Abstract

The present study was designed to evaluate the antioxidant activity of Rosa damascena on non-enzymic antioxidants in liver and kidney and other biochemical marker enzymes of kidney and serum in Streptozotocin (STZ) induced diabetic rats. The animals were divided into five groups of six animals each after the acclimatization period. Group I- (Normal control) Rats received standard pellet diet and distilled water. Group II- Diabetic control – Rats were injected with STZ at a concentration of 50mg/kg intraperitoneally. Group III & IV– Treated rats with the hydroethanolic flower extract of Rosa damascena at a concentration of 200 mg/kg and 400mg/kg body weight respectively. Group V- Treated rats with a standard drug Glibenclamide orally at a concentration 600μg/kg body weight. Non-enzymic antioxidants such as Vitamin C were assessed by the procedure given by Sadasivam and Theymoli, Vitamin E and A were measured by Rosenberg method and Bayfield and Cale method respectively. Reduced glutathione was estimated by Ellman method. Kidney marker enzymes such as serum urea were assessed by Natelson method and serum creatinine by the method of Owen. Data were reported as mean ± SD using the Statistical Package of Social Sciences (SPSS, Version 10.0 for windows). Analysis Of Variance (ANOVA) and Duncan’s Multiple Range Test (DMRT) were used and the values were considered statistically significant when p < 0.05. The level of Vitamin C, E, A and reduced glutathione were found to be significantly decreased in diabetic control rats when compared to normal control rats. Oral administration of hydroethanolic (HEE) flower extract of Rosa damascena at a dosage of 200 and 400 mg/kg body weight for 30 days showed a significant increase in the above non-enzymic antioxidants as compared to diabetic control rat. A significant increase in serum urea and creatinine was observed in diabetic control rats when compared to normal control group. Groups treated with plant extracts at both the concentrations (200 and 400 mg/kg body weight) and standard drug glibenclamide (600mg/kg body weight) showed significant decrease in serum urea and creatinine levels when compared to diabetic control group. The Results of the investigation suggested that the hydroethanolic flower extract of Rosa damascena exhibited not only antioxidant activity but also alters the hemodynamic changes in kidney to near normalcy level.
1. Introduction
Diabetes Mellitus is a disease caused by deficiency or diminished effectiveness of endogenous insulin. It is characterized by hyperglycemia, deranged metabolism and predominant by affecting the vasculature. Pancreatic diseases such as cystic fibrosis, chronic pancreatitis, endocrine disorders, drug induced corticosteroids, haemochromatosis and Diabetes insipidus under the genetic abnormality may be the causative agents for diabetes mellitus. Defects in insulin secretion or action or both are characterized by hyperglycemia often accompanied by glycosuria, polydipsia and polyuria\(^{(1)}\). During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS) in all tissues from glucose auto-oxidation and protein glycosylation\(^{(2)}\). The modern oral hypoglycemic agents produce undesirable side effects. Thus, alternative therapy is required which made to shift towards the different indigenous plants and herbal formulations. In the present study *Rosa damascena* is used to validate the antioxidant activity in Streptozotocin induced diabetic rats. *Rosa damascena* mill L, is commonly known as Damask rose\(^{(3)}\). It is one of the most important species of Rosaceae family. *Rosa damascena* (Rosaceae) is a deciduous shrub growing to 2.2 meters tall, the stems densely armed with stout, curved prickles and tuff bristles. The plant flowers have aromatic odour. The most therapeutic effects of *Rosa damascena* in ancient medicine include treatment of abdominal and chest pain, strengthening of the heart, treatment of menstrual bleeding, digestive problems and reduction of inflammation, especially of the neck\(^{(4)}\). Several components such as Terpenes, Glycosides, Flavonoids, and Anthocyanins\(^{(5)}\) were isolated from flowers, petals and hips (seed-pot) of *Rosa damascena*. It also contains carboxylic acid \(^{(6)}\), Myrcene, Vitamin C, Aempferol and Quercetin\(^{(7)}\). The medicinal functions of Rosaceae are partly attributed to their abundance of phenolic compounds\(^{(8)}\). Phenolics possess a wide range of pharmacological activities such as antioxidants, free-radical scavenging\(^{(9)}\), anticancer\(^{(10)}\), anti-inflammatory, antimutagenic\(^{(11)}\), and antidepressant\(^{(12)}\) activities.

