Effect of Troxerutin on Insulin Resistance Induced by Post-Natal Administration of Monosodium Glutamate: A Comparative Study with Rosiglitazone

V. Ranjith, M.K. Radika and C.V. Anuradha*

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar - 608 002, Tamil Nadu, India

Abstract: Administration of monosodium glutamate (MSG), a most abundant naturally occurring non-essential aminoacid, to new born rats induces hyperlipidemia, obesity and insulin resistance in the adult stage. In this study, we aimed to compare the beneficial effects of troxerutin (TROX), a semi-synthetic bioflavanoid from rutin, and rosiglitazone (RSG), peroxisome proliferator-activated receptor (PPAR)-γ agonist, on insulin sensitivity, oxidative and nitrosative stress and lipid abnormalities in liver of MSG-induced insulin resistant rat. TROX (150 mg/kg/b.w) and RSG (4 mg/kg/b.w) are used as therapeutic options. New born rats were received MSG (4mg/kg/b.w i.p for the first 10 alternate days) to induce insulin resistance. Rats were grouped as follows: Group I-CON received 0.9% saline i.p., Group II-MSG-received MSG alone, Group III-MSG+RSG-received MSG and treated with RSG, Group IV-MSG+TROX-received MSG and treated with TROX, Group V-CON+TROX-received saline and treated with TROX. RSG and TROX were dissolved in water and are given orally from the 21st day till the end the experimental period. After 3 months, assays were performed in plasma and liver. MSG-administered rats showed hyperglycemia, hyperinsulinemia, insulin resistance, oxidative damage, nitrosative stress, lipid accumulation and elevated serum aminotransferases. Increased expression of PPAR-α and γ and decreased expression of adiponectin were observed in them. The biochemical abnormalities associated with MSG administration were significantly reduced by RSG and TROX administration. Thus, TROX promotes insulin sensitivity in rats fed MSG possibly by modifying PPAR-γ and α in liver. With additional studies, TROX might be used as a functional drug or as an adjuvant in the management of insulin resistance.

Keywords: Monosodium glutamate, Troxerutin, Insulin resistance, Rosiglitazone, Peroxisome proliferator-activated receptor.

1. INTRODUCTION

Insulin resistance is characterized by a diminished ability of target tissues to respond to circulating insulin, resulting in hyperinsulinemia, hyperglycemia through enhanced gluconeogenesis and glucose output and increased free fatty acids (FFAs) due to reduced suppression of lipolysis [1]. Insulin resistance is a core pathogenic factor that contributes to a cluster of abnormalities like obesity, elevated blood pressure, hypertriglyceridemia and microalbuminria known as metabolic syndrome, which is strongly associated with development of type 2 diabetes (T2D) and cardiovascular disease [2]. T2D accounts for about 90-95% of all diagnosed cases of diabetes (WHO, 2006). It is estimated that by the year 2030, the total number of people worldwide with T2D might reach 366 million [3].

MSG is one of the most abundant naturally occurring non-essential aminoacid. It is used as a flavouring agent to increase palatability in meal [4]. Studies have report that consuming MSG will lead to Chinese restaurant syndrome, a collection of symptoms which includes headache, nausea, chest pain, sweating, numbness and overfeeding due to MSG lead to obesity, cancer, cardiovascular disease and age related degenerative diseases [5-8]. Administration of MSG has been shown to induce severe obesity with impairment of glucose homeostasis, leading to T2D in mice [9]. MSG consumption has increased throughout the world because of its use in cooking [10]. A study conducted by Sasaki et al. [11] reveals that the administration of monosodium glutamate (MSG) to newborn mice induces severe hyperlipidemia and diabetes mellitus and severe complications of obesity in adulthood.

Thiazolidinediones (TZDs) or glitazone class of drugs such as rosiglitazone (RSG), pioglitazone, ciglitazone have been used in the treatment of T2D as they improve tissue sensitivity towards insulin through activation of PPARs. RSG ameliorates insulin resistance and improves glucose and lipid metabolism in patients with T2D [12]. RSG has been shown to possess anti-inflammatory and anti-oxidant properties [13, 14].

