Research Article

GALLIC ACID POST-TREATMENT PROTECTS HYPERGLYCEMIA, HYPERLIPIDEMIA AND OXIDATIVE STRESS IN ALLOXAN-INDUCED TYPE-II DIABETIC RATS

Nelcy Joseph¹ and Karthikeyan Sivanesan²

¹Dept. of Pharmacology and Environmental Toxicology, Dr. ALM P.G. Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai-600 113.
²Associate Professor and HOD., Dept. of Pharmacology and Environmental Toxicology, Dr. ALM P.G. Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai-600 113. Tamil Nadu, India.

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ABSTRACT

Background: It is well recognized that oxidative stress is a vital factor that contributes pathological features like hyperglycemia, hyperlipidemia, microvascular and other complications in both type-I and II diabetes and hence several workers now focus on finding suitable alternative to mitigate diabetes by suppressing oxidative stress. Gallic acid, a phenolic acid found in several medicinal plants have been shown to exhibit anti-allergic, anti-inflammatory, anti-carcinogenic, anti-genotoxic, antioxidant and anti-hyperlipidemic properties in several animal models.

Objective: The objective of current study was to investigate the protective properties of gallic acid post-treatment against alloxan-induced hyperglycemia, hyperlipidemia and oxidative stress in rats.

Materials and Methods: Type-II diabetes was induced in male Wistar albino rats (150 to 170 gm) by administration of single dose of alloxan (120 mg/kg; i.p.) in group II and III rats. Gallic acid (50 mg/kg/day; i.p.) was administered in group III rats as post-treatment daily, for 21 days and group II were left without any treatment for this period. Group IV rats received saline instead of alloxan and were treated gallic acid (50 mg/kg/day; i.p.) daily, for 21 days. Control animals (group I) received saline throughout the study period. Serum glucose was investigated on day 12 and 22 after alloxan treatment. Additionally, serum lipid profiles (triglyceride, total cholesterol, HDL, LDL, VLDL-cholesterol) and markers of oxidative stress (LPO, SOD, CAT, GSH, GST) were also investigated.

Results: Hyperglycemia induced by alloxan alone treatment is evidenced by highly significant (p<0.001) 3 to 4-fold increase in fasting glucose on day 12 and 22 respectively. Hyperlipidemia was shown by significant increase (p<0.001) in serum triglycerides, cholesterol, VLDL and LDL and fall in HDL-cholesterol. Oxidative stress was evidenced by 7-fold increase in LPO (p<0.001) and decrease in SOD, CAT, GSH and GST levels (p<0.001) in serum. Above abnormalities induced by alloxan treatment was significantly protected (p<0.001) and reversed back towards normalcy in rats post-treated gallic acid in alloxan-induced type-II diabetic rats.