2. Materials and Methods

2.1 Chemicals
All chemicals and solvents used in this study were of analytical grade.

2.2 Plant material and Preparation of 50% hydroethanolic plant extract
Fresh flowers of *Rosa damascena* was collected from rose garden, Coimbatore, Tamilnadu, India. It was identified by the Botanical Survey of India (BSI), Tamilnadu Agricultural University (TNAU) (Plant identification no: BSI/SRL/5/23/09-10/Tech). The fresh flowers of *Rosa damascena* were collected, dried (shade dried) and pulverized using a mechanical grinder. The coarse powder of the flowers was cold macerated with hydroethanol for 72 hours. A pinkish brown crystals was obtained after evaporating the water portion to dryness under reduced pressure in a vacuum rotary evaporator. The crystals were suspended in distilled water and used for the study.

2.3 Procurement of animals
Male wistar rats weighing about 140 ± 20 grams were procured from PSG Institute of Medical Sciences and Research (CPCSEA No : 158/1999/CPCSEA) Coimbatore, Tamilnadu, India. All the procedures described were reviewed and approved by the Institutional Animal ethics committee (IAEC). The rats were grouped and housed in polycryllic cages and were maintained under standard laboratory conditions. Rats were acclimatized to animal house conditions with controlled temperature and humidity and they are fed with standard rat feed supplied by Hindustan lever ltd., Bangalore, Karnataka, India and filtered water *ad libitum*. 

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2.4 Induction of Diabetes
Streptozotocin was used to induce diabetes mellitus in normal rats through intraperitoneal injection. It was dissolved in ice cold 0.01M citrate buffer, pH 4.5 at a concentration of 50mg/kg and was injected to overnight fasting rats. Blood was collected through retro-orbital plexus after 72 hours of injection and the serum was separated by centrifugation at 2500rpm for 15 minutes. Blood glucose was then measured.

2.5 Experimental groups
The animals were divided into five groups of six animals each after the acclimatization period.

Group I - (Normal control) Rats received standard pellet diet and distilled water. Group II - (Diabetic control) The rats were made hyperglycemic by an intraperitoneal injection of Streptozotocin at a concentration of 50mg/kg body weight to overnight fasting rats.

Group III - Diabetic rats treated with oral administration of hydroethanolic flower extract of *Rosa damascena* at a concentration of 200 mg/kg body weight.

Group IV - Diabetic rats treated with oral administration of hydroethanolic flower extract of *Rosa damascena* at a concentration of 400 mg/kg body weight.

Group V - Diabetic rats treated with a standard drug Glibenclamide orally at a concentration 600μg/kg body weight.

The rats were fasted overnight and sacrificed by cervical decapitation at the end of experimental period (30 days). The liver and Kidney were excised and stored at 4ºC for the analysis. Serum was separated from the blood which was collected through cardiac puncture and was stored at 4ºC for further biochemical analysis.

2.6 Preparation of Tissue Homogenate
The tissues were homogenized in 0.1 m cold Tris HCl buffer (pH 7.4) using potter homogenizer fitted with teflon plunger. It was then centrifuged at 10,000 rpm for 20 minutes at 4ºC. The supernatant was used for enzyme assays.

2.7 Biochemical estimations
Non enzymic antioxidants such as Vitamin C was assessed by the procedure given by Sadasivam and Theymoli(13), Vitamin E by Rosenberg method(14), Vitamin A by Bayfield and Cale method(15). Reduced glutathione by Ellman method(16). Kidney marker enzymes such as serum urea was assessed by Natelson(17) method and serum creatinine by the method of Owen(18).

3 Statistical Analysis
Data were reported as mean ± SD by using the Statistical Package of Social Sciences (SPSS, Version 10.0 for windows). The data for all the parameters was analysed by using Analysis Of Variance (ANOVA) and the group means were compared by Duncan’s Multiple Range Test (DMRT). Values were considered statistically significant when p < 0.05(19).

4 Results

4.1 Non- Enzymic antioxidant effect of Hydroethanolic flower extract of *Rosa damascena* in Streptozotocin induced diabetic rats
The results show that (Table 4.1.1, 4.1.2, 4.1.3 and 4.1.4) the level of Vitamin C, E, A and reduced glutathione was found to be significantly decreased in diabetic control rats (group II) when
compared to normal control rats (group I) in both liver and kidney. Treatment with hydroethanolic flower extract of *Rosa damascena* at a concentration of 200mg/kg and 400mg/kg (Group III and IV respectively) in both the tissues showed a significant increase in non-enzymic antioxidant levels when compared to diabetic control rat (group II). Diabetic rats treated with a standard drug Glibenclamide at a dosage of 600µg/kg recouped the levels of non-enzymic antioxidants in both liver and kidney tissues.