Studies have revealed that (PPAR-γ and PPAR-α are the primary targets for the TZDs [15]. PPAR-α is highly expressed in liver, kidney, heart and plays an...
important role in controlling fatty acid oxidation during obesity and diabetes [16]. PPAR-γ is predominantly expressed in adipose tissue. Studies have shown that adipose-specific PPAR-γ null mice have elevated levels of circulating lipids, increased hepatic gluconeogenesis and insulin resistance [17]. Furthermore, studies have shown that insulin resistant, skeletal muscle-specific PPAR-γ null mice still responds to PPAR-γ agonists [18, 19]. Hence, PPAR-mediated approaches in the development of drug candidates have been shown to be effective for the treatment of T2D.

Troxerutin (TROX) (3’,4’,7-Tris [O-2-hydroxyethyl rutin]) a derivative of the natural bioflavanoid rutin, is found in many plants and can be easily extracted from Sophara japonica (Japanese pagoda tree). Using mouse models, researchers have demonstrated the vasoprotective [20], antioxidant and anti-inflammatory [21] properties of TROX. TROX has the ability to inhibit the upregulation of nuclear factor kappa B p65, inducible nitric oxide synthase and cyclooxygenase-2 expression and to suppress the serum aminotransferase levels in liver of mice [21]. Based on the pharmacological properties of TROX, this study was initiated to investigate whether TROX prevents MSG-induced insulin resistance by mediating PPARs in rat using RSG as the standard compound.

2.MATERIALS AND METHODS

2.1. Biochemicals, Kits, Primers, Antibody, Reagents and Solvents

TROX and primers for reverse transcriptase-PCR analysis were purchased from Sigma-Aldrich Pvt. Ltd., MO, USA. RSG (Rosicon) and MSG (Aginomoto) were obtained from the local pharmacy, Chidamabaram, Tamil Nadu, India. The kits for the assay of glucose, aspartate transaminase [AST] and alanine transaminase [ALT] were purchased from Agappe Diagnostics Pvt. Ltd., Kerala, India. Insulin assay kit was obtained from Accubind, Monobind Inc., CA, USA. TriZol reagent and RT-PCR kits were purchased from GeNei, Bangalore, India. The rest of the chemicals and solvents of analytical grade used in the study were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India or from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

2.2. Animals and Treatment

Male new born Wistar rats were used for the study. The new born rats received a subcutaneous injection of MSG (4g/kg in 0.9% saline per day) or 0.9% saline during the first 10 alternate days of life [22]. The animals were housed in a temperature-controlled room with a standard light - dark cycle and were allowed free access to water and standard pellet diet [Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd., Bangalore, India]. All the experimental procedures involving animals were approved by the Institutional Animal Ethical Committee (IAEC) (Register Number: 166/1999/CPCSEA/768). The animals were maintained under hygienic conditions and cared as per the guidelines of the Indian National Law on Animal Care and Use. After 10 days, the animals were assigned to five groups and maintained as follows:

- **Group I: CON** Rats received saline alone.
- **Group II: MSG** Rats received MSG alone (4 g/kg/b.w i.p for the first 10 days of life every alternate day).
- **Group III: MSG + RSG** Rats received MSG and treated with RSG (4 mg/kg/b.w).
- **Group IV: MSG + TROX** Rats received MSG and treated with TROX (150 mg/kg/b.w).
- **Group V: CON + TROX** Rats received saline and treated with TROX (150 mg/kg/b.w).

The dosage of RSG and TROX used in this study is based on a previous study [23, 24]. Food and water are provided *ad libitum*. Body weight was measured weekly.

At the end of 90 days, the animals were deprived of food overnight, anesthetized and then sacrificed by cervical dislocation. Blood was collected in heparinized tubes and centrifuged at 1500 × g for 15 min to separate plasma. The red blood cells were washed thrice in the saline and hemolysate was prepared in Tris HCl buffer. (0.1 M, pH 7.2). Liver tissue was excised, washed in ice-cold saline. Tissue homogenate were prepared in 0.1M Tris – HCl buffer, pH 7.4 using Potter – Elvehjam homogenizer and a Teflon pestle.

