

## Alleviating Effect of Gallic Acid on Dexamethasone Induced Insulin Resistance in Albino Mice.

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Available Online: 10<sup>th</sup> February, 2016

### ABSTRACT

Gallic acid is a naturally occurring phenolic compound and is well known for its prominent antioxidant potential. This study aims to evaluate the protective effect of gallic acid on dexamethasone-induced insulin resistance. Dexamethasone is a steroid, clinically used to treat numerous inflammatory conditions. Acute exposure to this drug is known to produce insulin resistance inducing prediabetic condition and hyperglycemia. In the current study, 30 albino mice were divided into 5 groups containing equal number of animals; one of the group was administered with dexamethasone 1 mg/kg i.p. for 28 days to induce insulin resistance where as dexamethasone and gallic acid of 50 mg/kg p.o. and 100 mg/kg p.o. respectively were administered to two groups. Saline and pioglitazone was given to normal and standard control groups respectively. On 29<sup>th</sup> day, the serum samples and excised pancreatic tissues were collected for biochemical estimations. The serum analysis revealed that treatment with gallic acid effectively reduced elevated glucose, LDL, VLDL and increased HDL levels in a dose dependent manner. Additionally, it was found to decrease the MDA levels and elevate the GSH levels in pancreas. In conclusion, gallic acid treatment in dexamethasone-induced insulin resistant mice exhibited a protective effect.

**Key words** Insulin resistance, Dexamethasone, Gallic acid, Hyperglycemia, Diabetes.

### INTRODUCTION

Diabetes is a global epidemic affecting millions of people worldwide. It is a metabolic syndrome, which can be partly prevented by effective changes in diet and lifestyle. It was observed that lifestyle changes in susceptible individuals were more preventive than using a renowned drug<sup>1</sup>. Insulin resistance is a predisposing factor of  $\beta$ -cell dysfunctioning and apoptosis, leading to type 2 diabetes and obesity<sup>2</sup>. Reduction in insulin sensitivity can be a probable consequence of elevated oxidative stress. Chronic disruption of redox balance may lead to stimulation of stress sensitive kinases like IKK $\beta$  which subsequently phosphorylates multiple substrates including insulin receptor and substrate proteins eventually leading to insulin resistance. Nonetheless, the protective effect of antioxidants by neutralizing the debilitating effect of free radicals is also evident against such insulin resistance<sup>3,4</sup>. The current study aims at assessing the protective effect of gallic acid against insulin resistance and type 2 diabetes in mice. Gallic acid is a phenolic acid, which is mostly found in different medicinal plants like *Terminalia chebula*, *Terminalia bellerica*, *Phyllanthus emblica* belonging to a traditional ayurvedic formulation known as Triphala<sup>5</sup>. The cytotoxic effect of triphala on various cancers is attributed to the presence of gallic acid in it<sup>6,7</sup>. Moreover, it has also shown to possess anti inflammatory and anti microbial activity<sup>8,9</sup>. To evaluate the effect of gallic acid in the current study, dexamethasone-induced insulin resistance in mice model was considered<sup>10</sup>.

### MATERIALS AND METHODS

Dexamethasone was procured from Ziska Pharmaceutical Ltd. Gallic Acid, thiobarbituric acid, Ellmann's reagent was purchased from Sigma-Aldrich (USA). Serum biochemical parameters were measured using commercially available diagnostic kits (Angstrom Biotech Pvt. Ltd). All the other chemicals and reagents used were of analytical grade.

#### Animals

30 Swiss albino mice (18-20g) were procured from National Institute of Nutrition, Hyderabad, India. Animals were housed in polypropylene cages and environmentally controlled room with 12hr light/dark cycle and had access to food and water *ad libitum*. Animals were acclimatized for a period of 7 days. All the experimental procedures adapted were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (320/CPCSEA, dated 03-01-2001) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee (GPRCP/IAEC/07/15/02/PCL/AE-4 MICE –M/F-30), G. Pulla Reddy College of Pharmacy, Hyderabad, India.