Conclusion: Our preliminary investigations demonstrate commendable protection on hyperglycemia, hyperlipidemia and oxidative stress by gallic acid post-treatment against alloxan-induced type-II diabetic rats. This alleviating property could be attributed to anti-hyperglycemic, anti-hyperlipidemic and antioxidant potentials of gallic acid. Further studies are warranted to understand the precise molecular mechanisms that govern the anti-diabetic potentials of gallic acid.
INTRODUCTION:
Diabetes is a heterogenous disorder, characterized by altered carbohydrate, lipid and protein metabolism, which is caused due to insufficient insulin secretion or its action or both. It is predicted that world-wide, around 430 million people would suffer due to diabetes by the year 2030 and it is a growing burden in developing countries. Several inter-connecting biochemical pathways and cellular signaling mechanisms such as i) increased flux through polyol pathway, ii) intracellular production of advanced glycation end products precursors, iii) protein kinase-C activation and iv) increased hexosamine pathway activity have been reported to operate in type-II diabetes. Further, diabetes is frequently reported to cause dyslipidemia, characterized by elevation of serum triglycerides associated with alterations in cholesterol levels and hence, monitoring serum lipid profiles is considered an important biomarker to recognize insulin resistance in patients suffering from this metabolic disorder. Oxidative stress is also an important factor that contributes microvascular complications in diabetes and several evidences have shown correlation between hyperglycemia and changes in redox homeostasis. Over production of reactive oxygen species (ROS) and decreased efficacy towards inhibition of free radical scavenging system are suggested to be the cause for enhanced oxidative stress in diabetes. Insulin is the mainstay for treatment of type-I and type II diabetes mellitus patients. Second generation sulfonylureas, biguanides, thiazolidinediones and few more drugs are advocated for treatment of type II diabetes. Adverse and toxic effects reported upon administration of these anti-diabetic drugs are severe hypoglycemia, IgE mediated cutaneous reactions, acidosis, liver toxicity, cholestatic jaundice, hemolytic anemia and generalized hypersensitivity reactions. Active principles extracted from natural sources have been tested for anti-diabetic properties globally and in India alone over 800 medicinal plants were tested for their alleviating potentials in experimental animals and humans. However, utility of these phytochemicals for therapeutic relief of hyperglycemia, hyperlipidemia and oxidative stress observed in type-II diabetes are questionable due to several reasons. Gallic acid (3,4,5-trihydroxy benzoic acid), a phenolic acid found in several medicinal plants, nuts, leaves and bark is used in foods, cosmetics and food packaging materials to prevent rancidity. Administration of gallic acid was shown to exhibit anti-allergic, anti-inflammatory, anti-carcinogenic and anti-genotoxic effects. It is also documented to protect against lipid peroxidation, glutathione depletion and protein carbonyl content in both in vitro and in vivo studies. However, the protective effects of gallic acid treatment against hyperlipidemia, hyperglycemia and oxidative stress against experimentally induced type-II diabetes have not been explored. Several studies have documented that parenteral administration of alloxan (2,4,5,6-tetraoxy pyrimidine; 2,4,5,6-pyrimidinetetraene) cause necrosis of insulin secreting beta cells in pancreas to form dialuric acid, which is postulated to generate reactive oxygen species (ROS) and superoxide radicals to induce oxidative stress in type-II diabetes. Oxidative stress is also an important factor that contributes microvascular complications in diabetes and several evidences have shown correlation between hyperglycemia and changes in redox homeostasis. In the current study, we investigated the alleviating potentials of gallic acid post-treatment against alloxan-induced type-II diabetic manifestations i.e., hyperglycemia, hyperlipidemia and oxidative stress, in rats.

MATERIALS AND METHODS

Animals
Approval of institutional animal ethical committee’s permission (IAEC No. 1/21/2015), was obtained prior to animal experimentation. Male Wistar albino rats (150 to 170 gm), procured from institutional animal house facility were used in this study. They were housed in polypropylene cages on husk beddings in controlled environmental conditions (temperature – 23 ± 4°C; humidity 50 to 70%; 12 h light/dark cycle) and fed standard pellet feed and water ad libitum.

Drugs, chemicals and diagnostic kits
Gallic acid, malondialdehyde (MDA) and pyrogallol were purchased from M/s Sigma-Aldrich Chemicals, USA. Alloxan was bought from SRL Chemicals Pvt. Ltd., Mumbai. Glucose, triglyceride and cholesterol diagnostic kits were procured from Rapid Diagnostics.
Pvt. Ltd., New Delhi and Robonik Pvt. Ltd., Mumbai. All the other chemicals used in various assays were obtained from local vendors and they were of analytical grade.

**Animal treatments and experimental protocol**

Four groups of rats were segregated at random (n=6 in each group). Type II diabetes was induced in group II and III rats by administration of single dose of alloxan (120 mg/kg b.w.; i.p.). While group II rats were left without any treatment 24 h after alloxan for a further period of 21 days, that of group III rats were post-treated gallic acid orally (50 mg/kg b.w./day; p.o.) for this duration. Group IV rats received gallic acid alone (50 mg/kg b.w./day; p.o.) for 21 days, 24 h after single oral administration of saline. The group I rats received saline throughout the study period and they served as control. Dosages for alloxan and gallic acid were selected as recommended by previous workers and they were dissolved in saline. Volumes of all administrations were maintained at 0.3 to 0.5 ml/100 gm b.w. of rats and were prepared just before use.

**Collection of serum**

Blood was collected (2 to 3 ml) by retro-orbital vein puncture after over-night fasting from mild ether anesthetized rats on days 1, 12 and 22 in clean dry tubes. It was allowed to clot at cold (15°C) for 20 min and then centrifuged (2,500 rpm for 15 min) for separation of serum, which was pipette out and stored separately in vials at cold (15°C) till analysis. All the biochemical analysis was performed within 24 h after collection of serum.

