



PHARMACOLOGICAL POTENTIAL OF *NYMPHAEA ALBA* FLOWER EXTRACT AGAINST STREPTOZOTOCIN INDUCED EXPERIMENTAL DIABETES MELLITUS MODEL

G. Karthikeyan*	Department of Pharmacology, Vinayaka Mission Kirupananda Variyar Medical College & Hospital, Salem-636 308, Tamil Nadu, India *Corresponding Author
S. Rajaram	Department of Pharmacology, Vinayaka Mission Kirupananda Variyar Medical College & Hospital, Salem-636 308, Tamil Nadu, India
S. Arulraja	Department of Pharmacology, Vivekananda dental college for women, Tiruchencode, Namakkal 637205, Tamil Nadu, India

ABSTRACT Diabetes mellitus is a rapidly increasing incidence in the developing and developed countries throughout the world. The present investigation was performed to assess the anti-diabetic activity of *Nymphaea alba* flower extract against streptozotocin (STZ) induced diabetic condition in rat model. *N. alba* flower extract was orally given to experimentally induced diabetic animals (at the concentration of 200, 400 and 800 mg/kg body weight). Treatment of *N. alba* flower extract significantly ($p < 0.05$) lowered the level of blood glucose, serum insulin and HbA1C levels in experimentally induced diabetic animals in a concentration dependent fashion. A significant ($p < 0.05$) reduction in the levels of antioxidant enzymes such as glutathione peroxidase, glutathione S transferase, catalase and superoxide dismutase were observed in experimental diabetic animal model. Upon treatment with *N. alba* flower extract the above said antioxidant enzyme levels were reverted back to normalcy in a dose dependent fashion. The present investigation revealed that *N. alba* flower extract exerted strongly antidiabetic action by restoring the antioxidant system and anti-hyperglycemic activity. Consequently, it might be used as a safer harmonizing substance in the diabetic conditions.

KEYWORDS : *Nymphaea alba*, streptozotocin, antioxidant enzymes, Serum insulin, HbA1C.

INTRODUCTION:

Diabetes mellitus is a prolonged metabolic syndrome with a fast growing incidence (Golbidi *et al.*, 2012). While physical inactivity and obesity are recognized to be chief risk factors for the progression of type 2 diabetes mellitus (T2DM), latest indication proposes that oxidative trauma may subsidize to the progression of T2DM through impairing insulin secretion or increasing insulin resistance (Montonen *et al.*, 2004). Medicinal plants and its components were deliberated as a substantial source of pharmaceutical products for keeping human wellbeing. From developed countries, about 65% to 80% of persons utilize traditional system of medicine, which has active mechanisms conquered from medicinal plants. The crude extracts use of medicinal parts and its phytochemicals, of known variety of properties such as anticancer, antimicrobial, etc., can be of remarkable importance in the treatments many human ailments. In contemporary existences, several investigations on medicinal plant extracts have been executed in numerous countries to validate such capability.

Nymphaea alba belongs to the family, Nymphaeaceae and almost all the species in its group of members are water plant, these leaves hang and sometimes it will immerse in aquatic environment (Abu-Zaida *et al.*, 2008) Phytochemical investigation of *N. alba* revealed that it has flavonoids, alkaloids, tannic acid, sterols, gallic acid, hydrolyzable tannins, glycosides as well as polyphenolic high molecular weight constituents. Whole portions of the *N. alba* have beneficial usages in native medicinal practices. It could be practiced as an antiscrophulatic, aphrodisiac, astringent, soothing, demulcent, cardiogenic, sedative as well as anti-inflammatory agent. Additional, it also having soothing and relaxing properties in the nervous structure, and is constructive in the action of anxiety, insomnia and related disorders. It has anticancer properties and has the ability to inhibit the oxidative stress in renal system. Though, so far, its consequence on antidiabetic action has not been examined. Consequently, the present investigation was designed to examine the prospective role of *N. alba* in chemically induced model of diabetes to evaluate the mechanism of action of the plant on their probing activities.

