EFFECT OF RESVERATROL WITH VITAMIN C COMBINATION TREATMENT ON STREPTOZOTOCIN INDUCED DIABETIC RAT RENAL IMPAIRMENT

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ABSTRACT

Objective: The present study was under taken effect of resveratrol with vitamin C combination treatment on streptozotocin induced diabetic rat renal impairment and to examine the effect of this combination on tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL6). Methods: Group-I: Normal control rats, Group-II: STZ induced diabetic rats, Group-III: diabetic rats treated with Resveratrol, Group-IV: diabetic rats treated with Vitamin C, Group V: diabetic rats treated with resveratrol and Vitamin C combination. Results: The present study strongly suggest that resveratrol with vitamin C combination treatment effectively prevent the diabetes associated renal damage by restoring the altered biochemical parameters, rectifies the architectural alterations and by reducing expression of TNF-α and IL-6 in the kidney. Conclusion: Resveratrol with vitamin C combination could be used as an adjuvant therapy with a conventional hypoglycemic regimen to treat diabetic complications.

KEYWORDS: Resveratrol, VitaminC, Streptozotocin, Diabetic nephropathy.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that is rapidly reaching epidemic proportions, and the World Health Organization (WHO) has predicted that by 2025, 300 million people will be affected worldwide. The incidence of diabetes mellitus (DM) is projected to dramatically increase worldwide in the coming decades, which will create an urgent need for
new therapies for diabetic complications. Among its major microvascular complications, life-threatening diabetic nephropathy is the leading cause of end stage renal disease (ESRD) in the Western world. Early in the twenty-first century, the total annual medical costs for managing prevalent patients with diabetic nephropathy in the United States reached nearly $17 billion.\[1\] Diabetic nephropathy is a microvascular complication of diabetic mellitus functionally characterized by proteinuria and albuminuria and pathologically by glomerular hypertrophy, mesangial expansion and tubulointerstitial fibrosis; these findings are closely related to the loss of renal function. Recent studies are reported that oxidative stress has been suggested to play an important role in the pathogenesis of diabetic nephropathy. Diabetic nephropathy is a state in which oxidative stress increases and antioxidant status is reduced, has been documented.\[2\] Recent studies have shown that inflammation, and more specifically pro-inflammatory cytokines, play a determinant role in the development of microvascular diabetic complications.\[3\] In 1991, it was first suggested that proinflammatory cytokines TNF-\(\alpha\) and interleukin-1 could significantly contribute to the pathogenesis of diabetic nephropathy.\[4\]

The effects of several antioxidants administered at the onset of experimental diabetes have been reported to prevent diabetic renal injury. Antioxidant therapy may be beneficial in preventing the development of diabetic nephropathy.\[5,6\] Resveratrol (3, 4’, 5-trihydroxystilbene) is a phytoalexin belongs to a class of polyphenolic compounds called stilbenes. Some types of plants produce Resveratrol and other stilbenes in response to stress, injury, fungal infection and ultraviolet (UV) radiation. It is found in the skins of certain red grapes, in peanuts, blueberries, some pines, and is produced in plants with the help of the enzyme stilbene synthase. Alone or in combination with other plant chemicals, resveratrol continues to be the focus of a great deal of ongoing research. Some research scientists believe that the combination of resveratrol and quercetin may not be the only synergistic possibility. Resveratrol and quercetin, vitamin E, vitamin C and selenium (a trace mineral) are being investigated together with this goal in mind.\[7\] Vitamin C or L-ascorbic acid is an essential nutrient for humans. It is found naturally in citrus fruits and many vegetables. Patients with diabetic nephropathy have reduced plasma and tissue ascorbic acid levels and therefore decreased antioxidant defense. If so, reduced ascorbic acid levels could be correctable by consumption of dietary vitamin C. Supplemental vitamin C normalized ascorbic acid level in rats with streptozotocin-induced diabetes and ascorbic acid deficiency.\[8\] The effects of several antioxidants at the onset of experimental diabetes have been reported to prevent
diabetic renal injury. Due to the strong implication of oxidative damage in the diabetic nephropathy, the present study was designed to counter the oxidative damage in the diabetic rat kidney.

