

## ANTIDIABETIC EFFICACY OF ETHANOLIC LEAF EXTRACT OF *NYMPHAEA ODORATA* IN ALLOXAN INDUCED DIABETIC MICE

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### ABSTRACT

The antidiabetic efficacy of ethanolic leaf extract of *Nymphaea odorata* was evaluated in alloxan induced Diabetic mice. In the present study, the animals were divided five groups as control, diabetes induced, glibenclamide treated and with *Nymphaea odorata* leaf extract of 300 and 600 mg/kg/b wt/day administered respectively. Effect of oral administration of plant extract of 300 and 600 mg/kg/b wt/day for 45 days on the level of body weight, blood glucose and activity of glucokinase, glucose-6-phosphatase, Fructose-6-phosphatase, and hexokinase were evaluated. When comparing with the values of the plant extract treated group with those of the control diabetic group the results indicated that the plant extract treated mice shown regain of body weight, significant decrease in the elevated blood glucose level and there is decrease in the activity of Glucose-6-phosphatase, fructose-6-phosphatase and increase in the activity of glucokinase and hexokinase. These results shown that *N.odorata* leaf extract possesses promising antidiabetic effect in alloxan induced diabetic mice.

**Keywords:** Antidiabetic, Alloxan, *Nymphaea odorata*, Blood glucose, Glycolytic enzymes

### INTRODUCTION

Diabetes mellitus is the failure of metabolic disorder characterized by altered carbohydrate, proteins and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action<sup>1</sup>. The World Health Organization (WHO) estimates that more than 220 million people worldwide have diabetes and this number is likely to more than double by 2030<sup>2</sup>. The occurrence and consequences associated with diabetes are found to be high in countries like India (31.7%), China (20.8%) and USA (17.7%). The rate is expected to rise to 79.4%, 42.3% and 30.3%, respectively, by 2030 in the above countries<sup>3</sup>. *Nymphaea odorata* (water-lily) is known by common names such as fragrant water-lily, American white-lily and Alligator Bonnet amongst many others<sup>4</sup>. Compounds such as tannins (tannic acids and gallic acids, anti-microbial), alkaloids (nymphaerine and nupharine) and glycosides (cardenolide and myricitrin) which are antiseptic, astringent and demulcent have been reportedly isolated from this plant<sup>5</sup>. The present study was necessary to carry out the antidiabetic efficacy of ethanolic leaf extract of *Nymphaea odorata*.

### MATERIALS AND METHODS

#### Preparation of plant extract

The fresh leaves of the *N. odorata* were shade dried for 6 days and reduced to powder by using dry grinder. This powder was packed into Soxhlet apparatus and extracted using absolute ethanol (40-50 °C). The extraction was carried out for 38 hrs till the total extraction was achieved. The extract obtained was dried at 45 °C in hot air oven till semisolid mass was obtained. The yield obtained was (4.5% w/w) and the extract was stored in a refrigerator at 4 °C until used.

#### Chemical

Alloxan monohydrate was purchased from Sigma-Aldrich, St.Louis, USA. All other reagents used were of analytical grade.

#### Animals

Laboratory bred 3 to 4 months adult virgin male albino mice weighing about 20 to 30 g are used under standard animal housing condition (temperature controlled 25 ± 2°C facility and maintained with 12 hrs light/dark cycle) with unlimited access to pellet diet "Gold Mohar" (Hindustan Lever Ltd., Mumbai) and water *ad libitum* throughout the study in the animal house, P.G. Department of Studies in Zoology, Karnatak University, Dharwad. Animals were randomly divided into control and four treatment groups (Distilled water vehicle is served as control). Each group consists of 10 mice housed in separate polypropylene cages containing sterile paddy husk as bedding material.

#### Induction of diabetes

Diabetes was induced in male Swiss Albino mice by intraperitoneal administration of alloxan monohydrate (Sigma-Aldrich Co., USA) in concentration of 150 mg/kg body weight dissolved in normal saline. Blood glucose was measured after 72 hour of alloxanisation by gluco-card-01 mini glucometer (Accu-check sensor) of ARKAY Factory, Inc. Japan, since alloxan is capable of producing fetal hypoglycemia as a result of massive pancreatic insulin release, mice were treated with 30 percent glucose solution orally at different time intervals after six hours of alloxan induction, and 5 percent glucose solution was kept in bottles in their cages for next 24 h to prevent hypoglycemia. Mice showing fasting blood glucose levels (>250 mg/dl) were selected for the study.

