Comparative evaluation of nephroprotective potential of resveratrol and piperine on nephrotic BALB/c mice

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Abstract:
Objective: The objective of this study was to evaluate the nephroprotective potential of resveratrol and piperine at same dose on cationic bovine serum albumin (cBSA) induced immune complex glomerulonephritis (ICGN) in BALB/c mice.

Materials and Methods: Female BALB/c mice were divided into five groups. Group I served as normal control (complete Freund’s adjuvant + Saline). Two weeks later, Groups II, III, IV, and V were administered cBSA (13 mg/kg) via the caudal vein 3 times/week every alternative day for 6 weeks to induce ICGN. Simultaneously, from the 3rd week, Groups III, IV were treated with resveratrol and piperine up to 6 weeks. Group V was treated with methylprednisolone considered as a reference standard.

Results: There was a significant decrease in albuminuria, serum creatinine, and blood urea nitrogen in Group IV animals when compared with Group III. In addition, Group III and IV have comparable results with cBSA treated animals. Concurrently, same groups showed significantly comparable variance in antioxidant enzymes, phagocytic index, and neutrophil adhesion assay. Group IV found to be more significant in IgG1 reduction than Group III.

Conclusion: The findings of this study well-demonstrated that piperine has potential immunomodulatory and anti-inflammatory activity than resveratrol; therefore, piperine needs special attention in autoimmunity and inflammation research.

Key words: Glomerulonephritis, nephroprotection, piperine, prednisolone, resveratrol

Resveratrol and piperine are bioactive compounds originated from natural sources used as a flavor, spice, and diluent. Piperine is a major alkaloid present in piper nigrum and piper longum. Constituents of piper species has in vitro inhibitory activity on enzymes responsible for leukotriene and prostaglandin biosynthesis, 5-lipoxygenase, and cyclooxygenase-1 respectively.[1] Similarly, resveratrol is a phytoalexin present in the skin of red grapes, peanuts and blueberries. Previous reports revealed that resveratrol has antioxidant, anti-aging, and cancer chemopreventive effects and seem to be beneficial for inflammatory diseases.[2] Therefore, the present study was performed for comparative evaluation of nephroprotective potential of both bioactive compounds at same dose on cationic bovine serum albumin (cBSA) induced immune complex glomerulonephritis (ICGN) in BALB/c mice.

Glomerulonephritis remains to represent a major cause of end-stage renal damage throughout the world. About 30–40% of patients develop progressive renal impairment that results in end-stage renal failure after 10–15 years.[3] It is categorized by accumulative immune deposits on the epithelial side of the glomerular capillary wall, consist of IgG, mainly IgG4 and IgG1 of antigens and membrane attack complex of complement c5b-9. The formation of subepithelial immune deposits and complement activation are together responsible for physiological impairment of the glomerular capillary wall causing severe proteinuria.[4] cBSA induced glomerulonephritis animal model closely related to human membrane nephritis.
and giving exact knowledge of the disease pathology and progression.

Thus, we opt for cBSA induced ICGN animal model, to postulate the daily administration of piperine and resveratrol may improve nephrotic disease symptoms through immunomodulatory and anti-inflammatory potentials.

**Materials and Methods**

**Chemicals and Reagents**
Piperine, resveratrol, and BSA were procured from Sigma-Aldrich, USA. Creatinine, blood urea nitrogen (BUN), uric acid, and albumin estimation kits were procured from Biosystems, India. All other chemicals used were of analytical grade and were purchased from local suppliers.

**Animals**
Specific pathogens free female 6-8 week old BALB/c mice weighing 20–25 g were purchased from the National Institute of Nutrition, Hyderabad. The animals were housed in polypropylene cages and maintained under controlled conditions of temperature (23–25°C), humidity (50–55%), and 12 h dark and light cycles. They were fed with chow diet and water ad libitum. All animal treatments were strictly carried out according to the guidelines and approval of the Institutional Animal Ethics Committee, LCP protocol no I/IAEC/LCP/035/2013/15.