However, the scientific data on resveratrol with vitamin C combination treatment on diabetic rat renal impairment is not available. So, in the present study we have made an attempt that effect of resveratrol with vitamin C combination treatment on streptozotocin induced diabetic rat renal impairment. The study also aimed to examine the effect of this combination on tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL6).

**MATERIAL AND METHODS**

**Preparation of STZ Solution**

STZ was dissolved in ice-cold citrate buffer of pH 4.5 and injected immediately within five minutes to avoid degradation.

**Preparation of Resveratrol and Vitamin C suspensions**

The Resveratrol suspension was prepared by dissolving Resveratrol in 0.5% carboxymethylcellulose solution. Vitamin C suspension also prepared in 0.5%CMC solution. All these drugs were administered in a constant volume of 0.5ml/100g body weight of rat.

**Experimental Animals**

Wistar rats (120-150 g) were obtained from QISCP (1921/PO/ReS/16/CPCSEA)+ animal house and housed three animals per cage with paddy husk as bedding. They were maintained in clean, sterile, polypropylene cages (Tarson). Animals were housed at temperature of 25±2°C and relative humidity of 30-60%. A 12:12 h light and dark cycle was followed. The animals had free access to food and demineralized drinking water ad libitum.

**Experimentally induced diabetes mellitus**

Male wistar rats (120-150 g) were fasted for overnight before challenging with single injection of freshly prepared STZ (50mg/kg) intraperitonially, and injected within 5 min of preparation to prevent degradation. After administration of STZ, the animals had free access to food and demineralized drinking water ad libitum. The development of hyperglycemia in rats was confirmed by fasting serum glucose estimation of 48 h on post STZ injection. The rats with fasting serum glucose level of above 200 mg/dl were considered diabetic and included in the study.
Care of Diabetic Animals
Since diabetic animals drink large amount of fluid and produce large volume of urine, the bedding is changed frequently, usually every day and, in some circumstances, more than once per day. Diabetic rats should have sufficient food and water; therefore only three diabetic rats have been housed per cage to avoid competition for food and water.

Collection of serum samples
The blood was drawn from the retro orbital plexus of the rats (fasted for overnight) under light ether anesthesia at 0 week, 2nd week, 4th week, 6th week, 8th week and 10th week.

The blood samples were allowed to clot for 30 minutes at room temperature and then they were centrifuged at 5000 rpm for 20 minutes. The resulting upper serum layer was collected in properly labeled, clean and dry micro-centrifuge tubes. The serum used as specimen, it should be free from hemolysis and was separated from the cells as soon as possible, to prevent glycolysis. The serum samples were stored at 2-8°C and analyzed immediately. This serum specimen is used for the estimation of different biochemical parameters by spectrophotometrically using standard kits (Span diagnostics, India)

PARAMETERS ANALYZED

Body Weight
The body weight of each animal was recorded daily.

Fasting Serum Glucose Estimation
Blood glucose levels were determined with a blood glucometer (ACCU-CHEK active Home diagnostics) at regular intervals. Blood glucose levels were also determined by GOD-POD method with the help of diagnostic reagent kit (Span diagnostic chemicals, India) at the end of the experiment.

Serum urea Estimation
Serum urea was estimated by DAM Method with the help of diagnostic reagent kit (Span diagnostic chemicals, India) at the end of the 10th week.

Serum Creatinine Estimation
Serum urea was estimated by Alkaline Picrate Method with the help of diagnostic reagent kit (Span diagnostic chemicals, India) at the end of the 10th week.
Serum Cholesterol Estimation
Serum Cholesterol was estimated by CHOD-PAP method for total cholesterol and PEG-
CHOD-PAP method for HDL cholesterol with the help of diagnostic reagent kit (Span
diagnostic chemicals, India) at the end of the 10th week.

Serum Albumin Estimation
Serum ALBUMIN was estimated with the help of diagnostic reagent kit (Span diagnostic
chemicals, India)) at the end of the 10th week.

HISTOPATHOLOGICAL EXAMINATIONS
10 weeks after the experiment animals from each group were randomly selected. A portion of
the kidneys were excised from ether anaesthetized rat fixed in 10% formalin and processed
for histological studies. Tissues were dehydrated through 70, 90 and 100% alcohol and
embedded in low melting point paraffin wax. Sections of 4µm thickness were cut and placed
serially on glass slide. The sections were deparaffinised in xylene and rehydrated through
100, 90 and 70% alcohol. Three continuous sections were made from each kidney tissue and
stained with hematoxylin and eosin for histological evaluation using light microscopy.