Materials and methods:

Chemicals and reagents:

Insulin kits, dimethyl sulfoxide (DMSO) and streptozotocin and were obtained from Sigma, USA. All the chemicals and reagents used in the experiments were analytical grade.

Preparation of the *N. alba* flower ethanolic extracts:

The *Nymphaea alba* flowers were collected and washed with distilled water followed by air-dry at the 4°C temperature for two days in the

shade condition and then it was coarsely powdered. Four grams of *N. alba* flowers were homogenized and extracted with ten volumes (v/v) of 70% (v/v) ethanol. The supernatant of the sample was collected through centrifugation at 12,000g for 5 min at 4°C and it was stored at -20 °C. The supernatant portion of the *Nymphaea alba* flowers extract was then filtered through whatman filter paper. The filtrate was collected instantaneously, concentrated and lyophilized and used for this investigation.

Experimental animals

The experimental rats used in this investigations were male Wistar albino strain weighing 150-250 grams. The animals were procured and maintained at central animal house facility, Vinayaka Mission Research Foundations (Deemed to be University) Salem. Animals were divided into several groups and maintained at well ventilated conditions. Animals were given standard food and water *ad libitum*. Ethical clearance letter number: IAEC/VMKVMC/03/2017

Acute oral toxicity study

Acute toxicity studies were done as per the OECD (2001) guidelines. Healthy male Wistar albino rats, overnight fasted, were separated into several group per dose consists of six animals in each group. The acute toxicity tested group were administered aqueous extract of *N. alba* flower in suspension at the dose of 5, 50, 300 and 2000 mg/kg/kg body weight. All above mentioned doses were given through gastric gavage. The post treated animals were perceived post dose at every one hour up to six hours, and thereafter every 24h for 14 days.

Induction of type 2 diabetes

Food with high fat content with the estimated energy of 5 kcal per g, comprising 35% protein, 5% carbohydrate and 60% calories from fat was given to the experimental animals (Reed *et al.*, 2000). Followed by the intraperitoneally administration of single dose of streptozotocin (30 mg/kg dissolved in 0.1 mol/L of citrate buffer with pH 4.5). The fasting blood glucose was should be more than 300 mg/100 ml later three days of streptozotocin administration, the type 2 diabetic model was established in streptozotocin induced rats successfully (Ebaid, 2014).

Biochemical parameters:

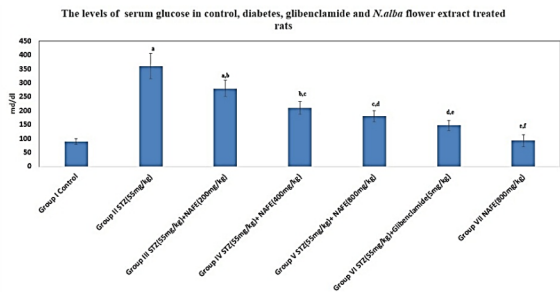
Serum glucose was estimated by Trinder (1969) method, serum insulin levels was assessed by the method of Herbert *et al.*, (1965), liver glutathione-S-transferase (GST) was estimated by the method of Habig *et al.*, (1974), quantification of liver glutathione peroxidase (GPx) was done by the method of Paglia and Valentine, (1967), liver superoxide dismutase (SOD) (Nishikimi *et al.*, 1972), and liver catalase (CAT) (Aebi, 1984).

RESULTS:

In figure 1, the level of blood glucose was presented. In the diabetic animals, the glucose levels were elevated. The treatment with *N.alba* flower extract interfere considerably with the glycemic condition in streptozotocin induced diabetic animals matched with the control group. All streptozotocin induced diabetic rats showed hyperglycemia four to five days of the streptozotocin administration, when related with the non-diabetic control and *N.alba* flower extract alone treated group of animals. The administration of *N.alba* flower extract (at the concentration of 200, 400 and 800 mg/kg body weight) considerably decrease the levels of fasting blood glucose compared with the diabetic control group in a dose dependent fashion ($p < 0.05$). Figure 2 reveals the levels of serum insulin in control and experimental animals. A decrease of the serum insulin levels of streptozotocin induced diabetic animals was revealed compared to control rats. Though, *N.alba* treated rats showed significant modification with the concentration insulin in streptozotocin treated group of animals in a dose dependent manner. On the other hand, a no significant changes were observed with respect to the levels of serum insulin when compared with *N.alba* alone treated animals with control animals.