2.3. Measurement of Glucose, Insulin and Insulin Sensitivity Indices

Plasma glucose and insulin were assayed using kits obtained from Agappe Diagnostics Pvt. Ltd., Kerala, India and Accubind, Monobind Inc., CA, USA respectively. Homeostatic model assessment value
(HOMA-IR) [25] and quantitative insulin sensitivity check index (QUICKI) [26] as two surrogate markers of insulin sensitivity, calculated using fasting glucose and insulin levels. The formulae for HOMA-IR and QUICKI are given below:

\[
\text{HOMA-IR} = \frac{\text{Fasting glucose (mg/dL)} \times \text{fasting insulin (μU/mL)}}{2430} - 1
\]

\[
\text{QUICKI} = \log \left( \frac{\text{Insulin (mU/L)}}{\text{Glucose (mg/dL)}} \right) - 2.4
\]

**2.4. Liver Injury, Oxidative Stress, Nitrite and Nitrosothiol**

To assess liver injury, the activities of AST and ALT were assayed in plasma using assay kits. Lipid peroxidation was assessed by quantifying thiobarbituric acid reactive substances (TBARS) [27] and lipid hydroperoxides [28] in plasma and liver. Protein damage was assessed by measuring the protein carbonyl (PC) content in plasma and liver [29]. The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and the levels of vitamins C and E and reduced glutathione (GSH) were measured by methods outlined elsewhere [30]. Plasma nitrite concentration, as an index of NO° was measured by the method of Rock et al. [31] using Griess reagent. Nitrosative stress was also assessed by measuring nitrosothiol [32] content in liver.

**2.5. Lipid Profile**

Lipids were extracted from the liver by the method of Folch et al. [33] using chloroform: ethanol mixture (2:1 v/v). The levels of cholesterol [34], TG [35] and the FFA [36] were determined in the extract.

**2.6. RNA Preparation and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Analysis**

Total cellular RNA was extracted from liver using TriZol reagent. The concentration and purity of RNA preparation were checked by measuring the absorbances at 260 and 280 nm. Total RNA (2.0 μg) was reverse transcribed to cDNA in a reaction mixture containing 1 μl of Oligo (dT) primer (0.2 μg/ml), 1 μl of RNase inhibitor (10 U/ml), 1 μl of 0.1 M DTT, 4 μl of RT buffer (5X), 2.0 μl of 30 mM dNTP mix (7.5 mM each), 0.5 μl of M-MuLV Reverse Transcriptase (50 U/μl) and 1 μl sterile water and kept at 37°C for 1 h and then heated at 95°C for 2 min. PCR amplification was performed in a mixture containing 1μl cDNA, 1 μl Taq DNA polymerase, 2μl 10X PCR buffer, 1μl 30mM dNTPs, 1μl each of 50pM reverse and forward primers and 13μl nuclease free water. Preliminary experiments were conducted with different cycles to determine the optimum conditions for the PCR amplification for each gene. The thermocycling conditions for all the genes were as follows: initial denaturation at 94°C for 4 min for all genes; template denaturation at 94°C for 1min for PPAR-α and PPAR-γ and at 95°C for 30 s for adiponectin; annealing at 60°C for 1min for PPAR-α, and PPAR-γ and at 59°C for 1min for adiponectin and extension at 60°C for 1min for PPAR-α, PPAR-γ and at 72°C for 30 s for adiponectin for 40 cycles. The sequences of primers used are given in Table 1. The reactions were run in triplicate for each sample. After PCR amplification, 5 μl of the reaction products were electrophoresed on 1.5% agarose gel with ethidium bromide for 20 min. The bands were visualized under UV light, captured and then quantified using Image J Software. The intensity of the bands for each gene was normalized with that of β-actin gene. The relative quantity was expressed as density ratio to β-actin for each gene.