**Assay of glucose and lipid parameters**

Glucose was quantified by using kit (Rapid Diagnostics Pvt. Ltd., New Delhi), as per manufacturer’s instructions. The serum triglyceride, total cholesterol and HDL-cholesterol were quantified using standard assay kits (Robonik Pvt. Ltd., Mumbai) as per manufacturer’s instructions. Very low density lipoprotein (VLDL)-cholesterol and low density lipoprotein (LDL)-cholesterol values were calculated by applying Friedewald formula, using the values of triglycerides, total cholesterol and HDL-cholesterol as follows:

\[
\text{VLDL-cholesterol} = \frac{\text{triglyceride}}{5}; \\
\text{LDL-Cholesterol} = \text{total cholesterol} - (\text{HDL} – \text{VLDL-Cholesterol})
\]

**Assay of lipid peroxidation (LPO)**

Malondialdehyde (MDA), the secondary product of LPO reacts with thiobarbituric acid to form pink chromogen (thiobarbituric acid-2-malondialdehyde adduct) and its optical density was measured in serum at 532 nm against blank as described by Ohkawa et al.

**Assay of Superoxide-dis-mutase (SOD) activity**

Pyrogallol, in presence of the mixture containing tris-HCl buffer and diethylene triamine penta acetic acid, undergoes rapid auto-oxidation to produce several intermediate products. The rate of inhibition of auto-oxidation by the enzyme present in serum was quantified at 420 nm to express the activity of SOD as detailed by Marklund and Marklund.

**Assay of Catalase (CAT) activity**

Dichromate placed in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide and serum to form perchromic acetate, whose optical density was measured at 570 nm to express the activity of CAT in serum as per the method of Sinha.

**Assay of reduced glutathione (GSH)**

p-nitrophenol released from 5-5’ dithio bis (2-nitrobenzoic acid) on reaction with reduced glutathione (GSH) in serum produce intense yellow color (Ellman’s reaction) with the extension coefficient (I/C log I/lo) of 13,600/m² pm⁻¹ at 412 nm, whose intensity was quantified as described previously by Beutler et al.

**Assay of Glutathione-s-transferase (GST)**

The change of absorbance in unit time, produced by addition of enzyme present in serum on its treatment with the reaction mixture containing 1-chloro-2,4-dinitro benzene and glutathione was measured at 340 nm for the expression of GST activity, as detailed by Habig et al.

**Total protein estimation in serum**

The total protein in serum was estimated by the standard method of Lowry et al.

**Statistical analysis**

Data were subjected to one-way analysis of variance (ANOVA). Tukey’s multiple comparison tests were performed to evaluate the significance of difference between means of various treatment groups using SPSS software (version-16). The values are expressed...
as mean ± S.D. and p value < 0.05 was considered significant.

RESULTS

Effect of gallic acid on alloxan-induced hyperglycemia

Hyperglycemia induced by alloxan alone treatment is clearly evidenced by a highly significant 4-fold and 3-fold increase (p<0.001) in serum glucose on days 12 and 22 respectively, as compared to saline treated control. Gallic acid post-treatment in rats receiving alloxan, show significant reduction (p<0.001) in glucose levels on day 12 and its level was almost near normal on day 22, revealing the hypoglycemic potentials of this treatment. Gallic acid alone treatment showed normal glucose levels on par with the control on all days of its evaluation (Figure – I).

Effect of gallic acid on alloxan-induced hyperlipidemia

Hyperlipidemia induced by alloxan is revealed by highly significant elevation in serum triglyceride, cholesterol, VLDL and LDL-cholesterol (p<0.001), accompanied by a highly significant fall (by around 80% of normal levels of control) in HDL-cholesterol. Above adversities induced by alloxan treatment was significantly reversed back (p<0.001; p<0.01) towards normalcy in rats receiving gallic acid post-treatment. These results reveal the anti-hyperlipidemic properties of gallic acid treatments against alloxan-induced hyperlipidemia. Gallic acid alone treatment did not produce any change in the above lipid parameters in serum and their values were similar to those of control (Table – I).

Table – I Lipid profile in serum of rats treated alloxan and gallic acid.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Triglycerides (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>159.61 ± 24.62</td>
<td>188.91 ± 3.82</td>
<td>35.47 ± 2.76</td>
<td>31.92 ± 4.92</td>
<td>121.50 ± 7.17</td>
</tr>
<tr>
<td>Group II</td>
<td>257.66 ± 33.59 &lt;***</td>
<td>285.30 ± 5.12 &lt;***</td>
<td>5.09 ± 0.87 &lt;***</td>
<td>51.53 ± 6.71 &lt;***</td>
<td>228.68 ± 5.97 &lt;***</td>
</tr>
<tr>
<td>Group III</td>
<td>193.85 ± 2.07 &lt;***</td>
<td>231.30 ± 14.36 a,b&lt;***</td>
<td>28.32 ± 4.69 &lt;***</td>
<td>38.77 ± 0.42&lt;***</td>
<td>164.21 ± 15.53 a,b&lt;***</td>
</tr>
<tr>
<td>Group IV</td>
<td>155.99 ± 19.11</td>
<td>184.45 ± 3.18</td>
<td>37.56 ± 2.74</td>
<td>31.19 ± 3.82</td>
<td>115.70 ± 6.76</td>
</tr>
</tbody>
</table>