Figure 3 displays the HbA1C levels in control, diabetic, glibenclamide treated experimental group of animals. Streptozotocin administered animals exhibited statistical significant weight loss matched with control animals the completion of the experimental period ($P < 0.05$). Glucose concentration in plasma as well as the levels of HbA1C in diabetic group animals were considerably lower ($P < 0.05$). Upon administration of *N.alba* extract the levels were reverted back near normalcy based on the dose dependent fashion. During poorly controlled or uncontrolled diabetes condition, glycated hemoglobin (HbA1C) was found to increase and it is straight proportional to the hyperglycemic state. Evidence showed that oxygen-derived free radicals induced by the condition of glycation and subsequently it leads to diabetic condition, therefore the HbA1C level is deliberated as one of the biomarkers for diabetes mellitus with respect to the degree of oxidative stress. Consequently, the estimation of HbA1C is thought to be a precise sensitive key for glycemic index. In the current study, streptozotocin induced diabetic rats exhibited elevated amount of HbA1C, matched with control group of animals. Treatment with *N.alba* reduced levels of HbA1C significantly ($P < 0.05$).

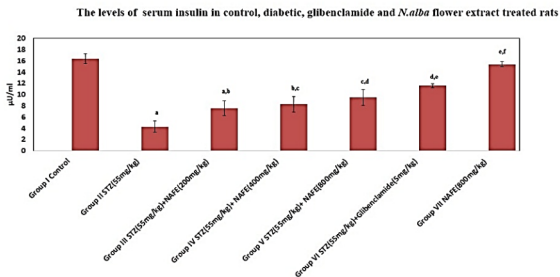
Figure 1.



Each bar expressed as mean + SD for six animals in each group a - Group I Vs Group II, III and IV; b - Group II Vs Group III and IV; c - Group III Vs Group IV d - Group IV Vs Group V; e - Group V Vs Group VI; F - Group VI Vs Group VII;

The significance at the level of $p < 0.05$

Figure 2.

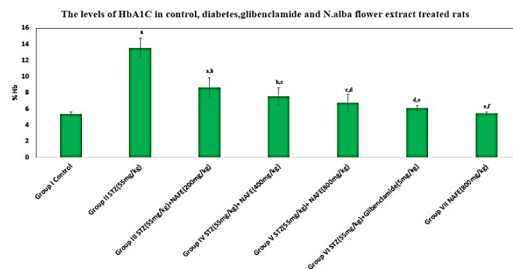


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The significance at the level of $p < 0.05$

Figure 3.



	GST (U/Gram Protein)	Glutathione peroxidase (U/Gram tissue)	CAT (U/Gram Protein)
Group I Control	3.52±0.5	6.2±0.2	2.80±0.4
Group II STZ(55mg/kg)	0.58±0.2 ^a	3.8±0.7 ^a	1.41±0.2 ^a
Group III STZ(55mg/kg)+NAFE(200 mg/kg)	0.87±0.1 ^b	4.6±0.3 ^b	1.63±0.2 ^b
Group IV STZ(55mg/kg)+NAFE(400mg/kg)	0.94±0.1 ^c	4.9±0.5 ^c	2.2±0.4 ^c
Group V STZ(55mg/kg)+NAFE(800mg/kg)	1.56±0.4 ^d	5.2±0.6 ^d	2.42±0.2 ^a
Group VI STZ(55mg/kg)+Glibenclamide(5mg/kg)	2.75±0.7 ^e	5.6±0.2 ^e	2.74±0.4 ^e
Group VII NAFE(800mg/kg)	3.74±0.8 ^f	5.9±0.3 ^f	2.86±0.3 ^f

Each bar expressed as mean + SD for six animals in each group a - Group I Vs Group II, III and IV; b - Group II Vs Group III and IV; c - Group III Vs Group IV d - Group IV Vs Group V; e - Group V Vs Group VI; F - Group VI Vs Group VII; The significance at the level of $p < 0.05$

Table 1 displays the antioxidant enzyme levels in control, diabetic, glibenclamide treated experimental group of animals. Streptozotocin administered animals exhibited statistical significant decrease with control animals the completion of the experimental period ($P < 0.05$). Upon administration of *N.alba* extract the levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and reduced glutathione were reverted back near normal in a dose dependent manner. In all the parameters there were no statistical significant changes were observed in group 6 and 7 when compared with control group.