**Preparation of Cationic Bovine Serum Albumin**
BSA was used as substrate to make charge-modified cBSA. An amount of 400 mg of BSA crystals were dissolved in 5 ml of 0.01M NaCl at room temperature. Above mixture was added to 8.8 ml of 2M anhydrous ethylenediamine. The pH was adjusted to 7.0 using 0.2M HCl. The reaction mixture was kept overnight at room temperature in a glass-stoppered flask and dialyzed against several changes of saline.[5]

**Experimental Design**
BALB/c female mice were divided into five groups, each consisting of six animals. Group I animals were treated with complete Freund’s adjuvant (CFA) + Saline. Groups II, III, IV, and V were subjected to intravenous injection of 13 mg/kg cBSA thrice weekly for 6 weeks to induce ICGN. Simultaneously, from the 3rd week, Group III and IV were treated with resveratrol (40 mg/kg, p.o.) and piperine (40 mg/kg, p.o.) up to 6 weeks; moreover, dose selection was based on previous studies.[6,7] Group V treated with methylprednisolone (MP) (12.5 mg/kg p.o). Mice were sacrificed at the end of 6th week.

**Induction of Experimental Immune Complex Glomerulonephritis in BALB/c Mice**
Female BALB/c mice were preimmunized with cBSA (0.2 mg emulsified in an equal volume of, CFA). Two weeks later, Groups II, III, IV, and V were injected with cBSA (13 mg/kg) through the caudal vein 3 times/week every alternate day for 6 weeks to induce glomerulonephritis.[8]

**Assessment of Induced Immune Complex Glomerulonephritis**

**Blood and urine metabolic data**
Blood and urine samples were collected and stored until assayed. Proteinuria was confirmed semi-quantitatively using URIT 2VPC (urine reagent strips, Nec Life, China). Urinary and serum concentrations of albumin and uric acid were quantitatively determined using albumin and uric acid kits (ERBA, Mumbai, Maharashtra, India). Serum creatinine was measured using an assay kit (ACCUREX biomedical Pvt. Ltd., Thane, Maharashtra, India). Concentrations of BUN were determined using BUN kit from biosystems, India. All the assays were performed in duplicate according to the manufacturer’s instructions.

**Antioxidant Enzyme Activity**
Kidney tissue samples were homogenized in 10 mmol Tris–HCl buffer (pH 7.4) and the supernatant were used for the measurement of total protein; lipid peroxidation (Thiobarbituric acid reactive substances [TBARS]); glutathione (GSH); superoxide dismutase (SOD), and catalase (CAT). Total protein was quantitatively estimated using Lowry et al., 1951 method.[9] Antioxidants such as SOD, CAT, GSH, and indicators of lipid peroxidation like TBARS[10] were assessed as per protocol.

**Biochemical Estimation**

**Serum IgG1 level**
The serum concentrations of IgG1 were measured using an enzyme-linked immune sorbent assay kit from Ray Biotech (Norcross GA, USA). Assay performed in duplicate according to manufacturer’s instructions.[14]

**Immunomodulatory Activity**
Neutrophil adhesion assay was done by the method described by Wilkinson, 1978 method.[15] Phagocytic index was determined by the method described by Gonda et al., 1990 method.[16]

**Statistical Analysis**
All result values are expressed as mean ± standard error of mean. Statistical analyses were performed using a one-way analysis of variance followed by Tukey’s multiple range test (GraphPad Prism 5.01, GraphPad Software, Inc.). P < 0.05 were considered statistically significant.

**Results**

**Blood and Urine Metabolic Data**

**Effect on albumin**
cBSA increased (P < 0.001) proteinuria in Group II (347.8 ± 10 mg/dl) as compared with Group I (47.13 ± 2.21 mg/dl) while piperine caused significant (P < 0.001) decrease in protein urea (113.9 ± 6.41 mg/dl) (Group IV) compared with cBSA control (Group II). The resveratrol-treated animals also showed a significant (P < 0.001) decrease in urinary protein level (240.9 ± 19.28 mg/dl) (Group III) when compared with Group II [Figure 1].

