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# Comparative study of nephroprotective effects of resveratrol and silymarin in diabetic rats; an experimental histopathologic study

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**ABSTRACT**

**Introduction:** Diabetes mellitus (DM) is distinguished as a serious health problem worldwide. The universal outbreak of DM because of urban life and alteration of lifestyle, day to day is increasing.
**Objectives:** The present investigation was designed to evaluate the nephroprotective effects of resveratrol (RSV) and silymarin (SM) on morphologic injury to renal tubular cells in adult male diabetic rats.
**Materials and Methods:** Twenty-five male Wistar rats randomly were designated into five groups (n = 5) including group I (control); rats received normal saline by gavage for 14 days. Group II; rats received a single injection of STZ at a dose of 60 mg/kg intraperitoneally and were also given isotonic saline orally for 14 days. Group III; Rats, after STZ injection, received 100 mg/kg of SM by gavage for 14 days. Group IV; Rats, after STZ injection, received 100 ml/kg of RSV by gavage for 14 days. Group V; rats, after STZ injection, received the combination of SM and RSV at a dose of 100 mg/kg by gavage for 14 days. The kidneys were removed immediately after sacrificing and prepared for morphological examination. Kidney sections were examined for the intensity of kidney damage (vacuolization, flattening, degeneration and necrosis).
**Results:** Significant differences were observed in types of morphologic injury to renal tubular cells (vacuolization, flattening, degeneration and necrosis) between groups ($P<0.05$). Significantly, both the SM and RSV reduced the injury of renal tubular cells in diabetic rats ($P<0.05$).
**Conclusion:** The findings of the present study indicated that although the protective effect of SM and RSV was more significant on necrosis and flattening, respectively, SM and RSV produced a nephroprotective impact on the injury of renal tubular cells in diabetic rats than their combination influences.

*Implication for health policy/practice/research/medical education:*
Our experimental study on 25 male Wistar rats indicated that resveratrol and silymarin can produce a nephroprotective impact on the injury of renal tubular cells in diabetic rats than their combination influences. Additionally, the protective effect of resveratrol and silymarin was more significant on necrosis and flattening, respectively.
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## Introduction

Diabetes mellitus (DM) is distinguished as a serious health problem worldwide. The universal outbreak of DM because of urban life and alteration of lifestyle, day to day is increasing. In regards to, over three decades ago, individuals with DM have doubled. DM complications can be related to the progression of end-stage renal disease, coronary vascular diseases, the disorders of visual, and involvement of limbs that extended morbidity and mortality in people (1-3). A serious factor in the advancement of micro- and macrovascular complications of DM is the oxidative stress process. The various agents have been considered in the enhanced free radical generation that hyperglycemia and hyperinsulinemia

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may play significant roles (4,5). Recently, novel natural composites, as a source of antioxidant agents, exert to exclude DM complications. Among various compounds, resveratrol (RSV) and silymarin (SM) possess a special place due to their therapeutic characteristics (6). RSV (3, 5, 4'-trihydroxystilbene) is a polyphenolic compound of phytoalexin that finds in food and herbal sources including peanuts, groundnuts, Itadori tea, grapevines, and red wines (7,8). The various properties such as anti-cancer, anti-inflammatory, antioxidant, cardioprotective, and neuroprotective are considered for RSV. The current studies have documented the effective impact of RSV in the improvement of obesity and DM (9). Also, several reports in animal models indicated that RSV regulates blood glucose levels by the elevated blood insulin levels, the prevention of hepatic glucose output and elevated peripheral glucose usage (10). It has been presented that RSV may be lucrative either in the recuperation of DM complications, such as diabetic nephropathy and diabetic neuropathy alone or in combination with other anti-diabetic medicines (10). One of impressive combination drugs may be SM. SM is a derived component of *Silybum marianum* plant with 70%-80% flavonolignans. Three structural components are known for SM: silibinin, silydianine and silychristine. Numerous pharmacological features have been reported from SM, referring to hepatoprotection, antibacterial, antiviral, antimutagenic, antiallergic, antineoplastic, antithrombotic anti-inflammatory, and vasodilatory actions (11). Different studies exhibited that SM is a safe component at higher doses, for this reason, it applies for the treatment of various diseases including cancer, burns, osteoporosis, arthritis, sepsis, and hypercholesterolemia. It has been displayed that SM is a therapeutic source for complications due to DM in several organs. In fact, flavonoids and other compounds present in SM are capable to stabilize the cell membrane and increase antioxidant enzyme (*superoxide dismutase* (*SOD*), glutathione peroxidase [GPX] and catalase [CAT]), subsequently, increased serum insulin and normalization of serum glucose in human and animal model (12,13).