**2.7. Statistical Analysis**

All the values were expressed as mean ± SD of 6 rats from each group and statistically evaluated by one way analysis of variance [ANOVA] followed by Duncan’s Multiple Range Test [DMRT] for multiple comparisons. A value of P<0.05 was considered statistically significant.

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene</th>
<th>Fwd 5'-&gt;Sequence-&gt;3'</th>
<th>Rev 5'-&gt;Sequence-&gt;3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PPAR-α</td>
<td>ttggttagcttcggcga</td>
<td>tcticctagctgccagca</td>
</tr>
<tr>
<td>2</td>
<td>PPAR-γ</td>
<td>gccagctccacgacgga</td>
<td>ttccaggtactcaacggacacc</td>
</tr>
<tr>
<td>3</td>
<td>Adiponectin</td>
<td>tcctcagatggggttgccac</td>
<td>atctctctgaccccttagg</td>
</tr>
<tr>
<td>4</td>
<td>β-actin</td>
<td>gagaagatgggccccaccac</td>
<td>cattacaatgcaatggag</td>
</tr>
</tbody>
</table>

PPAR-α: Peroxisome proliferator-activated receptor-α.
PPAR-γ: Peroxisome proliferator-activated receptor-γ.
### 3. RESULTS

#### 3.1. TROX and RSG Prevents Gain in Body Weight

Final body weight is presented in Table 2. Body weight of animals was increased during the experimental period. However, the mean final body weight of MSG (group II) was significantly lower than the control group (group I). The body weights of the groups (group III, IV, V) did not significantly differ from each other and from the control. Food and fluid intake during the experimental period did not vary between all the groups (data not shown).

#### 3.2. TROX and RSG Lowers Plasma Glucose and Insulin and Improves Insulin Sensitivity

Plasma glucose and insulin concentrations at the end of experimental period in MSG-treated rats were significantly higher by 43% and 55% respectively as compared to control rats. Higher HOMA-IR and reduced QUICKI values were observed in MSG treated groups confirming the presence of insulin resistance. TROX and RSG administration reduced the levels of plasma glucose and insulin and improved insulin sensitivity (Table 2).

#### 3.3. TROX and RSG Reduce Hepatocyte Damage

There was a marked increase in the levels of TBARS, LHP and PC content in circulation and liver of MSG-treated rats as compared to control, indicating damage to lipids and proteins. Administration of TROX and RSG prevented the increase in these indices (Table 3). The activities of enzymatic and non-enzymatic antioxidants are given in Figures 1 and 2. The activities of enzymatic antioxidants SOD, CAT and GPx were significantly lower in the hemolysate and liver by (36%, 23% and 78%) and (83%, 118% and 44.5%) respectively and non-enzymatic antioxidants such as GSH, vitamin C and E in plasma and liver by (64%, 43% and 49%) and (77%, 39% and 65%) respectively.

#### 3.4. TROX and RSG Lower Lipid Peroxidation and Protein Carbonyl Formation and Restore the Activities the Enzymatic and Non-Enzymatic Antioxidants

There was a marked increase in the levels of TBARS, LHP and PC content in circulation and liver of MSG-treated rats as compared to control, indicating damage to lipids and proteins. Administration of TROX and RSG prevented the increase in these indices (Table 4). The activities of enzymatic and non-enzymatic antioxidants are given in Figures 1 and 2. The activities of enzymatic antioxidants SOD, CAT and GPx were significantly lower in the hemolysate and liver by (36%, 23% and 78%) and (83%, 118% and 44.5%) respectively and non-enzymatic antioxidants such as GSH, vitamin C and E in plasma and liver by (64%, 43% and 49%) and (77%, 39% and 65%)