**Effect on creatinine**
Administration of cBSA increased (P < 0.001) serum creatinine (1.87 ± 0.07 mg/dl) in Group II when compared to Group I (0.28 ± 0.07 mg/dl). Treatment with piperine and resveratrol significantly (P < 0.001) decreased serum creatinine (1.01 ± 0.03 and 1.59 ± 0.02 mg/dl) (Group IV and III) compared with cBSA control (Group II). The standard MP treated animals also showed a significant (P < 0.001) decreased serum creatinine (0.508 ± 0.36 mg/dl) (Group V) when compared with cBSA control (Group II) [Figure 1].
Resveratrol and piperine on nephrotic BALB/c mice

Effect on uric acid
cBSA increased ($P < 0.001$) serum uric acid in Group II (5.97 ± 0.23 mg/dl) as compared to Group I (2.79 ± 0.18 mg/dl). Serum uric acid significantly ($P < 0.001$) decreased (4.57 ± 0.15, 3.51 ± 0.10 mg/dl) (Group III and IV) compared with cBSA control (Group II). Simultaneously, significant ($P < 0.001$) increase in uric acid levels in urine (0.33 ± 0.01, 0.45 ± 0.07 mg/24 h) (Group III, IV) compared with cBSA control (0.24 ± 0.02 mg/24 h) (Group II) [Table 1].

Effect on blood urea nitrogen
Administration of cBSA caused increased ($P < 0.001$) BUN in Group II (44.75 ± 0.87 mg/dl) when compared with Group I (26.24 ± 1.43 mg/dl). Resveratrol and piperine caused a significant ($P < 0.001$) decrease in BUN (39.16 ± 0.82, 33.88 ± 1.13 mg/dl) (Group III, IV) compared with cBSA control (Group II). MP treated animals shown a significant ($P < 0.001$) decreased serum BUN (28.80 ± 0.44 mg/dl) when compared with cBSA control (Group II) [Table 1].

Antioxidant Enzyme Activity

Effect on superoxide dismutase and catalase
Administration of cBSA decreased ($P < 0.001$) SOD (4.103 ± 0.16 U/mg protein) and CAT (0.07 ± 0.13 U/mg protein) activity as compared to Group I. Resveratrol and piperine shown significant ($P < 0.001$) increase SOD (5.195 ± 0.06, 8 ± 0.20 U/mg protein) (Group III, IV) compared with cBSA control. Significant ($P < 0.001$) increase in CAT (0.19 ± 0.03 U/mg protein) was observed in Group IV when compared with cBSA control (Group II) [Table 2].

Effect on glutathione
cBSA treatment decreased GSH (0.98 ± 0.05 µmol/mg protein) in Group II as compared to Group I (4.68 ± 0.20 µmol/mg protein). Resveratrol and piperine caused significant ($P < 0.001$) increase in GSH (2.02 ± 0.16, 3.16 ± 0.12 µmol/mg protein) (Group III, IV) compared with cBSA control (Group II). The standard MP treated animals also shown significant ($P < 0.001$) increase in GSH (4.03 ± 0.10 µmol/mg protein) (Group V) when compared with cBSA control [Table 2].

Effect on thiobarbituric acid reactive substances
Administration of cBSA increased ($P < 0.001$) TBARS (5.66 ± 0.11 nmol/mg protein) in Group II as compared to Group I (2.29 ± 0.11 nmol/mg protein). Resveratrol and piperine caused a significant ($P < 0.001$) decrease in TBARS (4.18 ± 0.18, 3.20 ± 0.16 nmol/mg protein) in all treatment groups when compared with cBSA control (Group II) [Table 2].

Biochemical Estimation

Effect on serum IgG1 level
cBSA increased ($P < 0.001$) IgG1 (0.16 ± 0.015 g/L) in Group II.
as compared to Group I (0.079 ± 0.02 g/L). Treatment with piperine shown significant (P < 0.01) decrease (0.120 ± 0.031) (Groups IV) when compared with cBSA control, whereas resveratrol (0.162 ± 0.03 g/L) (Group III) was to be inactive. The standard MP treated animals also shown significant (P < 0.001) decrease IgG1 (0.107 ± 0.034 g/L) (Group V) when compared with cBSA control [Table 3].