#### Experimental study design

After the completion of acclimatization period, 30 mice were randomly divided into five groups (n = 6) and the following treatment protocol was followed. Group I (Normal Control): Received vehicle orally. Group II (Disease Control): Received dexamethasone (1 mg/kg i.p.) for 28 days. Group III (Standard): Received pioglitazone (2 mg/kg/day, p.o.) and dexamethasone for 28

Table 1: Consolidated table representing various serum parameters like glucose, TG, TC, HDL, LDL, VLDL and effect of gallic acid (50 and 100 mg/kg.p.o.) on these parameters.

Groups	Serum glucose (mg / dL)	Triglyceride (mg/dL)	Total cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
N.C	63.6±1.7	94.6±4.2	66.1 ± 2.9	35.1±2.15	63.4±6.69	18.9±1.10
D.C	83.3±1.7 <sup>α</sup>	152±8.9 <sup>α</sup>	126.6±4.8 <sup>α</sup>	21±0.93 <sup>α</sup>	136.1±4.1 <sup>α</sup>	30.4±1.6 <sup>α</sup>
GA 50mg/kg	78.3±2.33	127.8±3.72 <sup>α,b</sup>	106.1±4.88 <sup>α,c</sup>	22.8±1.17 <sup>γ</sup>	109.2±4.51 <sup>γ</sup>	25.6±0.06 <sup>γ,c</sup>
GA 100mg/kg	73.5±1.62 <sup>β,b</sup>	118±4.31 <sup>β,a</sup>	94.5±6.13 <sup>β,a</sup>	32.2±1.08 <sup>a</sup>	88.76±5.06 <sup>γ,c</sup>	21.56±1.7 <sup>a</sup>
Std	72.83±1.54 <sup>c,b</sup>	118.59±1.98 <sup>γ,c</sup>	110.3±2.88 <sup>a</sup>	33.8±2.1 <sup>a</sup>	85.50±4.10 <sup>a</sup>	20.62±0.06 <sup>a</sup>

Data was expressed as Mean ± SEM (n = 6) and was analysed by One Way Analysis of Variance (ANOVA), followed by Tukey's test for the comparison of means.

<sup>α</sup>p<0.001, <sup>β</sup>p<0.01, <sup>γ</sup>p<0.05 when compared to normal control.

<sup>a</sup>p<0.001, <sup>b</sup>p<0.01, <sup>c</sup>p<0.05 when compared to disease control.

days. Group IV (Gallic acid 50 mg/kg): Received Gallic acid (50 mg/kg/day, p.o.) and dexamethasone for 28 days. Group V (Gallic acid 100 mg/kg): Received Gallic Acid (100 mg/kg/day, p.o) and dexamethasone for 28days. At the end of the experiment, the animals were anaesthetized using diethyl ether and blood samples were collected from retro-orbital plexus for the estimation of serum glucose levels, triglyceride levels, total cholesterol, High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL) and Very Low Density Lipoproteins (VLDL). The excised pancreatic tissue was subjected to estimation of malondialdehyde (MDA) and glutathione (GSH) levels.

#### Biochemical estimations

##### Serum analysis

Blood samples were centrifuged at 1500 rpm (4°C) for 10 minutes. The serum glucose levels were determined by routine GOD-POD method. Triglyceride, total cholesterol, HDL, LDL and VLDL levels were estimated by the following method provided with commercially available diagnostic kits (Angstrom biotech Pvt. Ltd). All the analysis was carried out using Biochemistry Auto Analyzer (Star 21 Plus), Rapid Diagnostic Pvt. Ltd.

##### Estimation of pancreatic malondialdehyde (MDA) levels

MDA levels were estimated by spectrophotometric method described by Ohkawa et al<sup>11</sup>. Briefly excised liver tissue was homogenized in 0.15 mol/L KCl using Remi homogenizer. 500 µl of this homogenate was then added to a mixture containing 200 µl of 8.1% SDS, 1.5ml of 20% acetic acid (pH 3.5) and equal volume of 0.8% thiobarbituric acid (TBA). The reaction mixture was heated at 95°C for 90 minutes. After cooling of the mixture, 1ml of distilled water and 5ml butanol/pyridine (15:1) solution was added. The resultant colored layer was separated and OD was measured at 532nm using spectrophotometer (UV 1700 Shimadzu).