### Table 2: Final Body Weight, Blood Glucose, Insulin, Homeostatic Model Assessment [HOMA] and Quantitative Insulin Check Index [QUICKI] of Experimental Animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>MSG</th>
<th>MSG +RSG</th>
<th>MSG+TROX</th>
<th>CON+TROX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight (g)</td>
<td>180.3±11.5</td>
<td>148.3±9.4a</td>
<td>174.8±10.9b</td>
<td>172.21±11.5b</td>
<td>182.13±11.2</td>
</tr>
<tr>
<td>Glucose(mg/dl)</td>
<td>78±5.7</td>
<td>12 2±11.8a</td>
<td>104±7.4b</td>
<td>95±6.5b</td>
<td>76±5.9</td>
</tr>
<tr>
<td>Insulin(μU/ml)</td>
<td>42±3.9</td>
<td>65±5.6c</td>
<td>46±3.7c</td>
<td>48±4.1c</td>
<td>40±4.3c</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.34±0.2</td>
<td>2.66±0.3a</td>
<td>1.96±0.1b</td>
<td>1.87±0.08b</td>
<td>1.25±0.02</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.28±0.03</td>
<td>0.25±0.02a</td>
<td>0.27±0.02b</td>
<td>0.27±0.01b</td>
<td>0.28±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 rats from each group.
CON-control rats (0.9% saline i.p); MSG-monosodium glutamate treated (4mg/g b.w i.p); MSG+RSG-monosodium glutamate treated and treated with rosiglitazone (4 mg/kg b.w/day); MSG+TROX-monosodium glutamate treated and treated with troxerutin (150 mg/kg b.w/day); CON+TROX-control rats treated with troxerutin (150 mg/kg b.w/day).

aSignificant as compared with control rats (P<0.05)(DMRT).
bSignificant as compared with MSG treated rats (P<0.05)(DMRT).

### Table 3: Liver Function Marker Enzymes in Plasma

<table>
<thead>
<tr>
<th>Groups</th>
<th>CON</th>
<th>MSG</th>
<th>MSG +RSG</th>
<th>MSG+TROX</th>
<th>CON+TROX</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>37.16±2.10</td>
<td>51.76±3.16a</td>
<td>43.72±2.28b</td>
<td>41.89±2.3b</td>
<td>38.25±2.27</td>
</tr>
<tr>
<td>ALT</td>
<td>56.27±3.16</td>
<td>74.16±4.23a</td>
<td>64.83±3.22b</td>
<td>63.52±3.28b</td>
<td>55.18±3.21</td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 rats from each group.
CON-control rats (0.9% saline i.p); MSG-monosodium glutamate treated (4mg/g b.w i.p); MSG+RSG-monosodium glutamate treated and treated with rosiglitazone (4 mg/kg b.w/day); MSG+TROX-monosodium glutamate treated and treated with troxerutin (150 mg/kg b.w/day); CON+TROX-control rats treated with troxerutin (150 mg/kg b.w/day).

aSignificant as compared with control rats (P<0.05)(DMRT).
bSignificant as compared with MSG treated rats (P<0.05)(DMRT).
A-IU/L; B=mg/g wet tissue.
respectively in the MSG-treated group as compared to control group. In TROX and RSG administered MSG-fed rats, the activities were significantly higher when compared to untreated MSG group (p<0.05).

3.5. TROX and RSG Increases Nitrite and Decreases the Nitrosothiol Levels

The levels of total nitrite and nitrosothiol in plasma and liver were determined to assess the nitrosative stress. Lower levels of nitrite (plasma: 8.23 and liver: 10.4) and higher levels of nitrosothiols (plasma: 16.7, liver: 62.18) were observed in MSG-fed rats as compared to control (nitrite (plasma: 13.5, liver: 15.2; nitrosothiol (plasma: 7.3, liver: 34.18)). TROX and RSG treatment abolished the nitrosative stress induced by MSG as indicated by the near normal levels of nitrite and nitrosothiol (Table 5).

3.6. TROX and RSG Increases the Expression of Adiponectin

RT-PCR analysis in liver of experimental animals showed decreased expression of adiponectin in MSG-fed rats as compared to control rats. Treatment with TROX and RSG resulted in higher expression of adiponectin when compared to TROX and RSG-supplemented MSG-treated rats. Figure 3 shows the band intensity of electrophoretogram of adiponectin normalized with β-actin and expressed as fold change with respect to control.

