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Anti-antioxidant activity of alcoholic extract of *Nelumbo nucifera* on streptozotozin induced diabetic albino wistar rats

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Abstract

The present study is to evaluate the *in-vivo* anti oxidant effect of hydro alcohol (50% aqueous ethanolic extract) extract of *Nelumbo nucifera* (G) flowers on streptozotocin-induced diabetes. Animals were induced for diabetes with streptozotocin (60 mg/kg of body weight, *i.p.*) and treated orally with hydro-alcoholic (50% aqueous ethanolic extract) extract of *Nelumbo nucifera* (G.) flowers in various doses (200 mg/kg and 400 mg/kg *p.o*). The effect of *Nelumbo nucifera* (G) flowers on diabetes was assessed from plasma glucose. At the end of the study, vital organs were dissected and estimated the parameters such as LPO, GSH, GPx and peroxidase. From the data obtained in this study it could be concluded that hydro alcoholic extract of *Nelumbo nucifera (G)* flowers seems to have potential value for the treatment of diabetic due to its Anti oxidant activity.

Key Words: Anti diabetic, Anti-oxidants, *Nelumbo nucifera*

Introduction

Chronic elevation of plasma glucose causes many complications in Diabetes mellitus. People with diabetes mellitus may develop characteristic micro vascular complications such as retinopathy, nephropathy and neuropathy (Ajit kar *et al.,* 1999). There is also an increased risk of macro vascular complications such as cardio vasculopathy, cerebro vasculopathy and peripheral vasculopathy. The long-term complications of diabetes, such as micro and macro vascular disease can be delayed or prevented with appropriate intervention, including lifestyle changes (David *et al*., 1986). Progressive damage to the eyes, kidney, nerves and large vessels possess a major threat to health and life of diabetic patients. Therefore, the prevention of complications due to the chronic hyperglycemia should be undertaken in order to alleviate diabetes vascular complications. Micro vascular complications of diabetes share a common pathophysiology that may be explained as a direct or indirect consequence of hyperglycemia-mediated overproduction of Reactive Oxygen Species (ROS) (Baynes, 1991). Micro vascular deterioration is preventable either by the inhibition of superoxide accumulation or by modulating the blood glucose levels, and among several micro vascular disorders. Enhanced glycosylation by elevated glucose concentration may induce the formation of oxygen-derived free radicals through protein glycosylation, which releases early and late glycosylation products, contributing to enhancement of oxidative stress (Konukoglu *et al*., 2002; Beisswenger *et al.,*1993)). In India, indigenous remedies have been used in the treatment of DM since the time of Charaka and Sushruta (6th century BC) (Grover & Vats, 2001). The ethno-botanical information reports about 800 plants that possess anti-diabetic potential (Alarcon-Aguilara *et al*., 1998).

N. nucifera Gaertn, (sacred lotus) is a large aquatic herb with stout, creeping rhizome. Almost all parts of the lotus plant are eaten as vegetable and also used in the indigenous system of medicine (Anonymous *et al*., 1992).

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They are used as astringent, cardiotonic, febrifuge, Hypotensive, resolvent, stomachic, styptic, tonic and vasodilator, immunomodulatory (Kuo *et al*., 2000).

The leaf extracts were used in the treatment of diarrhoea and is decocted with liquorice (*Glycyrrhiza* spp.) for the treatment of sunstroke. In particular, the leaves are known for diuretic and astringent properties, and are used to treat fever, sweating and strangury and as a styptic (Kuo *et al*., 2000). A decoction of the flowers is used in the treatment of premature ejaculation. The flowers are recommended as a cardiac tonic (Chopra *et al*., 1986). A decoction of the floral receptacle is used in the treatment of abdominal cramps, bloody discharges etc. It is used in treating bleeding gastric ulcers, excessive menstruation, post-partum haemorrhage (Brown *et al*., 1995). The stamens are used in treating urinary frequency, premature ejaculation, haemolysis, epistasis and uterine bleeding (Brown *et al*., 1995). The rhizome is considered nutritive, demulcent, diuretic and cholagogue and is used to treat piles, dyspepsia and diarrhoea (Kirtikar *et al*., 1975). A decoction of the fruit is used in the treatment of agitation, fever, heart complaints etc.