## Objectives

The present investigation was designed to evaluate the nephroprotective effects of RSV and SM on morphologic injury to renal tubular cells in adult male diabetic rats.

## Materials and Methods

### *Animals*

Twenty-five male Wistar rats with a mean body weight of 200-250 g in the Medical Plants Research Center in Shahrekord University of Medical Sciences were studied. All animals were kept in normal laboratory condition (temperature; 21-25°C and light cycle; 12 h dark-12 h light).

### *Induction of diabetes*

To induce diabetes, 60 mg/kg streptozotocin (STZ) (Sigma-Aldrich Co., St Louis, MO, USA) was dissolved in 0.1 M citrate buffer and injected to the rats after a fasting night intraperitoneally. Then, 72 hours after STZ injection, blood glucose was determined by glucometer and rats with blood glucose levels above 250 mg/dL were considered as diabetes.

### *Study design*

Rats randomly assigned into five groups, 5 rats for each group:

1. Group I (Control: Non-diabetic); Rats received normal saline by gavage for 14 days.
2. Group II (DM): Rats received a single injection of STZ at a dose of 60 mg/kg intraperitoneally and were also given isotonic saline orally for 14 days.
3. Group III (DM+ SM): Rats, after STZ injection, received 100 mg/kg of SM by gavage for 14 days.
4. Group IV (DM+ RSV): Rats, after STZ injection, received 100 ml/kg of RSV by gavage for 14 days.
5. Group V (DM+SM+RSV): Rats, after STZ injection, received the combination of SM and RSV at a dose of 100 mg/kg by gavage for 14 days.

### *Histopathological study*

For histopathological examination, kidney tissues were removed immediately after sacrificing and fixing with %10 formalin for morphological study. Then, the 2-3 µm-thick sections of renal tissues were prepared and stained with hematoxylin and eosin (H&E) for pathological evaluation. Kidney sections were examined by a light microscope for intensity of kidney damage by examination for degeneration, flattening and necrosis of renal tubular cells and also dilatation of tubular lumen. For statistical analysis and comparing among the groups we used a total of mean percent of four morphological variables, including vacuolization, flattening, degeneration, and necrosis.

### *Ethical issues*

All experimental protocols were conducted in compliance with the regulations of the Research Ethics Committee of the University and Iranian Ethical Guidelines for the use of animals in research. Additionally, all animal experiments were in accordance with protocols approved by the United States National Institutes of Health (NIH, 1978). This study was also approved and supported by Ethics Committee of NIMAD (National Institute for Medical Research Development; http://nimad.ac.ir) in Iran (Ethical code; IR.NIMAD.REC.1397.179).

### *Statistical analysis*

All parameters were summarized with mean and standard deviation (SD) and categorical variables are presented as percentage. One-way analysis of variance (ANOVA)

and post-hoc tests (Bonferroni test) were applied for comparison of mean values between groups. To calculate sample size and data analysis, SPSS version 21.0 software was used. Accordingly, *P* values of less than 0.05 were assumed to be significant.

## Results

As illustrated in Table 1, significant differences were observed in types of morphologic injury to renal tubular cells (vacuolization, flattening, degeneration, and necrosis) between various groups ($P<0.05$). In relation to the vacuolization variable in Table 2, the only difference between the control group and group V (DM+SM+RSV) was remarkable ($P=0.030$). The results of the comparison of the flattening variable between groups have been illustrated in Table 3. There was a significant difference between the control group and group V (DM+SM+RSV) ($P=0.008$). Additionally, a significant difference was observed between the RSV-treated diabetic group and the SM+ RSV-treated diabetic group ($P=0.030$). According to the comparison of the degeneration variable between groups showed in Table 4, significant differences were found between the SM+ RSV-treated diabetic group and groups of control and the RSV-treated diabetic group ($P<0.05$). The findings obtained from the comparison of necrosis variable between groups were revealed in Table 5. Significant relationships were observed between the control group and groups of II (DM group) and V (DM+SM+RSV). In addition, the relationships between group II (DM group) and groups of III (DM+SM) and IV (DM+ RSV) ($P<0.05$) were significant.