IMMUNOHISTOCHEMICAL ANALYSIS OF IL-6 AND TNF
Immunohistochemical detection of IL-6 and TNF-α in cold acetone fixed, paraffin embedded
kidney section was performed.

DOSE SELECTION AND TREATMENT
Doses of resveratrol and Vitamin C were selected based on previously reported
chemotherapeutic and toxicological properties. It has been reported that 10 mg/kg of
resveratrol and 0.9 g/kg of Vitamin C has beneficial effects against diabetes.

STATISTICAL ANALYSIS
The values are expressed as mean ± SEM. The data was analysed by using one way ANOVA
followed by Dunnett T3 test using Graph Pad Prism version 4. Statistical signficance was set
at P ≤ 0.05.

RESULTS AND DISCUSSION
Fig.1: depicted the body weight changes of different groups of rats during the experimental
period. The normal control rats (Group-I) were gained body weight during the experimental
period. STZ induced diabetic rats showed that severe loss of body weight throughout the
experimental period. Administration of resveratrol alone (Group III), vitamin C alone (Group IV) and resveratrol with vitamin C (Group V) combination to diabetic rats resulted in an increase in body weight compared to diabetic rats (Group II). The drug treated diabetic rats gained weight at a much higher rate compared to control. Results suggested that resveratrol alone, vitamin C alone and resveratrol with vitamin C treatment has positive effect on maintaining body weights in diabetic rats. Among the treatment groups resveratrol with vitamin C combination treated rats gained more weight.

![Body weight changes](image)

**Fig.1: Body weight changes during the experimental period.**

**Table 1: Biochemical Parameters.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood Glucose (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Serum urea (mg/dl)</th>
<th>Total serum cholesterol (mg/dl)</th>
<th>Serum HDL (mg/dl)</th>
<th>Serum albumin (gm %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>108.7±1.2*</td>
<td>0.42±0.09*</td>
<td>19.1±0.57</td>
<td>77.2±1.09</td>
<td>58.3±0.48*</td>
<td>2.4±0.03*</td>
</tr>
<tr>
<td>Group-II</td>
<td>394.9±3.7</td>
<td>1.76±0.03*</td>
<td>46.3±1.59</td>
<td>141.7 ± 0.93</td>
<td>40.9±0.73</td>
<td>3.8±0.03</td>
</tr>
<tr>
<td>Group-III</td>
<td>183.4±2.2*</td>
<td>0.99 ±0.04*</td>
<td>36.8±0.74*</td>
<td>114.9 ± 1.0*</td>
<td>52.2±0.68*</td>
<td>3.1±0.04</td>
</tr>
<tr>
<td>Group-IV</td>
<td>212±6.04*</td>
<td>1.13 ±0.0*</td>
<td>39.2±0.60*</td>
<td>119.1±0.66*</td>
<td>49.3±1.41*</td>
<td>3.3±0.06</td>
</tr>
<tr>
<td>Group-V</td>
<td>144.8±4.1*</td>
<td>0.65 ±0.03*</td>
<td>32.3±0.87*</td>
<td>105.6±0.97*</td>
<td>55.3±0.85*</td>
<td>2.7±0.09*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n=6.

* p ≤ 0.01 as compared to diabetic control

# p ≤ 0.01 as compared to resveratrol with vitamin C combination
Estimation of serum urea level

Increased levels of serum urea (46.34±1.59) was observed in diabetic rats and showed significant difference as compared normal control (19.18±0.57) (P<0.01). Significant differences was observed in resveratrol alone (Group III), vitamin C alone (Group IV) and resveratrol with vitamin C combination (Group V) treated rats as compared to diabetic control (Group II), (P<0.01). Reduction in serum urea levels was observed in resveratrol with vitamin C combination (32.39±0.87) (Group V) treated rats and showed significant difference as compared to resveratrol alone (Group III) and vitamin C alone (Group IV) (P<0.01) treated.

