



## EVALUATION OF PROTECTIVE EFFECTS OF ROSA DAMASCENA MILL.L AGAINST ALLOXAN INDUCED DIABETIC NEUROPATHY IN RATS

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### ABSTRACT

Diabetic neuropathy is damage to nerves in the body that occurs due to high blood sugar levels from diabetics. Diabetes mellitus (DM) is a chronic disease caused by insufficient production of insulin by pancreatic glands and decreases in absorption of glucose by the cells in the human systems and caused increase the concentration of glucose in blood. In this study we tried to evaluate the protective effects of *Rosa damascene* mill.L against alloxan induced diabetic neuropathy. The present study was design to evaluate the *Rosa damascene mill extract* of 200 mg/kg and 400 mg/kg/ p.o dose against Diabetic neuropathy in wistar rats. It was evaluated by Physical parameters: Body weight, pain-threshold level by Tail Flick Method, Hot plate Method, and Biochemical Estimations by Serum parameters: glucose, triglycerides, cholesterol, HDL, LDL levels. At the end of 5<sup>th</sup> week, diabetic rats were showed significant effect in tail flick latency by hot water tail immersion test and paw withdrawal response by hot plate test.

**Key words:** *Rosa damascene mill.L*, Rosaceae, alloxan induced diabetic

### INTRODUCTION

Today in many countries modern medicine has displaced plants with many synthetic products but almost 30% of pharmaceutical preparations are still obtained directly or indirectly from plants. The modern era has seen some decline in use of medicinal plants and their extracts as therapeutic agent, particularly in developed countries, many of which either been discarded by the medical profession or now given in the form of isolated compound<sup>1</sup>. The strategy of isolating the active principles from the medicinal plants and manufacturing a pharmaceutical preparation then became popular.

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Diabetes mellitus is a group of metabolic diseases characterized by high hyperglycemia urine hormone pancreas Normal glucose levels for men and women without diabetes are between 70-110mg/dL. Diabetic neuropathy is a painful complication involving progressive neuronal damage and dysfunction. It affects the sensory nerves, the autonomic nervous system and even the central nervous system (CNS). Diabetes mellitus (DM) is a chronic disease caused by insufficient production of insulin by pancreatic glands and decreases in absorption of glucose by the cells in the human systems and caused increases the concentration of glucose in blood. It is also produced due to the hereditary Characters. Due to increase glucose level in blood causes various deficiencies and hampers the normal Physiological effects of the human system like blood vessels and nerves system etc<sup>2,3</sup>

The effect of diabetes on the central nervous system has also been recorded in the form of changes in the structure of the blood brain barrier, neurophysiology, increased neuronal apoptosis and reduction in cognitive abilities. On the other hand, evidence challenging neuronal death by apoptosis and survival mechanisms in operations has also been reported. This information has accumulated by using a variety of *in vitro* and *in vivo* experimental models. The

neuropathies of the peripheral, central and autonomic nervous systems are known to be caused by hyperglycemia, a consequence of the deregulation of glucose in diabetes. Several *in vivo* models such as alloxan-induced diabetic rats, mice and Chinese hamsters have been used to study the pathogenesis of diabetic neuropathy because of their resemblance to human pathology. Many herbal products have been described for the use of diabetic neuropathy in ancient literature; herbal preparations alone or in combination with oral hypoglycemic agent sometimes produce a good therapeutic response in some resistant cases wherein the allopathic drug alone has failed to produce the satisfactory results<sup>4</sup>. *Rosa damascena* mill L, commonly known as Damask rose is known as Gole Mohammadi in Iran. It is one of the most important species of Rosaceae family. Rosaceae are well-known ornamental plants and have been referred to as the king of flowers. Several components were isolated from flowers, petals and hips (seed-pot) of *R. damascena* including terpenes, glycosides, flavonoids, and anthocyanins. This plant contains carboxylic acid, myrcene, vitamin C, kaempferol and quercetin. Flowers also contain a bitter principle, tanning matter, fatty oil and organic acids<sup>5</sup>. The present study is to evaluate Protective Effects of *Rosa damascena* millethanol extract against alloxan induced Diabetic Neuropathy in Rats<sup>6,7</sup>.

## MATERIALS AND METHODS

### Plant Material:

The plant material was collected from Dr. Y.S.R. Horticulture University, Horticulture Research Station, Sangareddy, Medak district, A.P. and authenticated by Dr. K.N. Reddy, Taxonomist, Lila Impex R&D centre, Vijayawada. The flowers were shade dried and ground into coarse powder.