**Table 2.** Comparison of morphological variable of vacuolization between groups

| Between groups comparison | Mean Difference(I-J) ± SE | *P* value |
|---|---|---|
| I vs. II | -19.25 ± 7.11 | 0.171 |
| I vs. III | -13.0 ± 7.11 | 0.890 |
| I vs. IV | -9.25 ± 7.11 | 0.999 |
| I vs. V | -25.50 ± 7.11 | 0.030* |
| II vs. III | 6.25 ± 6.57 | 0.999 |
| II vs. IV | 10.0 ± 6.57 | 0.999 |
| II vs. V | -6.25 ± 6.57 | 0.999 |
| III vs. IV | 3.75 ± 6.57 | 0.999 |
| III vs. V | -12.50 ± 6.57 | 0.785 |
| IV vs. V | -16.25 ± 6.57 | 0.271 |

* The significance level for *P* value is less than 0.05.

**Table 3.** Comparison of morphological variable of flattening between groups

| Between groups comparison | Mean Difference(I-J) ± SE | *P* value |
|---|---|---|
| I vs. II | -16.25 ± 5.28 | 0.080 |
| I vs. III | -12.50 ± 5.28 | 0.328 |
| I vs. IV | -5.00 ± 5.28 | 0.999 |
| I vs. V | -22.50 ± 5.28 | 0.008* |
| II vs. III | 3.75 ± 4.87 | 0.999 |
| II vs. IV | 11.25 ± 4.87 | 0.372 |
| II vs. V | -6.25 ± 4.87 | 0.999 |
| III vs. IV | 7.50 ± 4.87 | 0.999 |
| III vs. V | -10.0 ± 4.87 | 0.600 |
| IV vs. V | -17.50 ± 4.87 | 0.030* |

* The significance level for *P* value is less than 0.05.

## Discussion

The present study surveyed the comparative impact of SM and RSV in the improvement of kidney damage of diabetic rats. The findings showed significant differences in types of morphologic injury to renal tubular cells including vacuolization, flattening, degeneration, and necrosis between various groups. Furthermore, it was displayed that significantly both the SM and RSV reduced the injury of renal tubular cells in diabetic rats.

Similar to our results, the study by Giovannini et al demonstrated that received 0.23 µg/kg of RSV in rats accompanied with decreased renal failures including tubular cell necrosis, glomerular dysfunction, glomerular thrombosis, and cell infiltration (14). To examine RSV effect on oxidative stress and renal function in STZ-induced diabetic rats, it has been reported that renal glomerular and interstitial changes, polyuria, proteinuria, elevated serum creatinine, and BUN in addition to renal oxidative stress process attenuate through RSV. In fact, RSV act as a scavenger of hydrogen peroxide and superoxide anion due to STZ (15). The findings offered that RSV possesses properties of anti-atherogenic, anti-diabetic, antioxidant,

**Table 1.** Mean ± SD of vacuolization, flattening, degeneration, and necrosis of studied groups

| Groups | Injury | | | |
|---|---|---|---|---|
| | Vacuolization | Flattening | Degeneration | Necrosis |
| Control | 2.0 ± 1.0 | 0.00 ± 0.00 | 1.0 ± 1.0 | 0.00 ± 0.00 |
| DM | 21.25 ± 13.77 | 16.25 ± 11.09 | 21.25 ± 8.54 | 20.0 ± 7.07 |
| DM + SM | 15.0 ± 7.07 | 12.50 ± 2.89 | 10.50 ± 3.32 | 5.0 ± 0.0 |
| DM+ RSV | 11.25 ± 2.50 | 5.0 ± 0.0 | 11.25 ± 4.79 | 6.25 ± 2.5 |
| DM + SM + RSV | 27.50 ± 12.58 | 22.50 ± 9.57 | 32.50 ± 17.08 | 16.25 ± 8.54 |
| *P* value | 0.027* | 0.005* | 0.005* | 0.001* |

* The significance level for *P* value is less than 0.05.