![Fig.2: Serum urea levels within groups after 10 weeks.](image)

\* p ≤ 0.01 as compared to diabetic control

\# p ≤ 0.01 as compared to resveratrol with vitamin C combination

Estimation of serum albumin level

The serum albumin levels of diabetic rats showed (3.82±0.03) higher and significant difference was observed as compared to normal control (2.46±0.03) (P<0.01). The resveratrol alone (Group III), vitamin C alone (Group IV) and resveratrol with vitamin C combination (Group V) treated rats showed significant differences as compared to diabetic rats (Group II), (P<0.01). Increased serum albumin levels was reduced in resveratrol with vitamin C combination (2.76±0.09)(Group V) treated rats and showed significant difference as compared to resveratrol alone (Group III) and vitamin C alone (Group IV) (P<0.01) treated.
Estimation of serum total cholesterol level

Streptozotocin-induced diabetic rats showed increased levels of serum total cholesterol (141.7±0.93) as compared to normal control (77.24±1.09) (P<0.01). Furthermore significant difference (P<0.01) was observed in Group III, Group IV and Group V as compared to diabetic control (Group II) (P<0.01). In connection with the combination efficacy significant reduction of serum total cholesterol levels was observed (P<0.01) in Group V (105.60±0.97) treated rats and showed significant difference as compared to Group III (resveratrol alone) and Group IV (vitamin C alone).
Estimation of serum total cholesterol level
At the end of the 10th week, the serum total cholesterol level of the different groups of animals was shown in Table 2 and graphically represented in Fig. 5. Streptozotocin-induced diabetic rats showed increased levels of serum total cholesterol (141.7±0.93) as compared to normal control (77.24±1.09) (P<0.01). Furthermore, significant difference (P<0.01) was observed in Group III, Group IV and Group V as compared to diabetic control (Group II) (P<0.01). In connection with the combination efficacy, significant reduction of serum total cholesterol levels was observed (P<0.01) in Group V (105.60±0.97) treated rats and showed significant difference as compared to Group III (resveratrol alone) and Group IV (vitamin C alone).

Estimation of serum total cholesterol level

![Graph showing serum total cholesterol levels within groups after 10 weeks.](image)

* p ≤ 0.01 as compared to diabetic control
# p ≤ 0.01 as compared to resveratrol with vitamin C combination

Estimation of serum HDL level
At the end of the 10th week, serum HDL level of the different groups of animals was shown graphically represented in Fig. 6. Streptozotocin induced diabetic rats showed decreased levels of serum HDL (40.93±0.73) as compared to normal control (58.34±0.48) (P<0.01). The resveratrol alone (Group III), vitamin C alone (Group IV) and resveratrol with vitamin C combination (Group V) treated rats showed significant differences as compared to diabetic rats (Group II), (P<0.01). The serum HDL level of resveratrol with vitamin C combination (Group V) treated rats showed (55.30±0.85) increased levels of serum HDL and showed
significant difference as compared to resveratrol alone (Group III) and vitamin C alone (Group IV) (P<0.01).

**Estimation of serum HDL level**

![Figure 6: Serum HDL levels within groups after 10 weeks.](image)

* p ≤ 0.01 as compared to diabetic control
# p ≤ 0.01 as compared to resveratrol with vitamin C combination.

**HISTOPATHOLOGICAL CHANGES**

Histopathological examination of kidney tissue section of various experimental groups of animals was depicted in figure 7. The normal control rats (fig.7a) kidney section showed the normal cellular architectures like glomeruli, mesangial cells, glomerular basement membrane, capsular space, proximal convoluted tubules and distal convoluted tubules. The normal cellular architecture of kidney was preserved in control rats. The diabetic rat kidney section (fig.7b) showed marked alterations in cellular architecture and distinctive histopathological changes was observed like occasional ruptures, and a shorting at the brush border accompanied by the appearance of cytoplasmic debris and desquamated nuclei in the widened lumens of the tubules. In addition vacuolization, pyknotic nuclei and edema in the cells of tubules, thickened basement membranes were noted. The diabetic rats treated with resveratrol (fig.7c), vitamin C (fig.7d) and resveratrol with vitamin C combination (fig.7e) kidney sections showed noticeable improvement in the histopathological parameters like occasional ruptures, pyknotic nuclei’s, cytoplasmic debris, vacuolization, thickened basement membranes. There were no signs of appearance of cytoplasmic debris, desquamated nuclei, in resveratrol with
vitamin C combination treated diabetic rat kidneys and look like normal. The magnitude of kidney healing efficacy was observed much higher in diabetic rats treated with resveratrol with vitamin C combination compared with diabetic rats treated with resveratrol and vitamin C alone.