##### Estimation of pancreatic glutathione (GSH) levels

Reduced glutathione (GSH) level was measured in pancreas using the method described by Ellman<sup>12</sup>. The tissue homogenate was added with equal volume of 20% trichloroacetic acid containing 1mM EDTA and allowed to stand for 5 minutes. The mixture was subjected to centrifugation at 200 rpm for 10 minutes. 200 µl of this

mixture was added to a cuvette containing 1.8ml of the Ellman's reagent and OD was measured at 412 nm.

##### Statistical analysis

Results are expressed as Mean ± SEM. Statistical analysis was performed by one way ANOVA, followed by Tukey's test as post hoc test using Graphpad Prism 5.

## RESULTS

### Body weight

Body weight is the important hallmark for insulin resistance. As shown in Figure 1, the administration of dexamethasone significantly decreased the body weight from 14<sup>th</sup> day to 28<sup>th</sup> day as compared to normal control. Treatment with gallic acid (50 and 100 mg/kg p.o.) significantly increased the body weight as compared to disease control group and these changes were restored to the normal level with high dose. Similar changes were observed by treatment with pioglitazone. Data was expressed as Mean ± SEM (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey's test for the comparison of means. <sup>γ</sup>p<0.01 compared to normal control group; <sup>α</sup>p<0.001 compared to Disease control group

### Serum parameters

As shown in Table 1, induction of insulin resistance in disease control mice lead to significant increase in triglycerides (TG), total cholesterol (TC), LDL, VLDL and decrease of HDL levels when compared to normal control group. Treatment with gallic acid (50 and 100 mg/kg p.o.) has shown dose dependent reduction in LDL, VLDL levels. There was no dose dependent effect observed in TG and TC levels when compared to disease control group. However, the HDL levels were increased and restored to normal. Similar effects were observed by treatment with pioglitazone and these changes were restored to normal level in HDL and VLDL.

### Pancreatic MDA levels

As shown in Figure 2, induction of insulin resistance significantly increased MDA levels in pancreas of the disease control group when compared to the normal control group. Treatment with gallic acid (50 and 100 mg/kg p.o.) significantly decreased MDA levels when