3.7. TROX and RSG Differentially Affect the Expression of PPAR-α and γ

RT-PCR analysis in liver of experimental animals showed increased expression of PPAR-α and γ in MSG-treated rat as compared to control rats. RSG administration did not reduce the mRNA expression of PPAR-γ but reduced the mRNA expression of PPAR-α, whereas treatment with TROX resulted in decreased expression of PPAR-α and γ compared to MSG-treated rats. Figure 4 show the electrophoretogram of PPAR-α and γ normalized with β-actin and expressed as fold change with respect to control.

3.8. TROX and RSG Normalize Liver Lipids

Liver total lipid, cholesterol, TG and FFA were measured to determine lipid accumulation. Significant increase in the levels of cholesterol, TG and FFA (39%, 92%, 65% respectively) were observed in MSG-treated rats when compared to control. TROX and RSG prevented lipid accumulation in liver when given to MSG-fed rats (Table 6).

4.DISCUSSION

The major findings of the present study are 1. TROX administration improved insulin sensitivity, restored biochemical parameters, reduced lipid accumulation, oxidative and nitrosative stress and enhanced antioxidative potential in MSG-treated rats and 2. TROX upregulated adiponectin mRNA expression and modified PPAR-γ and α expression differently from RSG.

MSG is used as a taste maker in foods in many countries and this prompted us to study the effect of TROX on MSG-induced insulin resistant rat model. MSG consumption resulted in significant elevation of...
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Significant as compared with control rats (P<0.05) (DMRT).

Significant as compared with MSG treated rats (P<0.05) (DMRT).

Figure 1: Activities of enzymatic antioxidants in hemolysate and liver. Values are means ± SD of 6 rats from each group. CON-control rats (0.9% saline i.p); MSG-monosodium glutamate treated (4mg/g b.w i.p); MSG+RSG-monosodium glutamate treated and treated with rosiglitazone (4 mg/kg b.w/day); MSG+TROX-monosodium glutamate treated and treated with troxerutin (150 mg/kg b.w/day); CON+TROX-control rats treated with troxerutin (150 mg/kg b.w/day).
Significant as compared with control rats (P<0.05) (DMRT).

Significant as compared with MSG treated rats (P<0.05) (DMRT).

Figure 2: Levels of non-enzymatic antioxidants in plasma and liver. Values are means ± SD of 6 rats from each group. CON-control rats (0.9% saline i.p); MSG-monosodium glutamate treated (4mg/g b.w i.p); MSG+RSG-monosodium glutamate treated and treated with rosiglitazone (4 mg/kg b.w/day); MSG+TROX-monosodium glutamate treated and treated with troxerutin (150 mg/kg b.w/day); CON+TROX-control rats treated with troxerutin (150 mg/kg b.w/day).
Table 5: Nitrite and Nitrosothiol Levels in Plasma and Liver of Experimental Animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>CON</th>
<th>MSG</th>
<th>MSG +RSG</th>
<th>MSG+TROX</th>
<th>CON+TROX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>13.58±1.6</td>
<td>8.23±0.7a</td>
<td>12.48±1.1b</td>
<td>12.15±2.5b</td>
<td>13.86±1.4</td>
</tr>
<tr>
<td>Liver</td>
<td>15.2±1.2</td>
<td>10.4±1.6a</td>
<td>14.6±1.4c</td>
<td>14.7±1.5b</td>
<td>15.6±1.5</td>
</tr>
<tr>
<td>Nitrosothiols</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>7.3±0.8</td>
<td>16.7±1.2a</td>
<td>8.9±0.7b</td>
<td>8.4±0.6b</td>
<td>7.2±0.7</td>
</tr>
<tr>
<td>Liver</td>
<td>34.18±4.3</td>
<td>62.18±7.3a</td>
<td>54.84±4.5b</td>
<td>55.47±6.5b</td>
<td>36.18±4.5</td>
</tr>
</tbody>
</table>

Values are means ± SD of 6 rats from each group. CON-control rats (0.9% Saline i.p); MSG-monosodium glutamate treated (4mg/g b.w i.p); MSG+RSG-monosodium glutamate treated and treated with rosiglitazone (4 mg/kg b.w/day); MSG+TROX-monosodium glutamate treated and treated with troxerutin (150 mg/kg b.w/day); CON+TROX-control rats treated with troxerutin (150 mg/kg b.w/day). aSignificant as compared with control rats (P<0.05) (DMRT). bSignificant as compared with MSG treated rats (P<0.05) (DMRT).