Fig.7: a Normal histological appearance of kidney tissues of control rat (H&E), x 450 b. Histological appearance of kidney tissues of a diabetic rat. Occasional ruptures (→), shorting at the brush border (→), cytoplasmic debris (←), desquamated nuclei (←), vacuolization (←) pyknotic nuclei (←) in the cells of tubules and thickening of glomerular basement membrane (→), (H&E); x 450. c Histological appearance of
kidney tissues of resveratrol treated diabetic rat (H&E); x 450. d Histological appearance of kidney tissues vitamin C treated diabetic rat (H&E); x 450. e Histological appearance of kidney tissues resveratrol with vitamin C treated diabetic rat (H&E); x 450.

IMMUNOHISTOCHEMICAL EXAMINATION

Effect of drug treatment on expression of TNF-α: Immunohistochemical examination of TNF-α in the rat kidney tissue from various experimental group of animals were depicted in figure 8. Chromogen generated brown staining was an indication of immunostaining cells. It should be noted that almost brown satin over lapsed the condensed nuclei of renal tissues. Arrows indicate TNF-α immunopositive cells in kidney section of various group of experimental animals. In the kidney section of normal rats (fig.8a) were not shown any brown staining and brown spots. It indicates there was no expression in the control rat kidney. The diabetic rat kidney sections (fig.8b) showed more number of brown spots. This illustrates that diabetic rat kidney has more expression. The diabetic rats treated with resveratrol alone (fig.8c) and vitamin C (fig.8d) alone showed less brown spots. The diabetic rats treated with resveratrol with vitamin C combination (fig.8e) showed much less brown spots. The diabetic rats treated with resveratrol, vitamin C, and resveratrol with vitamin C combination showed less brown spots compared with diabetic control rat kidney.

The expression of TNF-α immunopositive cells in the kidney tissue of various groups of experimental rats after 10 weeks showed in the Table 2. Representative photomicrographs of immunohistochemical staining of TNF-α were presented as figure 8. The kidney section of normal control rats showed complete absence of immunostaining of TNF-α (fig.8a). A large amount of TNF-α immunopositive cells (28.9± 2.6) were detected in the kidney sections of diabetic rats (Group-II). In contrast, showed reduction of TNF-α immunopositivity (18.6 ±3.56, p ≤ 0.05) in resveratrol treated diabetic rats compared to diabetic control rats. The diabetic rats treated with vitamin C alone showed reduction of TNF-α immunopositivity (15.4 ±2.7, p ≤ 0.05) as compared to diabetic rats. Furthermore reduction of TNF-α immunopositivity (9.2 ±2.4, p ≤ 0.01) in resveratrol with vitamin C combination treated rats was observed as compared to diabetic rats.

The above observations showed that the treatment of diabetic rats with resveratrol, vitamin C and resveratrol with vitamin C combination reduces the expression of TNF-α in diabetic rats kidney, but resveratrol with vitamin C combination was effectively reduce the expression of
TNF-α in diabetic rat kidney which was significant compared to other resveratrol and vitamin C alone treated diabetic group.

Fig. 8: a. Expression of TNF-α in glomeruli, tubulointerstitium of control rat kidney (H&E); x 450. b. Expression of TNF-α in diabetic rat kidney (H&E); x 450. c. Expression of TNF-α in resveratrol treated diabetic rat kidney (H&E); x 450. d. Expression of TNF-α in vitamin C treated diabetic rat kidney (H&E); x 450. e. Expression of TNF-α in resveratrol with vitamin C treated diabetic rat kidney (H&E); x450. Arrow represents the expression of TNF-α in glomeruli, tubulointerstitium of kidney.
Effect of drug treatment on expression of IL-6

Immunohistochemical examination of IL-6 in the rat kidney tissue from various experimental group of animals were depicted in figure 9. Chromogen generated brown staining was an indication of immunostaining cells. It should be noted that almost brown satin over lapsed the condensed nuclei of renal tissues. Arrows indicate IL-6 immunopositive cells in kidney section of various groups of experimental animals. In the kidney section of normal rats (fig.9a) we did not show any brown staining and brown spots. It indicates there was no expression in the control rat kidney. The diabetic rats kidney sections (fig.9b) showed more number of brown spots. This illustrates that diabetic rat kidney has more expression. The diabetic rats treated with resveratrol alone (fig.9c) showed less brown spots and the diabetic rats treated with vitamin C (fig.9d) alone showed less brown spots. The diabetic rats treated with resveratrol with vitamin C combination (fig.9e) showed too less brown spots. The diabetic rats treated with resveratrol, vitamin C, and resveratrol with vitamin C combination showed less brown spots compared with diabetic control rat kidney.