#### Treatment

Animals were divided into five groups of ten mice each. Standard pellet diet and water was provided to the animals. Normal untreated mice given only vehicle (distilled water). Diabetic control mice given a single dose intraperitoneal injection of 150/mg/kg alloxan. Diabetic mice were given a single dose of glibenclamide (500 mg/kg b.wt) 1 ml with vehicle by oral administration daily for 45 days. Diabetic mice were given a single dose of ethanolic leaf extract of *Nymphaea odorata* leaves (300 mg /kg b.wt and 600mg/kg b.wt ) 1 ml with vehicle by oral administration daily for 45 days. All the experimental animals were sacrificed by cervical dislocation on the 46<sup>th</sup> day of termination. The blood was collected by tail prick and liver was dissected out and weighed to the near milligrams in digital weighing balance (vibra) were used for the Blood glucose level and glycolytic enzyme estimations.

#### Blood Glucose level and glycolytic enzymes

The Blood Glucose level by<sup>6</sup> glycolytic enzymes such as glucokinase<sup>7</sup>, Glucose-6-phosphatase<sup>8</sup>, Fructose-1-6-diphosphatase<sup>9</sup> Hexokinase<sup>10</sup> were estimated.

#### Statistical Analysis

Statistical significance between the control and experimental data were subjected to analysis of variance (ANOVA) together with Dunnett's test (P<0.05).

### RESULTS

#### Body weight

The results indicate that there was a significant decrease in the body weight of alloxan treated mice when compared with that of the normal control (Table 1). However, there was a significant increase

in the body weight of the mice treated with alloxan along with glibenclamide and plant extract of higher dose when compared with that of the alloxan treated control. Further, the results indicate that there was a recovery in the body weight of mice treated with alloxan along with glibenclamide and plant extract when compared with that of the alloxan treated control.

#### Organ weight

The results indicate that there was a significant decrease in the weight of the liver in the mice treated with alloxan when compared with that of the normal control (Table 2). However, there was a significant increase in the weight of the liver in the mice treated with alloxan along with glibenclamide and plant extract when compared with that of alloxan treated control. Further, the results indicate that there was a recovery in the weight of liver in the mice treated with

alloxan along with glibenclamide and plant extract when compared with that of alloxan treated control mice.

#### Blood Glucose level and glycolytic enzymes

The results indicate that administration of alloxan led to significant elevation in glucose level when compared with that of the normal control mice (Table 3). Further the plant extract leads to significant reduction in blood glucose level when compared to alloxan treated control group. The hyperglycemic activity of the plant extract was persistent throughout the period almost in comparable to that of the reference drug glibenclamide and there is decrease in the activity of Glucose-6-phosphatase, fructose-6-phosphatase and increase in the activity of glucokinase and hexokinase in plant extract treated mice. It depicts the antihyperglycemic potential of ethanolic leaf extract of *N. odorata*.

**Table 1: Effect of ethanolic leaf extract of *N. odorata* on body and organs weight in diabetic albino mice**

Groups	Treatment (mg/kg/d)	Change in body weight (g)	Relative organ weight /100 g body weight (Mean ± S.E) (g) liver
I	Normal Control	2.67±0.33	6.69±0.22
II	Alloxan Control (150)	-1.67±0.11*	5.61±0.21*
III	Alloxan Glibenclamide (500)	2.78±0.08*	6.59±0.31*
IV	Alloxan+N. odorata (300)	1.60 ±0.14	5.64±.0.28*
V	Alloxan+ <i>N. odorata</i> (600)	2.50±0.12*	6.48±0.21*

Values are mean± SEM of 10 animals \* Significant P ≤ 0.05 vs. Control

**Table 2: Effect of ethanolic leaf extract of *N. odorata* on blood glucose in diabetic albino mice**

Groups	Treatment (mg/kg/d)	Blood glucose level (mg/dl)	
		Initial (1 day)	Final (45days)
I	Normal Control	132.27±2.45	134.03±5.53
II	Alloxan Control (150)	362.26±3.56	254.80±5.46*
III	Alloxan+Glibenclamide (500)	280.28±3.38	162.50±6.42*
IV	Alloxan+N. odorata (300)	258.33±4.48	150.24±4.38*
V	Alloxan+ <i>N. odorata</i> (600)	244.31±4.52	148.45±5.58*