A-μmol/L; B-mg/g wet tissue; C-μmol/dl.

Figure 3: mRNA expression of adiponectin in liver. The band intensities of adiponectin are normalized with that of a β-actin and values are expressed as fold change with respect to control.

Figure 4: A and B: mRNA expression of PPAR-α and -γ in liver. The band intensities of adiponectin are normalized with that of a β-actin and values are expressed as fold change with respect to control.

blood glucose, hyperinsulinemia and insulin resistance. The altered values of HOMA and QUICKI suggest decline in whole body insulin sensitivity in MSG-treated rats. Machado et al. [9] observed that MSG can promote obesity and impair glucose homeostasis. MSG has been shown to induce obesity, despite hypophagia, normophagia cause distinct lesions of the hypothalamic arcute nucleus (ARC) which produce orexigenic or anorexigenic neuropeptides. ARC is the master central coordinator of energy homeostasis that adjusts feeding behaviour in response to peripheral signals [37]. The level of expression of these different neuropeptides is finely regulated notably by hormones such as leptin and insulin, both considered as satiety factors [38].

In the present study MSG-treated rats displayed low body weight, fat pad weight, hyperinsulinemia, glucose intolerance and higher plasma FFA and TG plasma
levels. The results obtained are similar to the changes reported by Nardelli et al. [5]. The rise in lipid level may induce defects in downstream insulin signaling in skeletal muscle and liver [39] via oxidative stress and by enhancing serine kinases. Administration of TROX to MSG-treated rats prevented fat accumulation and restored the levels of hepatic and plasma lipid concentrations, FFA, cholesterol, TG to normal and comparable to RSG treated group, possibly by inhibiting HMG-CoA reductase. RSG administration reduced TG, total cholesterol and FFA due to the decreased lipolysis of adipose tissue [40].

Plasma AST and ALT were measured to evaluate the degree of hepatocellular damage. MSG-administrated rats showed elevated levels of AST and ALT reflecting hepatocellular damage. TROX administration mitigated liver injury due to its hepatoprotective property. The values are normal as compared to RSG. RSG improved liver function and has potential in treating non-alcoholic fatty liver disease [41].

Oxidative stress is also associated with adiposity and insulin resistance in men and in those with MS [42]. Oxidative stress may be a primary factor in the etiology of obesity-induced insulin resistance and T2D. A previous study by Diniz et al. [43] reported that MSG administration showed increased hepatic oxidative stress and decreased the levels of antioxidants. Consistent with this, animals given MSG showed elevated levels of oxidative stress markers and reduced levels of antioxidants in the present study. TROX and RSG, being potent antioxidants reduced oxidative stress by replenishing the antioxidants when administered to MSG-treated groups [21, 44].

We also observed decline in NO levels and increase in S-nitrosothiol levels in MSG-treated rats. Peroxynitrite (ONOO\(^{-}\)) is a nitrosating agent and reaction between thiols especially GSH and ONOO\(^{-}\) produces S-nitrosothiol, a marker of nitrosative stress [45]. Both TROX and RSG upregulated NO bioavailability and reduced the levels of nitrosothiols.

MSG-treated rats resulted in increased mRNA expression of PPAR\(\gamma\) and \(\alpha\) in liver in this study [46]. Roman-Ramos et al. [47] have recently reported that MSG administration is associated with inflammatory state and steatosis in liver. MSG causes permanent damage to the neurons in the hypothalamic arcuate nucleus containing the growth hormone releasing hormone and the prolactin inhibiting dopamine neuron cell bodies which is associated with excess storage of fat and increased inflammation in tissues like liver. The rise in inflammation, dysregulated inflammatory profile, with multiple components derived from neuronal injury, visceral adipose tissue mass and liver abnormalities have been attributed to sustained activation of both PPAR-\(\gamma\) and \(\alpha\) by MSG [47].