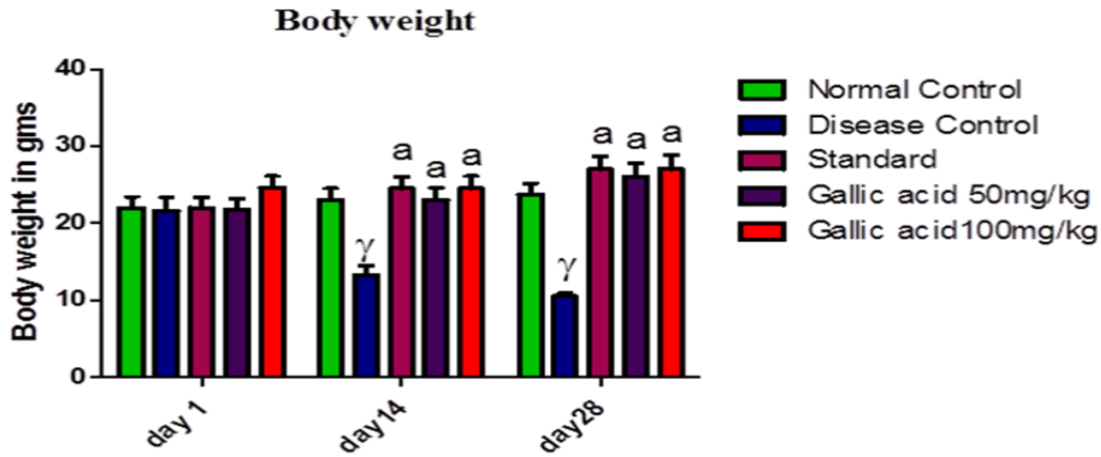


Figure 1: Effect of Gallic acid on body weight

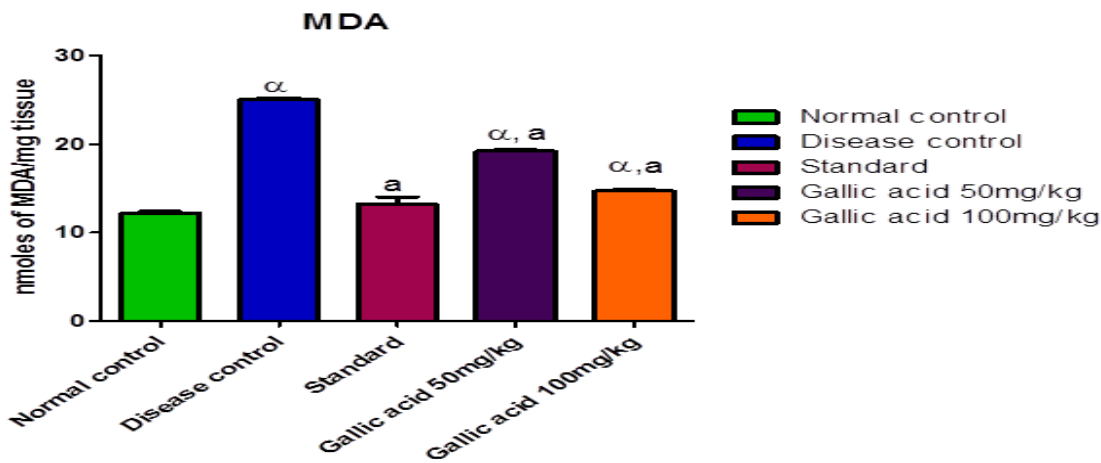


Figure 2: Effect of gallic acid (50 & 100mg/kg) on pancreatic MDA levels in dexamethasone-induced insulin resistance in mice.

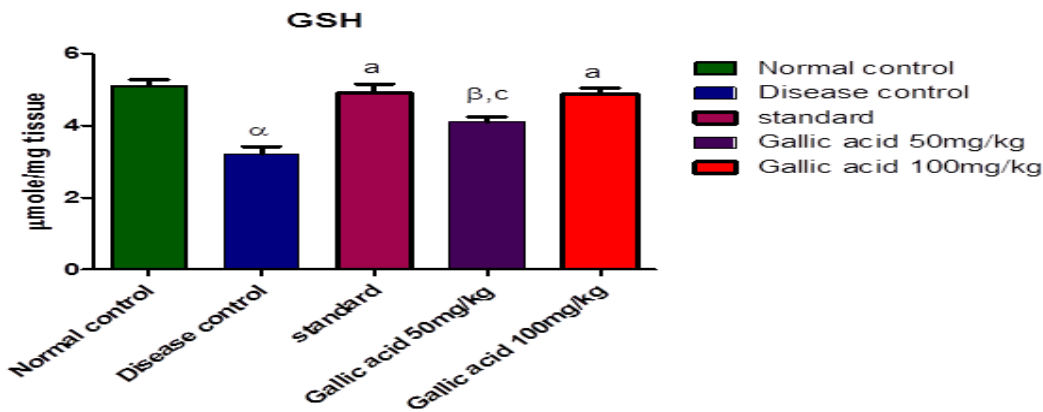


Figure 3: Effect of gallic acid (50& 100mg/kg) on GSH levels in dexamethasone-induced insulin resistance in mice.

compared to disease control group. In the pioglitazone treated group, the MDA levels were restored to normal levels as compared to disease control group. Data was expressed as Mean ± SEM (n=6). Data was analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey's test for the comparison of means. <sup>α</sup>p<0.001 compared to normal control group; <sup>a</sup>p<0.001 compared to Disease control group.