Table 4.1.1: Effect of *Rosa damascena* flower extracts on Vitamin C in liver and kidney of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vitamin C (mg / gm tissue)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Group – I</td>
<td>3.73 ± 0.81*</td>
<td>3.04 ± 0.13*</td>
<td></td>
</tr>
<tr>
<td>Group – II</td>
<td>1.51 ± 0.06</td>
<td>1.26 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Group – III</td>
<td>2.01 ± 0.07*</td>
<td>2.07 ± 0.16*</td>
<td></td>
</tr>
<tr>
<td>Group – IV</td>
<td>2.83 ± 0.14*</td>
<td>2.66 ± 0.13*</td>
<td></td>
</tr>
<tr>
<td>Group – V</td>
<td>3.82 ± 0.17*</td>
<td>3.18 ± 0.23*</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n=6) (p<0.05)
Group Comparison : G2 vs G1, G3,G4 and G5
Statistical Significance : * - P< 0.05, ns = not significant

Table 4.1.2: Effect of *Rosa damascena* flower extracts on Vitamin E in liver and kidney of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vitamin E (µg / gm tissue)</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Group – I</td>
<td>28.42 ± 1.51*</td>
<td>21.18 ± 1.71*</td>
<td></td>
</tr>
<tr>
<td>Group – II</td>
<td>15.37 ± 0.68</td>
<td>11.62 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>Group – III</td>
<td>26.06 ± 1.40*</td>
<td>19.17 ± 1.3*</td>
<td></td>
</tr>
<tr>
<td>Group – IV</td>
<td>27.37 ± 1.40*</td>
<td>20.16 ± 1.33*</td>
<td></td>
</tr>
<tr>
<td>Group – V</td>
<td>29.71 ± 1.36*</td>
<td>21.37 ± 1.48*</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n=6) (p<0.05)
Group Comparison : G2 vs G1, G3,G4 and G5
Statistical Significance : * - P< 0.05, ns = not significant
Table 4.1.3: Effect of Rosa damascena flower extracts on Vitamin A in liver and kidney of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vitamin A (µg / gm tissue)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Group – I</td>
<td>6.37 ± 0.33*</td>
<td>5.43 ± 0.35*</td>
</tr>
<tr>
<td>Group – II</td>
<td>3.21 ± 0.18</td>
<td>2.58 ± 0.14</td>
</tr>
<tr>
<td>Group – III</td>
<td>4.81 ± 0.28*</td>
<td>3.86 ± 0.17*</td>
</tr>
<tr>
<td>Group – IV</td>
<td>5.36 ± 0.31*</td>
<td>4.97 ± 0.23*</td>
</tr>
<tr>
<td>Group – V</td>
<td>6.5 ± 0.37*</td>
<td>5.74 ± 0.27*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n=6) (p<0.05)
Group Comparison: G2 vs G1, G3,G4 and G5
Statistical Significance: * - P< 0.05, ns = not significant

Table 4.1.4: Effect of Rosa damascena flower extracts on Reduced Glutathione in liver and kidney of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reduced glutathione (GSH) (mg/100 gm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Group – I</td>
<td>42.61 ± 2.11*</td>
<td>27.96 ± 1.37*</td>
</tr>
<tr>
<td>Group – II</td>
<td>19.34 ± 0.74</td>
<td>11.63 ± 0.34</td>
</tr>
<tr>
<td>Group – III</td>
<td>39.82 ± 1.66*</td>
<td>25.64 ± 1.58*</td>
</tr>
<tr>
<td>Group – IV</td>
<td>40.45 ± 2.24*</td>
<td>26.28 ± 1.48*</td>
</tr>
<tr>
<td>Group – V</td>
<td>43.36 ± 1.99*</td>
<td>28.61 ± 1.43*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n=6) (p<0.05)
Group Comparison: G2 vs G1, G3,G4 and G5
Statistical Significance: * - P< 0.05, ns = not significant

4.2 Kidney marker enzymes

Serum urea and creatinine levels in Streptozotocin induced diabetic rats was found to be elevated (Table 4.2.1) when compared to normal control rats (group I). A significant decrease in serum urea and creatinine level was found in the plant extract treated groups. Administration of 50%
hydroethanolic flower extract of *Rosa damascena* at a dosage of 200 and 400mg/kg body weight significantly decreased the levels of serum urea and creatinine as compared to the diabetic control rats (group II).

Table 4.2.1: Effect of *Rosa damascena* flower extracts on serum urea and creatinine of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea(mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I</td>
<td>37.81 ± 2.91*</td>
<td>3.81 ± 0.13*</td>
</tr>
<tr>
<td>Group – II</td>
<td>56.38 ± 2.91</td>
<td>7.43 ± 0.32</td>
</tr>
<tr>
<td>Group –III</td>
<td>35.62 ± 2.34*</td>
<td>1.97 ± 0.02*</td>
</tr>
<tr>
<td>Group – IV</td>
<td>36.13 ± 2.25*</td>
<td>2.47 ± 0.13*</td>
</tr>
<tr>
<td>Group – V</td>
<td>38.27 ± 2.33*</td>
<td>3.91 ± 0.09*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n=6) (p<0.05)
Group Comparison : G2 vs G1, G3,G4 and G5
Statistical Significance : * - P< 0.05, ns = not significant

5 Discussion

The changes in the level of non-enzymic antioxidants such as Vitamin C, Vitamin E, Vitamin A and Reduced glutathione (GSH) are important in cellular system in curtailing ROS. The levels of these non – enzymic antioxidants in the diabetic state and treated state were assessed and the results are discussed as follows.