**Table 4.** Comparison of morphological variable of degeneration between groups

| Between groups comparison | Mean Difference(I-J) ± SE | *P* value |
|---|---|---|
| I vs. II | -20.25 ± 7.06 | 0.124 |
| I vs. III | -9.50 ± 7.06 | 0.999 |
| I vs. IV | -10.25 ± 7.06 | 0.999 |
| I vs. V | -31.50 ± 7.06 | 0.005* |
| II vs. III | 10.75 ± 6.54 | 0.999 |
| II vs. IV | 10.00 ± 6.54 | 0.999 |
| II vs. V | -11.25 ± 6.54 | 0.999 |
| III vs. IV | -0.75 ± 6.54 | 0.999 |
| III vs. V | -22.0 ± 6.54 | 0.040* |
| IV vs. V | -21.25 ± 6.54 | 0.060 |

* The significance level for *P* value is less than 0.05.

**Table 5.** Comparison of morphological variable of necrosis between groups

| Between groups comparison | Mean Difference(I-J) ± SE | *P* value |
|---|---|---|
| I vs. II | -20.00 ±4.02 | 0.002* |
| I vs. III | -5.00 ±4.02 | 0.999 |
| I vs. IV | -6.25 ±4.02 | 0.999 |
| I vs. V | -16.25 ±4.02 | 0.012* |
| II vs. III | 15.00±3.72 | 0.012* |
| II vs. IV | 13.75±3.72 | 0.024* |
| II vs. V | 3.75±3.72 | 0.999 |
| III vs. IV | -1.25±3.72 | 0.999 |
| III vs. V | -11.25 ±3.72 | 0.091 |
| IV vs. V | -10.00 ±3.72 | 0.177 |

* The significance level for *P* value is less than 0.05.

and anti-obesity on obese rats with diabetes (16). RSV, as a polyphenolic compound, prevents damaging tubular epithelial cells by peroxynitrite scavenging and decreasing the levels of renal lipid peroxides and hydroperoxides (17, 18). In relation to SM, an animal model of renal ischemia-reperfusion injury indicated that treating mice with SM diminishes levels of BUN and creatinine. Furthermore, renal tubule cells failure and the number of apoptotic cells amend in mice receiving SM (19). Also, the findings obtained from the efficacy of SM on diabetic nephropathy detected that 60 and 120 mg/kg doses of SM declined blood glucose level, hemoglobin A1c concentration, uric acid, serum creatinine, and urine albumin. Histopathologically, the tubular epithelium damage and intertubular hemorrhage ameliorated by SM therapy (20). Soto et al investigated renoprotective impact of SM in rats with alloxan-caused DM. The outcomes of their study illustrated that SM elevates the renal activity and expression of antioxidant enzymes (*SOD*, GPX and CAT) and restitutes renal morphology (21). It has been revealed that SM has the ability to improve proteinuria in type 2 diabetes patients via its antioxidant and anti-inflammatory properties. SM can also elevate protein and nucleic acid synthesis and contribute to the regeneration of the renal cells (22).

The oxidative stress process is one of the threat agents in early diabetic and later development. The increased glucose during diabetes involves in the advanced glycation end-products generation and reactive oxygen species release that leads to renal dysfunction including tubular atrophy, glomerular hypertrophy, podocytes dysfunction, thickening of glomerular basement membranes, interstitial fibrosis and etcetera (23). Therefore, it is offered that the use of various antioxidants such as SM and RSV is a treatment strategy for complications due to DM.

## Conclusion

In conclusion, the findings of the present study indicated that SM and RSV produced a nephroprotective impact on the injury of renal tubular cells in diabetic rats than their combination influences. Although, the protective effect of SM and RSV was more significant on necrosis and flattening, respectively.

## Authors' contribution

EG and HN designed the research. AHD conducted the animal study. NH and PeN supervised the study. BY prepared the final draft of the article. PaN analyzed the data. HN studied the pathologies. HN also edited the manuscript. All authors read and signed the final paper.

## Conflicts of interest

The authors declare that they have no competing interests.

## Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors. This study was conducted in the animal lab of Shahrekord University of Medical Sciences and supervised by the supervision of the AHD.

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