Values are mean ± SEM of 10 animal \* Significant P ≤ 0.05 vs. Control

**Table 3: Effect of ethanolic leaf extract of *N.odorata* on glycolytic enzymes in diabetic albino mice**

Groups	Treatment (mg/kg/d)	Glycolytic Enzymes			
		Glucokinase <sup>a</sup>	Glucose-6-phosphatase <sup>b</sup>	Fructose-6-di-phosphatase <sup>c</sup>	Hexokinase <sup>d</sup>
I	Normal Control	128.52±4.38	0.168±0.01	0.242±0.02	402.67±6.62
II	Alloxan Control (150)	99.01±6.9*	0.278±0.02*	0.380±0.03*	88.36±5.12*
III	Alloxan+Glibenclamide (500)	126.48±6.38*	0.159±0.01*	0.248±0.01*	122.13±8.17*
IV	Alloxan+N. odorata (300)	102.3±5.08*	0.230±0.01*	0.266±0.04*	68.42±6.18*
V	Alloxan+ <i>N. odorata</i> (600)	125.48±7.18*	0.188±0.02*	0.272±0.03*	72.33±6.17*

a moles of glucose phosphorylated/g/h. b moles of pi liberated/min/mg.

c moles of pi liberated/min/mg. d nmoles of glucose -6- phosphate formed /min

Values are mean ± SEM of 10 animals \* Significant P ≤ 0.05 vs. Control.

#### DISCUSSION

In the present study the body weight of the control mice were found to be increased when compared with that of the initial body weight. The results of the present study on body weight indicate that alloxan treatment leads to significant decrease in body weight<sup>11</sup>.

This was recovered by treatment of ethanolic leaf extract of *Nymphaea odorata* for 45 days. These results are almost in similar with standard drug glibenclamide. The loss of body weight was expected in diabetic mice as the diabetes affects the protein synthesis in the body<sup>12, 7</sup>. Alloxan has been reported to cause a massive reduction of β-cells of the islets of langerhans and induce hyperglycemia<sup>13</sup>. Therefore, the findings of the present study indicate that the mice treated with plant extract showed revitalization in the body weight might be due to protein synthesis

in the body<sup>7</sup>. This ability to protect the body weight loss could be due to plant extracts antidiabetic activity.

Diabetes mellitus is a group of syndrome characterized by hyperglycemia and altered metabolism of carbohydrates, lipids and proteins. In the present study there was a hyperglycemia in alloxan treated mice showing Diabetes mellitus might be due to glycogenolysis or gluconeogenesis.

In animals, it can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan, streptozotocin, and anti-insulin serum. Alloxan causes massive destruction of the β cells of islets of Langerhans. The hyperglycemia and diabetes were imputed to the selective destruction of pancreatic β cells that secrete insulin and <sup>14, 15</sup>. Abnormalities in the regulation of peroxide and transition metal metabolism are postulated to result in the

development of the disease as well as its long term complication<sup>16</sup>. Similar reports of reduction in blood glucose also reported such as *Artemisia herba*<sup>17</sup> in alloxan induced Diabetes mellitus. Similar results have been reported on the hypoglycemic activity of plant extract *Terminlia catappa*<sup>18-21</sup>.

The antidiabetic effect of *N. odorata* extract could be linked to more than one mechanism. The possible mechanism includes the stimulation of  $\beta$  cells and subsequent release of insulin and activation of the insulin receptors. The plants antihyperglycemic action may be by stimulation of pancreatic secretion of insulin. In this context a number of other plants have also been reported to have antihyperglycemic and insulin release stimulatory effect<sup>22-25</sup>. Hence, in the present study the *N odorata* extract also may act as a hepatoprotective agent so this evidently, improves the function of liver and maintains glucose uptake, enhanced transport of blood glucose to peripheral tissue and utilization, which may be another mechanism of action.

Although *N odorata* plant has been used as a traditional plant treatment in ayurveda medicine in India and North American continent detailed scientific investigation on efficiency and mechanism of its action are yet to be done. The present study indicates the scientific basis for its use. As we know Diabetes mellitus is a metabolic disorder characterized by insufficient insulin secretion and efforts have continued to seek for insulin like molecules from synthetic or plant sources for treatment of diabetes and reported to have hypoglycemic effect. The *N. odorata* (water lily) is known by common names such as fragrant water lily, alligator bonnet among others<sup>4</sup>. It belongs to family; *Nymphaeaceae* which consisting of six genera and seventy species<sup>5</sup>. The leaves and roots are used to treat coughs, tuberculosis, remove pimples from face and skin<sup>26</sup>. In the present study ethanolic extract of *Nymphaea odorata* is used which exhibited antihyperglycemic activity at a dose level of 300 mg and 600 mg/kg treatment for 45 days. It is deep-rooted that after glucose loading in glucose tolerance test, along with blood glucose, blood insulin level is also increases. Insulin brings down blood glucose in 45 days by different mechanisms. The present results with extract and glibenclamide are in support of this. In the case of the aqueous extract and drug treated groups, the glucose level was not increased like the alloxan induced diabetic control group, giving an indication regarding the supportive action of the extracts<sup>27</sup>, fraction and drug in the glucose utilization. Glibenclamide is reported to enhance the activity of  $\beta$  cells of the pancreas resulting in secretion of larger amount of insulin which in turn bring down glucose level.