### Preparation of Extract:

The powdered flowers (1000 Gms) of *Rosa damascene mill* was extracted continuously with ethanol at 60 °C using Soxhlet apparatus for about 48 hours. The extract was concentrated under reduced pressure on a Rotary Vacuum Evaporator to a constant mass and to yield a solid (9.6% w/w). The extract was stored in a refrigerator until the initiation of study.

### Phytochemical screening:

The ethanolic extract of flowers of *Rosa damascene mill* was subjected to various qualitative chemical tests to detect the chemical constituents present in it in the following manner<sup>9, 10, 11, 12</sup>.

### Test for Alkaloids

#### Dragendroff's Test:

0.5 gm of the crude dried powder was warmed with 10 ml. of 2% sulphuric acid for 2 minutes and filtered. The solution was used for the tests. 1 ml. portion was treated with a few drops of Dragendroff's reagent, no colour precipitate was observed showing the absence of alkaloids.

#### Wagners test:

To 1 ml of the solution, 1 ml. of Wagner's reagent (iodine in potassium iodide) was added. No colour precipitate was observed indicating that alkaloids are absent.

### Test for Saponins

#### Salkowski test:

A small amount of extract was taken in a test tube containing 1 ml. chloroform and added 5 to 6 drops of conc. sulphuric acid. Blood red color in lower layer was formed indicating the presence of steroidal saponins.

#### Antimony trichloride test:

A small amount of extract was taken in a test tube containing 1 ml. of chloroform and added 3 ml. of antimonytrichloride followed by heating. Color change was observed (purple to violet color) indicating the presence of steroidal saponins.

### Test for Tannins

About 0.5 Gms. of the extract was stirred with about 10 ml. of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml. of the extract in methanol. Blue-green precipitate was formed indicating the presence of tannins.

### Test for Flavonoids

#### Shinoda test:

A small amount of the extract was taken in a test tube containing 1 ml. of ethanol and added a mixture containing magnesium ribbon and concentrated hydrochloric acid. Red color was observed indicating the presence of flavonoids.

A small amount of extract was taken in a test tube containing 1 ml. of ethanol and added 1 ml. of lead acetate solution. Colored precipitate was formed indicating that flavonoids are present.

### Test for Steroids

#### Libermann-burchard test:

A small amount of extract was taken in a test tube containing 1 ml. of chloroform and added 4 to 5 drops of acetic anhydride and 4 to 5 drops of conc sulphuric acid. Blue color was observed. It indicates that steroids are present.

A small amount of extract was taken in a test tube containing 1 ml. of chloroform and added 3 ml. of antimonytrichloride followed by

heating. Color change was observed (purple to violate color). It indicates that steroids are present.

**Test for Anthracene glycosides**

A small amount of extract was taken in a test tube containing 1 ml. of ethanol and added 0.5 ml. of potassium hydroxide solution. Violate color was observed indicating that anthracene glycosides are present.

**Test for Amino acids**

Ninhydrin test:

A small amount of extract was taken in a test tube containing 1 ml. of ethanol and added ninhydrin reagent, followed by heating. Purple color was observed, indicating that amino acids are present.

**Pharmacological screening**

**Chemicals and reagents:**

Normal Saline, Alloxan, Glucose kit, Triglyceride kit, HDL kit, LDL kit, Cholesterol kit, Ethanol, Glimperide used in the study are of analytical grade.

**Experimental animals:**

Healthy male Wistar rats (200-250g) were housed in CPCSEA approved animal house ingroups of four in polypropylene cages. They were maintained at 25 ± 2° C, relative humidity of 45 to 55% and under standard environmental conditions. The animals had free access to food and water *ad libitum*. All the procedures were performed in accordance with the Institutional Animal Ethical Committee constituted as per the directions of the CPCSEA.

**Acute toxicity studies:**

Acute toxicity study was performed as per OECD guidelines 423. The animals were

randomly divided into 5 groups and were orally supplemented graded doses (200, 400, 800, 1600 or 3200gm per kg body weight) of methanol extract and were observed for behavioral changes and mortality till 72 hour and LD50 was calculated.