PPAR-\(\alpha\) modulates genes involved in FA uptake, activation of acyl-coA esters, mitochondrial \(\beta\)-oxidation and ketone body synthesis. PPAR-\(\alpha\)-deficient mice fed a high-fat diet display a massive accumulation of lipids in liver, highlighting the crucial role that PPAR-\(\alpha\) plays in lipid metabolism.

PPAR-\(\gamma\) activation modulates the expression of adipokines such as leptin and adiponectin. PPAR-\(\gamma\) activation promotes glucose utilization in tissues and consequently decreases gluconeogenesis in the liver.
and improves glucose oxidation in muscle. Improved insulin sensitivity and reduction in blood glucose in TROX and RSG treated groups may be due to reduced hepatic glucose production and improved glucose disposal to peripheral tissues. RSG, a synthetic PPAR-γ ligand, significantly increases insulin sensitivity by up-regulating the expression of genes involved in glucose uptake of adipocytes and lipid storage of adipocytes [48]. However, it has also been known that continued activation of PPAR-γ by TZDs is not beneficial and can have side effects by promoting liver steatosis, by inducing the expression of hepatic lipogenic genes in mice [49]. Though it has many beneficial effects, people using RSG chronically have experienced a number of serious side effects on lipid profile thereby leads to heart attack, stroke and hepatotoxicity which led to its ban in Europe and also in India from December 2010 [50, 51]. Weight gain (averaging 7.3%) was the major side effect of RSG [52]. For these reasons, we examined the effect of TROX, a natural bioflavanoid as insulin sensitizing agent in this model and compared this outcome with that of RSG.

RSG and TROX decreased the expression of PPARα in MSG-treated group. However, the effects of RSG and TROX were different with respect to PPARγ expression. In TROX+MSG treated animals, PPARγ and α activation was reduced while in RSG+MSG treated animals PPARγ was increased and PPARα was reduced. As antidiabetic agents, glitazones are primarily used for their effect on glucose homeostasis. Nevertheless, these drugs exert hypotriglyceridemic actions in rodents by increasing lipolysis and clearance of TG-rich lipoproteins [48]. In the present study, we demonstrate that administration of RSG to MSG-treated animals results in reduced expression of PPAR-α alone, whereas there was no further increase in PPAR-γ activation. TROX administration alleviates MSG-induced insulin resistance by decreasing the expression of PPAR-γ and α. In the present study, we demonstrate that administration of TROX alleviates MSG-induced insulin resistance by decreasing the expression of PPAR-γ and α.

MSG-treated rats showed reduced mRNA expression of adiponectin. RSG (TZD) enhanced the expression of adiponectin via binding to PPAR-γ which in turn enhances the expression of the adiponectin gene [53]. In MSG+RSG rats, administration of RSG would have affected the adiponectin mRNA through PPAR-γ activation. However, in MSG alone treated rats, the adiponectin mRNA level was not altered, although PPAR-γ was activated. In one study, MSG administration did not produce any difference in adiponectin mRNA expression in adipose tissue and serum concentration as compared to control rats, but MSG administration caused increased expression of PPAR-γ in adipose tissue [47]. This suggests that there may be other mechanisms that reduce the adiponectin mRNA level in MSG rats (probably due to inflammatory changes) Adiponectin stimulates PPARα in the liver, where AdipoR2 is mainly expressed. Adiponectin enhances suppression of hepatic glucose production. RSG increases adiponectin secretion in cell lines, animal models and in human subjects. The plasma levels of adiponectin in normal, obese and T2D subjects are increased following RSG treatment [54].

**CONCLUSION**

In conclusion, the current study indicates that supplementation of TROX in MSG-treated rats can prevent the development of hyperglycemia and hyperinsulinemia and reduce oxidative stress. It also increases adiponectin levels in liver. Thus, TROX may have beneficial effects in the management of insulin resistance and T2D.

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