**Discussion**

In this study, cBSA induced glomerulonephritis animal model shown severe renal function damage, which was manifested by the severe proteinuria, serum creatinine, BUN and uric acid production. Moreover, development of ICGN is always consistent with the extent and grade of proteinuria.[17] In the present investigation, severe proteinuria was observed cBSA treated group, whereas administration piperine and resveratrol reduced it manifestly. On the other hand, when piperine compared with resveratrol potential effects was noticed [Figure 1]. This consequence might be influenced by reduced immune complex deposition on the glomerular basement membrane.

It has been well-known that antioxidant enzymes such as SOD, CAT, GSH-reductase, and GSH-peroxidase are the markers for oxidative stress-induced tissue damage.[18] Oxidative stress also plays an important role in the pathophysiology of ICGN. SOD is a very effective defense enzyme that catalyzes the dismutation of superoxide to hydrogen peroxide, which can be further detoxified through the action of CATs and peroxidases. A poorer level of SOD in glomerulonephritis results in a decline scavenger reaction of superoxide and causes renal tissue to become more vulnerable to oxidative stress.[19] cBSA administration results in the enhanced formation of reactive oxygen species which attack the cellular macromolecules, disrupt epithelial cell integrity leads to membrane damage. Reduced SOD and CAT activities after cBSA administration have been suggesting that oxidative stress is one of the causes of renal damage. The administration piperine and resveratrol to nephrotic mice showed an increase in SOD and CAT activities might be due the antioxidant property of these phytochemical. Moreover, piperine showed significantly increased antioxidant enzymes activities than resveratrol [Table 2], which reveals the antioxidant potential of piperine in autoimmune nephritis.

One of the most important intracellular antioxidant systems is GSH redox cycle. GSH has a protective role against oxygen free radical damage by providing reducing equivalents for several enzymes. Reduced GSH was reported to guard the cells from cytotoxic damage usually through lipid peroxidation.[20] Earlier studies revealed that GSH level has been reduced in tissues when antioxidant was neutralized by liberated oxygen-derived free radicals.[21] In this study, the levels of GSH were lower after cBSA injection. An explanation for GSH depletion after cBSA administration is increased consumption of GSH for nonenzymatic removal of oxygen-radicals. Surprisingly, in piperine treated animals there was remarkable prevention in the depleted GSH caused by cBSA. This effect might be...
influenced by the hydroxyl radical scavenging activities of piperine.[11]

Mononuclear phagocytic system (MPS) is part of immune system that consists of phagocytic cells and has been helping in the clearance of immune complexes.[14] Administration of piperine shown more significant potentiation in the macrophage phagocytic activity as compared to resveratrol [Table 3]. Therefore, piperine may have a substantial stimulatory influence on the cells of MPS. Neutrophil has a potential to digest cellular debris and exogenous particulate matter and provide a vital step in the healing process under homeostatic condition.[12] In the present investigation, administration piperine showed mild enhancement in neutrophil adhesion to the nylon fiber when compared with resveratrol, although it was not statistically significant.

BALB/c mice having Th2 subset; however, Th2 subset response plays a vital role in IgG1 secretion and subepithelial immune deposition during the development of ICGN.[9] The result of this study showed that piperine significantly decreases serum IgG1 in the cBSA induced nephrotic mice compared to resveratrol [Table 3]. This effect has been accomplished because of immunomodulatory potential of piperine.[20] Moreover, in the present investigation, MP treated animals shown significant outcome because of the multidisciplinary mechanism of corticosteroid.[21] However, there is pharmacological evidence of piperine as a bioavailability enhancer for certain drugs.[25] Therefore, in future, piperine should co-administered with MP to get synergetic action with regard to use as a pharmaceutical or dietary supplement.

Conclusion

The findings of this study significantly revealed that piperine has potential immunomodulatory and anti-inflammatory activity than resveratrol. Therefore, piperine needs special attention in autoimmunity and inflammation research. Moreover, our research is in progress in a mouse model of experimental autoimmune nephritis to confirm precise molecular mechanism involved in the nephroprotective activity of piperine.

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Conflicts of Interest

There are no conflicts of interest.

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