**Oral Glucose tolerance test (OGTT)**

Rats were fasted overnight and divided into five groups with 6 animals in each group. Group-I received distilled water, to serve as control. Group-II animals were treated with Glimperid (1 mg / kg) to serve as standard. Group-III animals were treated with *Rosa damascenamill* extract (500mg/kg, B.wt), The groups control, standard and test were treated with drugs 30 minutes prior to the glucose load (2.5 g/kg). Blood samples were collected at 15, 30, 45, 60, 75, 90 and 120 min after glucose loading. Serum was separated and glucose levels were measured immediately (Li et al., 2005).

**Anti diabetic study:**

In the present study, diabetes was induced by single intra-peritoneal injection of alloxan (125mg/kg) (Katsumata *et al.*, 1992)). The alloxan was freshly prepared by dissolving 125mg of alloxan in 1ml of normal saline solution. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. 72 hours after injection of alloxan, fasting plasma blood glucose was estimated. Animals with plasma glucose of > 140mg/dl were included in groups II-V. The rats were divided into five groups consisting of five rats in each group; the animals were treated for 28 days.

**Treatment schedule for antidiabetic activity**

Group No.	Treatment	Purpose
I	No treatment	To serve as normal control
II	Alloxan + Distilled water (125mg/kg i.p)	To serve as disease control
III	Glimperide (1mg/kg.)	To serve as standard
IV	Ethanollic extract of <i>Rosa damascenamill</i> (200mg/kg,)	To study the antidiabetic effect of <i>Rosa damascenamill</i>
V	<i>Ethanollic extract of Rosa damascenemill s</i> (400mg/kg,)	To study the Antidiabetic effect of <i>Rosa damascenamill</i>

**Collection of blood sample**

The blood samples were drawn on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube after a fast of 12 hrs and the blood was centrifuged (2,500 rpm/10min) to get serum. The serum was used for biochemical estimation of blood glucose, triglycerides, cholesterol, HDL-cholesterol and before collection of blood sample that day we carried out the estimation of analgesic activity by using Eddy’s hotplate method and Tail immersion method for diabetic neuropathy.

**Biochemical Estimations**

Serum analytical methods

- Estimation of serum glucose
- Estimation of triglyceride(TG)
- Estimation of total cholesterol(TC)
- HDL estimation
- Estimation of LOW density lipoprotein (LDL) and Estimation of body weight

**Eddy’s hot plate method**

Animals were placed on a hot plate maintained at a temperature of 55±0.5°C. The

latency to flick the hind paw or lick or jump from the hot plate was the reaction time. The reaction time was noted.

**Tail immersion method**

About 5 cm of the tail of each of the rats was dipped into a water bath containing warm water maintained at the temperature of 55 ± 0.5°C and the period of tolerance to the pain (PRT), i.e the time taken for the rat to flick its tail was recorded for all the rats.

**Statistical analysis**

The results were expressed as mean ± SEM. Statistical analysis was performed by One-way analysis of variance (ANOVA) test for multiple comparisons followed by Turkey-Kramer test. Statistical significance was set accordingly.

**RESULTS**

**Phytochemical Screening**

The preliminary phytochemical studies RDE received that presence of Phenols, Flavanoids, Steroids, Glycosides, terpinoids and absence of alkaloids as shown table

Name of Component	Result
Test for phenols	+
Flavanoids	+
Saponins	+
Steroids	+
Glycosides	+
Terpinoids	+
Alkalods	-

**Acute toxicity studies**

The pet ether, chloroform, ethyl acetate, and aqueous fractions of ethanol extract of *Rosa damascena* (RDE) did not show any mortality and toxic manifestations up to the dose of 3200 mg/kg. b.w. Further dosing was not performed to estimate the LD<sub>50</sub> (lethal dose) value. According to the OECD guidelines for the acute toxicity, an LD<sub>50</sub> dose of 2000 mg/kg and above is categorized as unclassified and hence the drug is found to be safe. Based on the acute toxicity studies, the dose 200 mg/kg of the fractions has been selected as the therapeutic dose. In animals treated with alloxan (G-II) (125 mg/kg i.p) a significant increase in the serum glucose, Triglyceride, Cholesterol, LDL levels and decreased HDL was observed on the 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day, when compared to the normal group (G-I), Group-III treated with standard drug