DISCUSSION:

Cells have developed various enzymic and non enzymic antioxidant systems, could act in synergistic manner from which it might safeguard the body system from the damages induced by free radicals. During the progression of diabetes mellitus, numerous antioxidant systems were lowered which include precise antioxidants such as vitamin C and E in serum/plasma levels. In addition to that increased plasma oxidisability was observed in type 2 diabetes mellitus condition. Furthermore, other antioxidant systems for instances superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) have been reported as decreased in the diabetic condition. A reduction in the endothelial production of nitric oxide (NO) has also been revealed in type 2 diabetics mellitus, which apart from doubtful from vascular antioxidant protection, may contribute for the deficiency in the anti-atherogenic signaling part of N (Arulselvan and Subramanian,2007)

The cellular system can be protected from the toxic consequences of lipid peroxidation through glutathione. The reduced glutathione level was reported in diabetes complaint (Baynes *et al*, 1999). During the free radical induced cellular damage reduced glutathione doing the

free radical scavenging activity and the reduced GSH content could modify the effect of antioxidant systems. Due to the decreased level of GSH content, the activity of glutathione-S-transferase (GST) was decreased since it acts as a substrate for the action of GST (Rathore *et al*, 2000). The reduction of GSH content in tissues characterizes rises in the usage owing to oxidative stress caused by streptozotocin. In the current investigation, a substantial reduction in the level of GSH in tissues of streptozotocin induced diabetic animals. *N.alba* flower extract treated streptozotocin induced diabetic animals showed elevated levels of GSH in rat tissues. From the results it could be concluded that the *N.alba* may upsurge the biosynthesis of glutathione or decrease the oxidative damage or together.

There are two main scavenging enzymes, superoxide dismutase (SOD) and catalase (CAT) are Play a major role in the removal of the toxic substances generated through free radicals induced by streptozotocin. The CAT and SOD activities were statistically reduced in diabetic conditions. The enzyme superoxide dismutase alters the superoxide radicals into hydrogen peroxide (H₂O₂) as well as molecular oxygen. Catalase enzyme safeguard the tissues from extremely reactive hydroxyl radical through catalyzing the decrease of hydrogen peroxides. The reduction of CAT and SOD actions might consequence from the inactivation by glycation of the enzyme (S^ozmen *et al*,2001). *N.alba* flower extract treated streptozotocin induced diabetic animals exhibited proliferation in the actions of CAT and SOD to near-normal levels. These outcomes exposed that *N.alba* flower extract may encompass a free radical trapping action and preclude cellular damages caused by action of free radicals. Alongside with glutathione, the glutathione peroxidase catalyzed the decrease of hydrogen peroxide into nontoxic metabolic substances. In diabetes condition, there is a reduced in the levels of GSH that reduced the actions of GPx (Sayed *et al*, 2012). Glutathione, a co-substrate for GPx and tripeptide purposes as a trapper of free radicals generation. NADPH essential for GSH formation was utilized by the polyol pathway which is conspicuous in chronic hyperglycemic circumstances, there occurs a reduction of GSH subsequent in dropped GPx action (Lorenzi, 2007). Numerous investigations exhibited that antioxidants from natural sources have been proposed to have valuable properties in the action against oxidative stress associated disease (Arulselvan and Subramanian, 2007; Prathapan, *et al*, 2012). In conclusion, these findings revealed that *N.alba* flower extract evidently abridged hyperglycemia condition and related oxidative problems in streptozotocin induced diabetic animals, reduced glucose level, and augmented antioxidants markers including glutathione peroxidase (GPx), glutathione S transferase (GST), catalase (CAT) and superoxide dismutase (SOD).

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