*Pancreatic GSH levels*

As shown in Figure 3, induction of insulin resistance significantly decreased the pancreatic GSH levels in disease control group when compared to normal control group. Treatment with gallic acid (50 mg/kg p.o.) significantly increased the GSH levels when compared to disease control group. However, these changes were restored normal levels with high dose (100 mg/kg) of gallic

acid treated group and pioglitazone treated group. Data was expressed as Mean  $\pm$  SEM (n=6). Data was analyzed by one way Analysis of Variance (ANOVA) followed by Tukey's test for the comparison of means. <sup>a</sup>p<0.001, <sup>b</sup>p<0.01 compared to normal control group; <sup>a</sup>p<0.001, <sup>c</sup>p<0.05 compared to Disease control group.

## DISCUSSION

Flavonoids are phenolic compounds found in plants; the antioxidant activity of many plant extracts is primarily attributed by such compounds. Gallic acid is a similar phenolic compound occurring naturally in many plants<sup>13</sup>. It is considered as a potent free radical scavenger, which can protect the cell from oxidative damage<sup>14,15</sup>. A similar study reveals the protective effect of gallic acid against (type 1) diabetic induced cardiac dysfunction, which clearly shows the lipid lowering and anti hyperglycemic potential of gallic acid<sup>16</sup>. Dexamethasone is a steroid derivative which has a prominent anti inflammatory effect and is used in the treatment of various inflammatory conditions like asthma, chronic obstructive lung disease, skin and rheumatic problems. Clinical use of dexamethasone has shown modulating effect on blood glucose and body weight<sup>17,18</sup>. These changes may be accredited to insulin resistance produced by steroids. The current study successfully demonstrates the effect of gallic acid on insulin resistance. Dexamethasone significantly increased serum glucose levels in disease control group, which was affected by oral administration of gallic acid for 28 days, thereby reducing the elevated serum glucose levels in a dose dependent manner (p<0.01). Moreover, changes in body weight were also observed. Treatment with gallic acid not only prevents weight loss but also slightly increases the body weight than normal. Studies using streptozotocin as an inducer shows the preventive effect of gallic acid against destruction of pancreatic islets cell resembling type 1 diabetes<sup>19</sup>. Furthermore, the current study attempts to evaluate insulin resistance by dexamethasone, which forms an appropriate pre diabetic model. Treatment with gallic acid had a notable impact on lipid profile including triglycerides, total cholesterol, LDL, VLDL which was effectively reduced near to normal levels in a dose dependent manner, whereas a significant increase in the HDL levels was observed. Gallic acid at a dose of 100 mg/kg demonstrated better effect than standard drug (pioglitazone). Normal cell has a natural balance of antioxidant system which counters the harmful effect of radicals; once the balance is disrupted it causes oxidative stress eventually damaging the cell and organelles. Such an oxidative stress can be estimated by marker such as malondialdehyde (MDA)<sup>20</sup>. In our study, the elevated MDA levels were effectively curbed close to normal upon treatment with gallic acid in a dose dependent manner. Glutathione (GSH) is an endogenous free radical scavenger. In states of oxidative stress, endogenous antioxidants tends to deplete the concentration of GSH in a cell, which can act as a good marker. Clinical investigation on diabetic subjects has shown a decline in GSH levels, reflecting oxidative stress due to chronic hyperglycemia<sup>21</sup>. In accordance to it, treatment with gallic

acid could maintain the elevated levels of GSH dose dependently. Hence, gallic acid nullifies the unwanted (hyperglycemia, altered lipid profile) effects of dexamethasone owing to its antioxidant properties.

## CONCLUSION

Administration of dexamethasone hampered various biochemical parameters in mice. Treatment with gallic acid exhibited protective effect against insulin resistance so produced by dexamethasone.

## ACKNOWLEDGEMENT

We sincerely acknowledge the management of G. Pulla Reddy College of Pharmacy (GPRCP) for all the grants and support in conduct of our research. We extend our sincere gratitude to Dr. Veeresh, Professor and H.O.D, Department of Pharmacology, GPRCP, for his much needed guidance.

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