Materials and methods

Animals and Experimental Design

Wistar albino rats (150-200gms) were obtained from the animal house, Carism, Sastra University. Rats were maintained on standard pellet diet and tap water *ad libitum*. They were kept in clean cages under 12 hrs light/dark cycle and room temperature 22-24^{o}C and were acclimatized to the environment for 2 weeks prior to experimental use. This study was conducted according to the guidelines approved by the Institutional Animal Ethics Committee.27/SASTRA/IAEC/RPP.

Diabetes was induced by injection of a single intra-peritoneal dose of Streptozotocin (freshly prepared in 0.01M citrate buffer, pH 4.5). Overnight fasted rats were injected with Streptozotocin (STZ; 60 mg/kg body wt., *i.p*) to induce diabetes. Diabetic was confirmed by glucose estimation. Animal with plasma glucose level > 200 mg / dl were selected for the study. Diabetic induced animals were grouped for further study. After 7 days of STZ induction, treatment was started. First group received only distilled water (Disease control). Second and third group received hydro-alcohol extract of *Nelumbo nucifera* 200 mg/kg B. Wt. and 400mg/kg, B. Wt. orally respectively. In each group, body weight, food intake and water intake, blood glucose levels, urinary volume were measured (Hazem *et al.,* 2007). At the end of the study organ was collected, and checked enzymatic and non-enzymatic anti oxidant activity.

Determination of antioxidant activity in vivo anti oxidant activity

At end of the study, the animals were sacrificed by decapitation. Eyeball, Liver, sciatic nerve and kidney were removed, weighed and homogenized immediately with Elvenjan homogenizer fitted with Teflon plunger, in ice-cold Tris buffer. The suspension was centrifuged at 2500 rpm for 10 min and clear supernatant was used for the following estimations . Total Protein (Lowry O.H *et al.,* 1951), Estimation of GSH (Van Dooran *et al.,* 1987*)*, Estimation of lipid peroxide (Uchiyama *et al*., 1979), Estimation of Glutathione peroxidase (Wendel *et al*., 1981), and estimation of peroxidase (Okhawa *et al.,* 1979).

Results and discussion

Streptozotocin (STZ) is known to induce not only diabetes but also develop diabetic complication similar to early stage complication of humans. For the study of antidiabetic agents, STZ induced hyperglycemia in rodents is considered to be a good perliminiary screening model. It is a potent methylating agent for DNA and acts as nitric oxide donor in pancreatic cells. β cells are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes (Kelvin & Moss,1992). The streptozotocin injected rats developed not only diabetes as indicated by increased fasting blood glucose values but also showed external signs of symptoms. Earlier symptoms of micro vascular

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complication such as Retinopathy, formation of cataract, body weight loss, polyphagia, dried dark coloured stool, neuropathy accompanied with loss in sensation and nephropathy associated with high urine output was observed after 10 days of induction of Diabetes (Halim Eshrat *et al*., 2002). Table 1 show that the serum glucose level of Diabetes induced animals was found to be increased significantly in diseased rats from 2nd week onwards. The increment of glucose level was found to be extended up to 9th week. No significant decrease in glucose level was observed up to 9th week against 4th week (treatment starts) of animals in diseased rats. The Diabetic animals were administered with extract from 5th week onwards. This drug was found to decrease the level of glucose significantly ($p<0.05$). The lower dose of extract itself exhibits its activity and the effect was observed to be dose dependently. The hypoglycemic activity of *N.nucifera* has been reported earlier by (Mukherjee *et al.,* 1997).