Values presented are mean ± S.D of 6 nos. of rats in each treatment group. Group I – control; Group II – alloxan alone (120 mg/kg; i.p.); Group III – alloxan (120 mg/kg ;i.p) + gallic acid (40 mg/kg/day for 21 days; p.o); Group IV – gallic acid alone (40 mg/kg/day for 21 days; p.o). Multiple comparisons were performed by Tukey’s test. a – Group I compared to Groups II and III; b – Group II compared to Group III. ** p < 0.01; *** p < 0.001.
**Effect of gallic acid on alloxan-induced oxidative stress**

The MDA levels were increased by almost 7-folds (p<0.001) of normal levels of control in serum of alloxan alone treated diabetic rats. Oxidative stress induced by alloxan is further shown by almost 50% fall in the activities of antioxidant enzymes SOD and CAT (p<0.001) as compared to saline treated control.

Gallic acid post-treatment in rats receiving alloxan decreased the MDA levels and increased the SOD and CAT activities (p<0.001) almost towards the normal levels of control, revealing its antioxidant potentials. There was no change in the status of MDA, SOD and CAT in rats treated gallic acid alone and their respective values resembled those of saline treated control (Figure – II).

*Figure – II Effect of gallic acid treatment on serum LPO, SOD and CAT in alloxan-induced diabetic rats*

Values presented are mean ± S.D of 6 nos. of rats in each treatment group. Group I – control; Group II – alloxan alone (120 mg/kg; i.p.); Group III – alloxan (120mg/kg ;i.p.) + gallic acid (40 mg/kg/day for 21 days; p.o); Group IV – gallic acid alone (40 mg/kg/day for 21 days; p.o). Multiple comparison was performed by Tukey’s test. a – Group I compared to Groups II and III; b – Group II compared to Group III. *** p < 0.001.
The oxidative stress induced by alloxan is further evidenced by a highly significant fall in the levels of the secondary antioxidant biomarkers GSH (p<0.001) and GST (p<0.01) as compared to saline treated control. Additionally, this treatment also produced a fall in serum protein highly significantly (p<0.001).

Gallic acid post-treatment in rats receiving alloxan significantly reversed back the fall in levels of GSH, GST and protein in serum to near normalcy. Gallic acid alone treatment did not produce any change in the status of these parameters and they were on par with the saline treated control rats (Table – II).

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>GSH</th>
<th>GST</th>
<th>Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>24.10 ± 2.88</td>
<td>18.62 ± 1.96</td>
<td>7.46 ± 0.58</td>
</tr>
<tr>
<td>Group II</td>
<td>17.30 ± 2.59 **</td>
<td>10.47 ± 2.86 ***</td>
<td>3.37 ± 0.30 ***</td>
</tr>
<tr>
<td>Group III</td>
<td>20.93 ± 2.28 b**</td>
<td>15.72 ± 1.21 b**</td>
<td>5.05 ± 0.68 a,b***</td>
</tr>
<tr>
<td>Group IV</td>
<td>22.93 ± 2.37</td>
<td>18.15 ± 1.89</td>
<td>8.35 ± 0.48</td>
</tr>
</tbody>
</table>

Values presented are mean ± S.D of 6 nos. of rats in each treatment group. Group I – Control; Group II – alloxan alone (120 mg/kg; i.p.); Group III – alloxan (120 mg/kg; i.p.) + gallic acid (40 mg/kg/day for 21 days; p.o.); Group IV – gallic acid alone (40 mg/kg/day; daily for 21 days; p.o.). GSH-reduced glutathione (μM of GSH/mg protein); GST-glutathione-s-transferase (μM of CDNB utilized/min/mg protein); total protein (gm/dL). Multiple comparisons were performed by Tukey’s test. a – Group I compared to Groups II and III; b – Group II compared to Group III. ** p < 0.01; *** p < 0.001.

DISCUSSION

Alloxan inhibits insulin secretion by selectively blocking the activity of glucokinase and destruction of beta cells of pancreas to induce hyperglycemia\(^1\)\(^2\)\(^3\). It is postulated that alloxan exhibits biphasic effect, initially by showing high blood glucose few days after its administration and maintaining sustained glucose level in the second phase, depending upon nutritional status and physiological adaptability\(^2\)\(^4\). In the current study, alloxan treatment initially produce a 4-fold increase in serum glucose on day 12 and thereafter its level fell down to 3-folds of normal levels of control on day 22, exhibiting biphasic effect and these results are in agreement with the above report.

