c] B	6.8± 0.10 [c] B	7.2± 0.06[b] [a] A	6.8± 0.10 [b] C	6.8 ± 0.10[b] B
DRD	7.7 ± 0.07[a] C	5.4± 0.1 [e] D	7.1± 0.02[dc] A	7.2± 0.06[b] B	7.2± 0.06 [b]B	7.2± 0.06 [b]B	7.4± 0.02 [a] A	7.3± 0.02 [a] B

Each value represent mean ± SD

Values with non-identical superscripted (a, b, c, d, e& f) are considered as significantly different (p<0.05) among the same group of rabbits, Values with non-

identical superscripted (A, B, C, D, E& F) are considered as significantly different (p<0.05) among the different groups of rabbits, N(number of animals)=3.

C=Control ;D=Diabetic; I=Diabetic after treated with glbcimide;DR= Diabetic after treated with resveratrol; DRD= Diabetic after treated with derivatives.

The results showed significant increase in the level of Serum Albumin which is asign of renal dysfunction in the diabetic rabbits when compared with control rabbits.

The diabetic rabbits treated with resveratrol and its derivative decrease levels of Serum Albumin significantly (p<0.05). The results showed significant decrease in the level of serum urea of rabbits treated with resveratrol and its derivative (p<0.05). Resveratrol derivative and resveratrol show significant value after one week of treatment 3.8±0.2 and 3.7±0.18 respectively as shown in table(3) .

The effects of Oral administration of an aqueous extract of *A. squamosa*leaves (300 mg/kg body weight) of rats for 30 days were examined in the plasma, liver and kidney tissues of control and experimental groups. It shows significant decrease to diabetic on blood albumin level (p<0.05)(Kaleem*et al*,.2008).

Table (3): The effect of resveratrol and its derivative on serum albumin concentration
mg/dL of rabbits' groups with weeks.

Groups	Treatment periods							
	WK0	**PT**	**Wk1**	**Wk2**	**Wk3**	**Wk4**	**Wk5**	**Wk6**
C	4.4± 0.1 [a] A	4.3± 0.07 [a] A	4.3± 0.07 [a] A	4.2± 0.05 [a] A	4.4± 0.10 [a] A	4.2± 0.05 [a] A	4.2± 0.05 [a] A	4.3± 0.08 [a] A
D	4.2± 0.1 [a] A	2.9± 0.15 [b] B	2.9± 0.22 [b] B	2.6± 0.15 [b] B	2.7± 0.17 [b] B	2.9± 0.22 [b] B	2.9± 0.1 B	2.7± 0.18 [b] B
DI	4.3 ± 0.09 [a]A	3.0± 0.15 [b]B	3.9± 0.23[a] A	4.5± 0.25 [a] A	4.4± 0.20 [a] A	4.2± 0.04 [a] A	4.4± 0.1 [a] A	4.1 ±0.02[a] A
DR	4.2± 0.05 [a]A	3.1± 0.03 [b]B	3.7± 0.1[a] A	3.8± 0.20[a] A	4.0± 0.10 [a] A	4.4± 0.2 [a] A	4.4± 0.1 [a] A	4.2± 0.05 [a]A
DRD	4.2 ± 0.06 [a]A	2.9± 0.21 [b]B	4.2± 0.05 [a] A	4.3± 0.08 [a] A	4.3± 0.07 [a] A	4.4± 0.02 [a] A	4.4± 0.06 [a]A	4.3± 0.10 [a]A

Each value represent mean ± SD

Values with non-identical superscripted (a, b, c, d, e& f) are considered as significantly different (p<0.05) among the same group of rabbits, Values with non-identical superscripted (A, B, C, D, E& F) are considered as significantly different (p<0.05) among the different groups of rabbits, N(number of animals)=3.

C=Control ;D=Diabetic; I=Diabetic after treated with glbcimide;DR= Diabetic after treated with resveratrol; DRD= Diabetic after treated with derivative.