The expression of IL-6 immuno positive cells in the kidney tissue of various groups of experimental rats after 10 weeks showed in the Table 2. Representative photomicrographs of immunohistochemical staining of IL-6 were presented as figure 9. The kidney section of normal control rats showed complete absence of immunostaining of IL-6 (fig.9a). A large amount of IL-6 immunopositive cells (22.8±0.4) were detected in the kidney sections of diabetic control rats (Group-II). In contrast, showed reduction of IL-6 immunopositivity (15.5 ±1.6, p ≤ 0.05) in resveratrol treated diabetic rats compared to diabetic rats. The diabetic rats treated with vitamin C alone showed reduction of IL-6 immunopositivity (12.4 ±1.5, p ≤ 0.05) compared to diabetic rats. Furthermore reduction of IL-6 immunopositivity (9.0 ±0.4, p ≤ 0.01) in resveratrol with vitamin C combination treated rats was observed compared to diabetic rats.

The above observations show that the treatment of diabetic rats with resveratrol, vitamin C and resveratrol with vitamin C combination reduces the expression of IL-6 in diabetic rats kidney, but resveratrol with vitamin C combination was effectively reduce the expression of IL-6 in diabetic rat kidney which was significant compared to other resveratrol and vitamin C alone treated diabetic group.
Fig. 9: a. Expression of IL-6 in glomeruli, tubulointerstitium of control rat kidney (H&E); x 450. b. Expression of IL-6 in diabetic rat kidney (H&E); x 450. c. Expression of IL-6 in resveratrol treated diabetic rat kidney (H&E); x 450. d. Expression of IL-6 in vitamin C treated diabetic rat kidney (H&E); x 450. e. Expression of IL-6 in resveratrol with vitamin C treated diabetic rat kidney (H&E); x 450. Arrow represents the expression of IL-6 in glomeruli, tubulointerstitium of kidney.
Table 2: The percentage of immunopositive cells of TNF-α and IL-6

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α (%)</th>
<th>IL-6 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-II</td>
<td>28.9 ± 2.6</td>
<td>22.8 ± 0.4</td>
</tr>
<tr>
<td>Group-III</td>
<td>18.6 ±3.56*#</td>
<td>15.5±1.6*#</td>
</tr>
<tr>
<td>Group-IV</td>
<td>15.4 ±2.7*#</td>
<td>12.4 ±1.5*#</td>
</tr>
<tr>
<td>Group-V</td>
<td>9.2 ±2.4*</td>
<td>9.0 ±0.4*</td>
</tr>
</tbody>
</table>

* p ≤ 0.05 as compared to Group-II
# p ≤ 0.01 as compared to Group-V

The expression of TNF-α and IL-6 was not detectable in the kidney of normal control group of rats. Approximately, 200 cells were counted for field, nine felids were examined per slide and six slides were examined per group.

DISCUSSION

In the present study, the groups of normal rats have showed gain in the body weight and fasting blood glucose, serum urea, serum cholesterol, serum creatinine, serum albumin and serum HDL levels were maintained in the normal range.

STZ-injected rats demonstrated typical characteristics of diabetes mellitus such as hyperglycemia, polyurea and growth retardation. It has also been observed that significantly increased fasting blood glucose, serum albumin, serum creatinine, serum urea, serum cholesterol levels and showed significantly decreased levels of serum HDL.

The improvement in body weight gain in diabetic rats supplemented with resveratrol, vitamin C and resveratrol with vitamin C combination highlight the blood glucose homeostasis which in turn promotes the body weight gain.

The elevated blood glucose levels observed in the diabetic rats was significantly decreased in resveratrol, vitamin C and resveratrol with vitamin C combination treated groups suggesting insulin stimulatory effect of resveratrol, vitamin C and resveratrol with vitamin C combination from the remnant β-cells. This was further evidenced that these drugs have anti diabetic property.