The characteristic features of lipid abnormalities in type-II diabetes and its associated risk of coronary heart diseases are increase in serum VLDL and LDL-cholesterol and decrease in HDL-cholesterol. Hyperlipidemia, marked as elevation in serum total lipids, triglycerides, VLDL and LDL-cholesterol and fall in HDL-cholesterol levels have been reported previously in alloxan treated rats\(^2\)\(^5\)\(^6\). Moreover, insulin deficiency induced by alloxan treatment is reported to elevate plasma triglyceride levels by increasing activity of the enzyme lipoproteinlipase, leading to augmentation of lipid metabolism and mobilization of free fatty acids from liver and adipose tissues\(^3\). The hypercholesterolemia and hypertriglyceridemia along with fall in HDL-cholesterol observed in alloxan treated diabetic rats in our current study are in concurrence with the above reports.

Experimental evidences have shown that oxidative stress impairs glucose uptake in muscles and adipose tissues in diabetic condition and decrease insulin secretion from beta cells of pancreas\(^2\)\(^8\). It is postulated that pancreatic beta cells contain low levels of SOD, CAT and GPx, which could be overwhelmed by redox imbalance, resulting in enhanced ROS production. And hence, it requires an environment rich in oxygen in order to generate more insulin. ROS production during diabetes might precipitate oxidative stress culminating in suppression of insulin synthesis\(^1\). Oxidative stress, the outcome of increased ROS...
production in alloxan-induced type-II diabetic rats is shown by 7-fold increase in MDA level (which is a vital biomarker of lipid peroxidation and oxidative stress), associated with the fall in SOD and CAT and our current results are in accordance with above reports. Fall in reduced glutathione (GSH) levels and GST activity in serum further confirms the oxidative stress induced by alloxan and our present results are in agreement with previous report\textsuperscript{28}. Diabetics is often associated with impairment of protein metabolism and its excretion in urine (microalbuminurea) in humans\textsuperscript{30}. In view of this report, the observation of fall in serum protein in alloxan treated diabetic rats could be attributed to excretion of proteins through urine as a consequence of impaired protein metabolism.

In the current investigation, post-treatment of gallic acid significantly mitigated the increase in serum glucose levels, alterations in lipid profiles and oxidative stress and reversed these adversities towards normalcy in alloxan-induced diabetic rats. These results illustrate the protective capability of gallic acid post-treatments against alloxan-induced hyperglycemia, hyperlipidemia and imbalance in oxidant-antioxidant homeostasis. Supplementation of gallic acid in obesity induced diabetic rats was shown to reduce the increase in body weight, triglycerides, cholesterol and increased the status of SOD, CAT and GSH in rats\textsuperscript{31} and our present results are in agreement with this report. Phenolic phytochemicals containing gallic acid was shown to suppress the synthesis and secretion of VLDL and decrease the levels of triglycerides in diabetic rats\textsuperscript{32}. Ho \textit{et al.},\textsuperscript{33} reported the anti-diabetic, anti-inflammatory, anti-carcinogenic and antioxidant potentials of gallic acid extracted from plant sources. Further, it is also reported to stimulate GLUT-4 translocation and glucose transport in dose-dependent manner and this is presumed to be the rationale for maintenance of normal glucose level in adipose tissues and muscles\textsuperscript{34}. In the light of above reports, it could be suggested that gallic acid post-treatment protects alloxan-induced hyperglycemia, hyperlipidemia and oxidative stress by stimulating GLUT-4 translocation and by enhancing glucose uptake. However, it is suggested that further studies are needed to understand the precise molecular mechanism and potential benefits of gallic acid against alloxan-induced type-II diabetes.

**CONCLUSION**

Our preliminary investigations demonstrate commendable restoration of hyperglycemia, hyperlipidemia and oxidant-antioxidant homeostasis towards normalcy upon gallic acid post-treatment against alloxan-induced type-II diabetic rats. These alleviating potentials of gallic acid could be attributed to its i) anti-hypoglycemic, ii) anti-hyperlipidemic and iii) antioxidant properties. Gallic acid may be a preferable agent for protection of type-II diabetes and its associated complication. Further studies are warranted to understand the precise molecular mechanisms that govern the anti-diabetic potentials of gallic acid.

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**Conflict of Interest:**

The authors declare that there is no conflict of interest in this study.

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