Lipid per oxidation (LPO) in Diabetic micro vascular complications

The level of TBARS was found to increase in diseased rats by 24.8%. Similarly, neither lower dose nor higher dose of drug treatment was found to decrease the level of TBARS significantly. However, some percentage of activity has been exhibited by the extract. The percentage was calculated for lower dose as 10.4% and for higher dose as 19.1%. This might be due to the antioxidant effect of *Nelumbo nucifera*. Earlier references showed that *Nelumbo nucifera* extract is having antilipid peroxidative in nature. *Nelumbo nucifera* seeds contain alkaloids, saponins, phenolics and carbohydrates, mostly these compounds possess free radical scavenging and antioxidant activity (Tripathi *et al.,* 1996). Several studies have revealed that a major part of the antioxidant activity may be from compounds such as flavonoids, isoflavones, flavones, anthocyanin, catechin and other phenolics (Kähkönen *et al.,* 1999) with mechanisms involving both free radical scavenging and metal chelation (Lien *et al.,* 1999). Though no much significant difference have been observed in eye tissue of STZ administered rats, ($p<0.05$) but significant difference have been observed in liver tissue. Moreover a negative correlation ($R=-0.880$) has been observed between GPx and LPO of liver tissue confirms that the decreased lipid per oxidation might be due to the antioxidant activity of *Nelumbo nucifera*. This result confirms the toxicity of STZ. However, lower dose of extract itself exhibits significant protection in liver tissue ($p<0.05$). Likewise, in the kidney a negative correlation has been observed between GSH and LPO of kidney tissue, which reveals that the increased GSH has decreased the LPO of kidney tissue. The significantly decreased lipid peroxidation of sciatic nerve in treated groups confirms a significant protection in *Nelumbo nucifera* treated animals ($p<0.05$).

GSH in Diabetic micro vascular complication

Administration of Streptozotozin was found to decrease the level of GSH in eye, liver, kidney and sciatic nerve. This might be due to the utilization of GSH in diseased condition to protect the organ from damage caused by Streptozotozin. Earlier references showed that there is a greater degree of lipid peroxidation caused by Streptozotozin (Kostić *et al.,* 2007).

On treating animals with extract at lower dose, no much significant difference has been observed. But at higher dose it was found to be increased significantly ($p<0.05$), and return back to normal level. *i.e.* no much significant difference between normal and higher dose treated rats of liver and eye. However, in kidney and sciatic nerve at the lower dose itself the drug was found to exhibits a better activity. The results confines that the major complications developed by STZ which includes Diabetic nephropathy and neuropathy can be prevented by extract at lower dose. The diabetic retinopathy developed by STZ is prevented by *Nelumbo nucifera* at higher dose. These results should be further confirmed by carrying out various specific markers. This might be due to the free radical scavenging and antioxidant activity of *Nelumbo nucifera*.

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Table.1 Effect of treatment with hydro alcoholic extract of Nelumbo nucifera on the plasma cholesterol level (mg/ dl) in STZ induced diabetic rats.

Groups	Fasting plasma cholesterol level (mg/dl)					
	0 week	2 week	4 week	6 week	8 week	9 week
Normal control	80.25±2.5^{d}	100.25±0.3^{b}	114.75±0.5^{c}	123.25±0.25^{c}	133.75±4.6^{c}	148.39±0.26^{c}
Disease Control (STZ) 60 mg/kg, *i.p.*	86.7±0.25^{d}	265.6±5.6^{b}	442.1±3.5^{b}	556.03±0.64^{ab}	658.25±35^{a}	687.16±0.65^{a}
HANN- 200 mg/kg, *p.o*.	100.02±0.36^{d}	275±3.0^{c}	463.4±0.32^{b}	258.24±5.6^{b}	128.56±.64^{b}	94.68±3.4^{d}
HANN-400 mg/kg, *p.o,*	101.9±2.6^{d}	291.8±3.1^{c}	465.2±3.2^{b}	236.69±5.9^{b}	116.84±26^{b}	92.56±2.6^{d}

The results are expressed as mean ± S.E. for six animals in each group and statistical significance was calculated by ANOVA followed by Duncan test. Values not sharing common alphabets (a,b,c) are differ significantly at p<.0.05

Table 2. Effect of Hydro Alcoholic Extract of Nelumbo nucifera on organ weight in STZ induced Diabetic rats

Organ weight (gram)				
Group	Eye	Liver	Kidney	Sciatic nerve
Normal control	0.2525 ± 0.02^{ns}	9.0375 ± 0.9^{ns}	1.805 ± 0.5^{ns}	0.0475 ± 0.001^{ns}
Disease Control (STZ)60 mg/	0.245 ± 0.02^{ns}	8.24 ± 0.8^{ns}	1.825 ± 0.5^{ns}	0.0175 ± 0.002^{ns}
HANN-200 mg/kg/p.o	0.236 ± 0.02^{ns}	8.702 ± 0.9^{ns}	1.826 ± 0.5^{ns}	0.18 ± 0.002^{ns}
HANN-400 mg/kg/p.o	0.223 ± 0.02^{ns}	8.986 ± 0.9^{ns}	2.03 ± 0.8^{ns}	0.043 ± 0.001^{ns}

The results are expressed as mean ± S.E for six animals in each group and statistical significance was calculated by ANOVA followed by Duncan test. ns – Non-significant.