Liver functions as a monitor and plays a principle role in the maintenance of blood glucose level and hence, it is of interest to examine the possible role of *N. odorata*. Ethanolic leaf extract on principle enzymes of carbohydrate metabolism in liver. Liver is the organ involved in glucose homeostasis. It is the main site for glycolysis a process where glucose is degraded and gluconeogenesis, where glucose is synthesized from lactate, amino acids and glycerol. These are the two important complementary events that balance the glucose load in our body<sup>28</sup>. Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes. Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver<sup>29</sup>. Increase in liver glycogen can be brought about by an increase in glycogenesis and or decrease in glycogenolysis<sup>30</sup>. Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increase hepatic glycolysis by increasing the activity and amount of several keys enzymes including glucokinase and phosphofructokinase. Hexokinase catalyses the conversion of glucose to glucose-6-phosphate and plays a central role in the maintenance of glucose homeostasis. In the liver, this above enzyme is an important regulator of glucose storage and disposal<sup>30</sup>. Hexokinase is the main enzyme catalyzing glucose phosphorylation. Impairment of hexokinase activity suggests the impaired oxidation of glucose via glycolysis leading to its accumulation resulting in hyperglycemia.

In the present study the hexokinase and glucokinase activity in the liver was found to be decreased in alloxan induced diabetic mice

which might be due to insulin deficiency, insulin stimulates the activities of hexokinase. The deficiency of insulin in mice treated with alloxan might be due to damage of  $\beta$  cells of pancreases or reported to inhibit the activity of glucokinase directly. Treatment with *N odorata* ethanolic leaf extract elevated the activity of hexokinase in the liver. *N odorata* extract may stimulate insulin secretion which activates hexokinase, thereby increasing utilization of glucose leading to decreased blood sugar levels. It has been reported that the *N odorata* extract contains tannins (tannic acids and gallic acids), alkaloids (nymphaerine and nupharine), glycosides (cardenolide and myricitrin) which are antiseptic, astringent and demulcent have been reportedly isolated from this plant<sup>5</sup>. The antidiabetic effect of the ethanolic leaf extract might be due to action of any one or more of these compounds which stimulate the secretion of insulin leading to activation of glucokinase enzymes in the liver. Similar results are reported in rats treated with hydroethanolic extract of *Nymphaea stellata* flower, the activity of hexokinase and LDH was increased<sup>21</sup> it has been suggested that treatment with *Hemidesmus indicus* or *Lagerstroemia speciosa* L. extract increased the activity of hexokinase in the liver<sup>8</sup>.

Glucose-6-phosphatase and Fructose 1-6-diphosphatase are important regulatory enzymes in gluconeogenesis. Liver being the main organ responsible for maintaining the homeostasis of the blood glucose<sup>32</sup>. Insulin decrease gluconeogenesis by decreasing the activities of key enzymes such as glucose-6-phosphatase, fructose-1-6-diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase<sup>33</sup>. In the present study increased activities of glucose-6-phosphatase and fructose-6-diphosphatase were observed in the liver of alloxan induced diabetic mice. In *N. odorata* ethanolic leaf extract and glibenclamide drug treated mice, showed significant decrease in the two enzymes (glucose-6-phosphatase and fructose-6-diphosphatase) seen significantly reduced in liver. This might be due to increased insulin secretion which is responsible for the repression of the gluconeogenic principle enzymes.

The present observation provide evidence that ethanolic leaf extract of *N.odorata* exhibited antidiabetic or hypoglycemic activity on experimental diabetic mice might be due to enhancing the peripheral utilization of glucose by correcting the impaired liver or kidney glycolysis and by suppression of its gluconeogenic activity similar to that of insulin. This effect might be due to the presence of tannins (tannic acids and gallic acids), alkaloids (nymphaerine and nupharine) and glycosides (cardenolide and myricitrin) and other constituents present in the leaves which could act synergically or independently in enhancing the activity of glycolytic and gluconeogenic enzymes. However, Further, comprehensive chemical and pharmacological investigation should be carried out to isolate the active compound and appropriate elucidation of its mechanism.

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