WK0=Zero week; PT=Pretreatment; WK1=First week; WK2=Second week; WK3=Third week; WK4=Fourth week; WK5=Fifth week; WK6=Sixth week.

The results showed significant increase in the level of Serum Globulin which is a sign of renal dysfunction in the diabetic rabbits when compared to control rabbits. The diabetic rabbits treated with the resveratrol and its derivative showed significant decrease levels of Serum Albumin ($p<0.05$). The results showed significant decrease in the level of serum Globulin of rabbits treated with resveratrol and its derivative ($p<0.05$).Resveratrol derivative and resveratrol showed significant value after one week of treatment 2.2±0.10and 2.2±0.10 respectively as shown in table (3).

The effects of Oral administration of an aqueous extract of *A. squamosa*leaves (300 mg/kg body weight) of rats for 30 days were examined in the plasma, liver and kidney tissues of control and experimental groups.It shows show significant decrease to diabetic on blood albumin/globulin level ($p<0.05$)(Kaleem*et al*,.2008).

Table (4): The effect of resveratrol and its derivative on serum Globulin concentration
mg/dL of rabbits' groups with weeks.

Groups	Treatment periods							
	WK0	**PT**	**Wk1**	**Wk2**	**Wk3**	**Wk4**	**Wk5**	**Wk6**
C	2.6± 0.15 ^{a}A	2.8± 0.20 ^{a}A	2.6± 0.15 ^{a}A	2.5± 0.13 ^{a}A	2.5± 0.13 ^{a} A	2.5± 0.13 ^{a}A	2.5± 0.13 ^{a}A	2.5± 0.13 ^{a}A
D	2.4± 0.10 ^{a}A	1.9± 0.23 ^{b}B	1.9± 0.23 ^{C} B	2± 0.05 ^{c} B	2.2± 0.10 ^{b} A	1.8± 0.20 ^{b}B	2.2± 0.5 A	1.9 ± 0.22 ^{b}B
DI	2.5 ± 0.13 ^{a}A	2.0± 0.05 ^{b}B	2.1± 0.06^{bc} B	2.6± 0.12 ^{a}A	2.5± 0.13 ^{a} A	2.4± 0.10 ^{a}A	2.5± 0.13 ^{a}A	2.6 ± 0.15 ^{a}A
DR	2.5± 0.13 ^{a}A	1.9± 0.23 ^{b} C	2.2± 0.10^{b} B	2.2± 0.10^{bc} B	2.3± 0.08 ^{ab}AB	2.4± 0.10 ^{a}A	2.5± 0.13 ^{a}A	2.5 ± 0.13 ^{a}A
DRD	2.4 ± 0.10 ^{a}A	2.0± 0.05 ^{b}B	2.4± 0.1 $^{a\ b}$ A	2.4± 0.1 ^{a}A	2.4± 0.10 ^{ab} A	2.4± 0.10 ^{a}A	2.6± 0.15 ^{a}A	2.5± 0.13 ^{a}A

Each value represent mean ± SD

Values with non-identical superscripted (a, b, c, d, e& f) are considered as significantly different ($p<0.05$) among the same group of rabbits, Values with non-identical superscripted (A, B, C, D, E& F) are considered as significantly different ($p<0.05$) among the different groups of rabbits, N(number of animals)=3.

C=Control ;D=Diabetic; I=Diabetic after treated with glbcimide;DR= Diabetic after treated with resveratrol; DRD = Diabetic after treated with derivative.

Conclusion: The significant anti-diabetic effect of resveratrol may be due to the presence of phenolics, the presence of hydroxyl as shown in resveratrol and carboxyl groups as shown in derivative (1), increase number of carbon chains as shown in derivative (2), presence of amine groups as shown in derivative 3 and heterocyclic amide rings as shown in derivative 4 or their synergistic properties. Further studies are to be carried out to investigate the anti-diabetic principle present in these compounds may be used as a phytomedicine for anti-diabetic treatment in future.

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