Table.3 Effect of Hydro Alcoholic Extract of Nelumbo nucifera (HANN) on antioxidant enzymes of Streptozotozin induced Diabetic micro vascular complication in eye.

Eye				
Group	LPO	GSH	GPx	Peroxidase
	Mean ±S.D	Mean±S.D	Mean±S.D	Mean±S.D
Control	9604.23 ±1084.27^{a}	5373.20 ±324.10^{b}	138.04±12.10^{c}	56.66 ±3.052^{b}
Disease Control (STZ) 60mg/kg, *i.p*.	11990.57±2583.00^{a}	2061.28 ±845.90^{a}	18.32±2.52^{a}	25.01±4.29^{a}
HANN-200 mg/kg/p.o	10875.35 114.74^{a}	3223.21±476.93^{a}	62.49±4.077^{b}	50.43±12.27^{b}
Treatment 2 HANN-400 mg/kg/*p.o*	9829.59±1932.35^{a}	4802.47±694.25^{b}	123.04±19.88^{c}	43.06±4.00^{b}

The results are expressed as mean ± S.E for six animals in each group and statistical significance was calculated by ANOVA followed by Duncan test. Values are Mean ± SD. Values not sharing common alphabets (a,b,c) are differ significantly at p<0.05.

Table.3a Effect of Hydro Alcoholic Extract of Nelumbo nucifera (HANN) on antioxidant enzymes of Streptozotozin induced Diabetic micro vascular complication in liver.

Liver				
Group	LPO	GSH	GPx	Peroxidase
	Mean±S.D	Mean±S.D	Mean±S.D	Mean±S.D
Control	643.30±76.13^{a}	4577.86±452.90^{b}	3.22±0.24^{b}	8.59±1.07^{b}
Disease Control (STZ) 60 mg/kg, *i.p.*	3300.91±725.12^{b}	2470.27±385.01^{a}	1.68±0.38^{a}	5.12±2.75^{a}
HANN-200 mg/kg/*p.o*	1394.92±268.19^{a}	3474.80±371.02^{a}	2.53±0.29^{b}	3.88±2.02^{a}
HANN-400 mg/kg/*p.o*	946.16±148.37^{a}	4616.78±1182.96^{b}	2.79±0.67S^{b}	3.60±0.35^{a}

The results are expressed as mean ± S.E.M. for six animals in each group and statistical significance was calculated by ANOVA followed by Duncan test. Values not sharing common alphabets (a,b,c) are differ significantly at p<.0.05.

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Table 3b. Effect of Hydro Alcoholic Extract of Nelumbo nucifera (HANN) on antioxidant enzymes of Streptozotozin induced Diabetic micro vascular complication in kidney.

Kidney				
Group	LPO	GSH	GPx	Peroxidase
	Mean ±S.D	Mean ±S.D	Mean ±S.D	Mean ±S.D
Control	113.74±88.29 [a]	253117.7± 5818.84[b]	1.75±.056[c]	287.43±46.66[a]
Disease Control (STZ) 60 mg/kg, *i.p*.	3350.58±447.58[c]	135822.9±14824.44[a]	1.25±.913[a]	287.43±46.66[a]
HANN-200 mg/kg/*p.o*	1878.55±208.68[b]	198425.9±39983.09[b]	1.32±0.171[ab]	828.59±304.71[b]
HANN-400 mg/kg/*p.o*	1408.85±78.38[ab]	249939.1±22767.80[b]	1.47±.027[b]	459.23±98.85[a]

The results are expressed as mean ± S.E.M. for six animals in each group and statistical significance was calculated by ANOVA followed by Duncan test. Values not sharing common alphabets (a,b,c) are differ significantly at p<.0.05.

Table 3c. Effect of Hydro Alcoholic Extract of Nelumbo nucifera (HANN) on antioxidant enzymes of Streptozotozin induced Diabetic micro vascular complication in kidney.