Urea is the main end product of protein catabolism in the body. Accumulation of urea nitrogen in experimental diabetes may be due to enhanced break down of both liver and plasma proteins. The oral administration of resveratrol, vitamin C and resveratrol with vitamin C combination to the diabetic rats significantly decreased the altered levels of blood.
urea suggesting the prophylactic role of resveratrol, vitamin C and resveratrol with vitamin C combination in the protein metabolism.

Creatinine is a byproduct of the breakdown of creatine and phosphocreatine, which are considered as an energy storage compounds in muscle. The serum creatinine values depend on the ability of the kidney to excrete creatinine. Creatinine concentration often used as a variable not only to assess impairment of kidney function but also as clinical end point to detect treatment related toxic effects of compound on the kidney in the experimental animals. In the present study the oral treatment with resveratrol, vitamin C, resveratrol with vitamin C combination significantly reduced the serum creatinine levels. Therefore it may be concluded that early changes occurred in diabetic rats were significantly improved by the oral administration of resveratrol, vitamin C and resveratrol with vitamin C combination.

Increased levels of serum creatinine, serum urea, indicates progressive renal damage which is taken as an index of altered GFR in diabetic nephropathy. There have been reports that decreased GFR is associated with the formation of reactive oxygen intermediates.

Treatment with resveratrol, vitamin C and resveratrol with vitamin C combination significantly reduced the increased serum cholesterol levels in the diabetic rats suggesting that these drugs strongly improved the lipid metabolism.

Treatment with resveratrol, vitamin C and resveratrol with vitamin C combination significantly reduced the increased serum albumin levels. But the treatment of resveratrol with vitamin C combination reduced the increased fasting blood glucose, serum albumin, serum creatinine, and serum urea and serum cholesterol levels in the diabetic rats near to the control and significant compared to the other resveratrol, vitamin C alone treated diabetic rats.

The diabetic rat kidney section showed marked alterations in cellular architecture and distinctive histopathological changes were observed. Histopathological examination results revealed that the diabetic rats treated with resveratrol, vitamin C and resveratrol with vitamin C combination kidney sections showed noticeable improvement in the histopathological parameters. But the combination resveratrol with vitamin C treatment showed a marked improvement and looks like a normal as compared to the other resveratrol and vitamin C alone treated rats and this combination might be much more effective in the renal damage.
The finding observed in histopathology of kidney gave a strong support to the biochemical parameters observed in this study.

Recent studies have shown that inflammation, and more specifically pro-inflammatory cytokines, play a determinant role in the development of microvascular diabetic complications.[10] Inflammatory cytokines, mainly IL-1, IL-6, and IL-18, as well as TNF-α, are involved in the development and progression of diabetic nephropathy.[11] Immunohistochemical results demonstrated that the diabetic rats showed more number of immunopositive cells and have much expression of TNF-α and IL-6. The diabetic rats treated with resveratrol, vitamin C and resveratrol with vitamin C combination showed less number of immunopositive cells and reduced the expression of TNF-α, IL-6 compared to diabetic rats. Resveratrol with vitamin C combination showed much less number of immunopositive cells and effectively reduced the expression of TNF-α, IL-6 compared to resveratrol and vitamin C alone treated rats. This immunohistochemical results also gave a strong support to the biochemical and histopathological reports.

This results indicating that TNF-α, IL-6 have a prime role in the pathophysiology of diabetic renal impairment. A marked improvement in renal function was observed in the treated rats. Finally the total results’ indicating that resveratrol with vitamin C combination was much more effective in the renal damage compared to resveratrol and vitamin C alone. This may be due to antioxidant property of resveratrol and vitamin C.

**CONCLUSION**

The present study strongly suggest that resveratrol with vitamin C combination treatment effectively prevent the diabetes associated renal damage by restoring the altered biochemical parameters, rectifies the architectural alterations and by reducing expression of TNF-α and IL-6 in the kidney. Finally the total result indicating, that resveratrol with vitamin C combination was effective when compared to resveratrol and vitamin C alone. These results imply that resveratrol with vitamin C combination could be used as an adjuvant therapy with a conventional hypoglycemic regimen to treat diabetic complications. Further studies are necessary to ascertain the use of resveratrol with vitamin C combination in diabetic complications.
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