(Glimipiride drug(10 mg/kg)) showed a significant decrease in serum glucose, Triglyceride, Cholesterol, LDL and increased HDL levels on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day, when compared to the diabetic control group (G-II). On administration of *Rosa damascena mill L* .extract groups (G-IV and G-V), the blood glucose Triglyceride, Cholesterol, LDL levels were decreased and increased HDL levels on 7<sup>th</sup>, and 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day, when compared to the control group (G-II) . Administration of *Rosa damascena mill L* groups ( G-V) have shown a more significant effect on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day, when compared to group (G-IV). The rats induced with alloxan (G-II) a significant decrease in body weight was observed on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day, when compared to the normal group(G-I). Group-III, receiving standard drug

(Glimiperide drug(1 mg/kg) , G-IV and G-V showed a significant increase in body weight on 14<sup>th</sup> ,21<sup>st</sup> and 28<sup>th</sup> day , when compared to the control group (G-II).

#### Eddy's hot plate method and tail immersion method

The rats induced with alloxan (G-II) a significant decrease in latency time was observed on 7<sup>th</sup>, 14<sup>th</sup> ,21<sup>st</sup> and 28<sup>th</sup> day, when compared to the normal group(G-I). Group-III, Group IV and Group-v showed a significant increase in latency time on 21<sup>st</sup>, 28<sup>th</sup> and 35<sup>th</sup> day, when compared to the control group (G-II). Administration of *Rosa damascena mill L* groups ( G-V) have shown a more significant increase in latency time on ,21<sup>st</sup> 28<sup>th</sup> and 35<sup>th</sup> day, when compared to group (G-IV).

#### DISCUSSION

Diabetic neuropathy is a painful complication involving progressive neuronal damage and dysfunction. It affects the sensory nerves, the autonomic nervous system and even the central nervous system (CNS).

Alloxan is a cyclic urea compound, which induces permanent diabetes. It is a highly reactive molecule, which produces free radical damage to beta islet cells & causes cell death. When islets are exposed in vitro to alloxan, it exhibits exceptional beta cell specificity, the other islets cells remaining largely unaffected by both its inhibitory and cytotoxic effects. Alloxan is a specific toxic substance that destroys the  $\beta$  cells provoking a state of primary deficiency of insulin without affecting other islet types (Dunn et al., 1943; Goldener et al., 1964). The damage occurs in nerves; hence, alloxan was selected to induce diabetes in the present study. Almost all the flavonoids having potential for antidiabetic activity but they are limited in usage on account of deprived solubility and bioavailability<sup>21, 22, 24</sup>.

In the present study, preliminary phytochemical screening of extracts showed the presence of flavonoids, steroids, terpenoids and alkaloids. Antidiabetic activity and protect against their neuropathy of extracts may be due to its high content of flavonoids and steroids (Feng et al., 1998) Flavanoids usually reduction of aldose reductase, regeneration of pancreatic cells to enhance the insulin releases<sup>26</sup>. Literature survey revealed flavonoids and phenols are effective antihyperglycemic agents which can regenerate the damaged  $\beta$  cells in alloxan induced diabetic rats (Chakrabortiet al., 2003; Manickamet al., 1997) and produse analgesic action and also plant contain rich amount steroids. Steroids also showed analgesic activity, in the present study.

Similar mechanisms may be considered responsible for the hypoglycemic action and their protect against the neuropathy shown by of *Rosa damascena mill* in diabetic rats<sup>28, 29, 30</sup>.

#### CONCLUSION

From this study, we can state that the ethanolic extract of *Rosa damascena mill.L* has beneficial effects on blood glucose levels as well as improving the hypoglycemic action and their protect against the neuropathy. The diabetic neuropathy activity of *Rosa damascene mill.L* may be attributed to the active ingredients present in the drug. So *Rosa damascena mill.L* has shown significant increase in body weight, increase in grip strength and pain sensitivity. This indicates its protective role against damage to the neurons. Therefore, it can be concluded that *Rosa damascena mill.L* has significant anti-diabetic and neuroprotective effects in experimental animals.

#### ACKNOWLEDGEMENT

The authors are sincerely thankful to the management of C.E.S College of Pharmacy, Chinnatekur, Kurnool, and Andhra Pradesh, India for providing the facilities to carry out this research work.

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**How to cite this article:**

H.G Raghavendra, G.Lakshmikanth, N.Ravinaik, Y.Samatha, **Evaluation of protective effects of *rosa damascena mill.l* against alloxan induced diabetic neuropathy in rats**, 6 (3): 2798 – 2804 (2015)

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