Sciatic nerve				
Group	LPO	GSH	GPx	Peroxidase
	Mean±S.D	Mean±S.D	Mean±S.D	Mean±S.D
Control	1933.2012±398.46[a]	662110.6±16465.62[d]	0.0235±.001[b]	7669.50±564.42[a]
Disease Control (STZ) 60 mg/kg, *i.p*	5142.1693±839.45[b]	177660.8±48122.43[a]	0.0223±.003[ab]	9264.24±1092.82[b]
HANN-200 mg/kg/p.o	2777.40±268.84[a]	383603.4±14030.89[b]	0.0208±0.0098[a]	7584.58±718.78[a]
HANN-400 mg/kg/*p.o*	2203.71±290.52[a]	521248.3±15979.73[c]	.0225±.001[b]	7361.08±835.90[a]

The results are expressed as mean ± S.E. for six animals in each group and statistical significance was calculated by ANOVA followed by Duncan test. Values not sharing common alphabets (a,b,c) are differ significantly at p<.0.05.

Moreover, a negative correlation has been observed between GSH and LPO of sciatic nerve, which further confirms the antioxidant activity of NN and protection exhibited by it. The increased level of GSH by *Nelumbo nucifera* has been reported earlier by (Dongmei Yang *et al.,* 2007*).*

GPx in Diabetic micro vascular complications

Administration of Streptozotozin was found to decrease the level of GPx in eye, liver, kidney and sciatic nerve. (Dongmei Yang *et al.,* 2007) has observed similar results. This might be due to the utilization of GPx by animals to protect the damage caused by streptozotozin.

On treating animal with extract dose dependent effect was observed. High dose of drug can protect the eye and sciatic nerve from damage caused by STZ and brings back the GPx level to normal value. Likewise in kidney and liver tissue the lower dose of drug itself protect the damage caused by extract. From these results, it has been observed that the extract at lower dose is exhibits antioxidant activity. Moreover a positive correlation was observed between the GSH and GPx value (R=0.887). These results further confirm the antioxidant activity of *Nelumbo nucifera*. Earlier references showed that *Nelumbo nucifera* extract exhibits antioxidant activity with increasing level of GPx.

Peroxidase in micro vascular complications

Peroxidase level was found to be decreased in eye and liver but increased in kidney and static nerve of diseased condition. Though no earlier references have shown these effects in STZ induced micro vascular complications, this might be a new finding in our study. Moreover, the decreased concentration of enzyme might be due to the utilization of Peroxidase to scavenge the large amount of hydrogen peroxide formed during STZ administration. In kidney and sciatic nerve, the damage caused by STZ might be scavenged by large utilization of GSH and GPx. This might decrease the utilization of Peroxidase by the tissue to protect the organ results in decreased level of the same enzyme.

Increased level of Peroxidase in liver and eye was observed at the lower dose. This might be due to the antioxidant activity of *Nelumbo nucifera*. The extract either may increase the level of synthesis of Peroxidase or it may scavenge the Peroxidase by its own thereby prevent the decrement of Peroxidase level. The significant effect was observed even at lower dose of extract. Earlier references showed that

the *Nelumbo nucifera* extract can scavenge the hydrogen peroxide in *in-vitro* studies (Dongmei Yang , *et al.,* 2007*).* This reference further confirms our present result.

In kidney and sciatic nerve, the increased peroxidase in diseased rats was found to be decreased in treatment. This decrement might not be due to the absence of antioxidant activity but mainly due to the increased utilization of peroxidase by these organs. Different results observed in liver and eye with kidney and sciatic nerve furnish that the *Nelumbo nucifera* extract does not exhibits its activity by increasing the synthesis of Peroxidase but through the scavenging activity of NN from damage caused by hydrogen peroxide.

Histopathological studies reveals that kidney of diseased group is having acute tubular necrotic changes and liver shows severe central venous congestion with minimal periportal inflammations. In eye, retinal blood vessels with basement membrane thickening were observed. In sciatic nerve, Myelinated nerve fibre loss was seen. In treated group with high dose shows, there was a decrease in basement membrane thickening in retina and decrease in myelinated nerve fiber loss, when compared with diseased control.

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