Vitamin C is an important water soluble antioxidant in biological fluids and an essential micronutrient required for the normal metabolic functioning of the body. Vitamin C at high doses has been shown to reduce the accumulation of sorbitol in the erythrocytes of diabetes and to inhibit the glycosylation of proteins\(^ {20}\). Transport of Vitamin C into cell is facilitated by insulin. Many diabetics do not have enough intracellular Vitamin C. Therefore a relative Vitamin C deficiency exists in many diabetics despite adequate dietary consumption\(^ {21}\). The present study showed that Hydroethanolic extract of *Rosa damascena* significantly increases the antioxidant level in experimental treated rats which may be due to the presence of potent antioxidant capacity in the extracts.

Enhanced level of Vitamin E or tocopherols in plant extracts treated groups is based on their ability to donate phenolic hydrogens to lipid radicals. Vitamin E protects PUFA from being oxidized\(^ {22}\). Vitamin E exhibits a protective role on the oxidative stress in hypertension\(^ {23}\). Vitamin E inhibits smooth muscle cell proliferation as it plays an important role in the development of atherosclerosis and inhibits oxidation of carbondioxide by free radicals\(^ {24}\).

Vitamin A and carotenoids prevent lipid peroxidation by scavenging free radicals and other reactive oxygen species produced during diabetes which may be present in the plant extracts. This increases the level of Vitamin A in the diabetic rats treated with 50% hydroethanolic flower extract of *Rosa damascena* at a dosage of 200 and 400mg/kg bodyweight. This Vitamin has been implicated as a biological factor in reducing the incidence of cancer. In humans, Vitamin A and β – carotene reverse
precancerous leucoplakia and reduce the occurrence of pathological micronuclei even on continuous exposure to mutagens from betal – nuts and tobacco chewing\(^{(25)}\).

Vitamin A, a fat soluble Vitamin plays a role in trapping peroxyl radicals in tissues at low partial pressure of oxygen. Short term supplementation of Vitamin A along with Vitamin E and C significantly reduced lipid peroxide levels in coronary heart disease\(^{(26)}\).

Reduced glutathione is a major intracellular redox buffer that may approach concentrations up to 10 mM\(^{(27)}\). GSH detoxification system is one of the defense systems against free radicals and carcinogens. In diabetic patients there is a marked decrease in the level of reduced glutathione\(^{(28)}\). Hyperglycemia is found to be an indirect cause of GSH depletion. As GSH is an important antioxidant molecule its depletion leads to the increase of oxidative stress.

In the present study there was an increased level of reduced glutathione in plant extract treated groups which implies that the plant extracts may has an enhanced amount of GSH activity which plays a role in coordinating the body’s antioxidant defense processes. Reduced glutathione, synthesized mainly in the liver is an important non-enzymic antioxidant in the antioxidant defense system. The depletion of GSH content may also lower the GST activity as GSH is required as a substrate for GST activity\(^{(29)}\).

Elevated glycosylated protein, glucose and renal damage were found in diabetes mellitus due to metabolic defects in the regulation of blood glucose as well as due to increased oxidative stress\(^{(30)}\). The enhanced serum urea and creatinine levels in diabetic control rats may be due to the damages in the glomeruli. After 30 days treatment of diabetic control rats by an oral administration of 50% hydroethanolic flower extract of *Rosa damascena* at the dosages of 200 and 400 mg/kg body weight reduced the level of these substances. Such a decrease in level of serum urea and creatinine indicate that the plant extracts has an ability to enhance the renal function.

Hence from the present study it was found that Hydroethanolic extract of *Rosa damascena* may be responsible to exert such an antioxidant activity under the stress of diabetes mellitus.

6 Summary and Conclusion

We suggest that the mode of action of hydroethanolic flower extract of *Rosa damascena* may be due to the presence of phytochemicals which might have increased the nonenzymic antioxidant levels and kidney marker enzymes in Streptozotocin induced diabetic animals. An effective treatment was also found with a standard drug Glibenclamide at a concentration of 600 \(\mu\)g/kg body weight. Further aspects include the pharmacological investigations and mechanism of action of the active components present in the plant.

References


