Review Article

Hepatoprotective and Antioxidant Properties in *Nelumbo nucifera* (Lotus)

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ABSTRACT

Nelumbo nucifera an aquatic perennial plant belonging to Nelumbonaceae family has its traditional use as a medicinal herb & as "Sacred Worshipped Flower" dating back to 'The Pharoic Egyptians' found along the banks of river Nile. The large population of its species is mainly found in Asian countries restricted to shades of red through pink to pure white in contrast to American kind which is yellow in colour. The Chinese Lotus Research in Wuhan is the leading Asian lotus breeding research centre in the world with 300 ponds growing 500 lotus varieties. It has been found by many researchers that this perennial aquatic plant has several phytochemical and pharmacological activities such as anti-pyretic, hypoglycaemic, immunomodulatory, psychopharmacological, spermatorrhoea, haematemesis, antioxidant, antiviral, anti-diarrheal, anti-inflammatory, anti-cancer & hepatoprotective activities.

Keywords: *Nelumbo nucifera*, antioxidants, free-radical scavenging, hepatoprotective.

INTRODUCTION

The lotus (*Nelumbo nucifera* Gaertn; 2n=16) or the sacred lotus of Nelumbonaeceae family which is now placed in the mono-generic family. Nymphaeaceae, is an aquatic medicinal plant[1,3]. The fossil records reveal that there were 8 species of *Nelumbo nucifera* worldwide 15 million years ago[3,5]. At present, the count has been reduced to only two species of its kind namely;

(a) Asian Lotus or ***Nelumbo nucifera***(synonyms *Nelumbium speciosum* , *Nelumbo speciosa*) found in China & India.

(b) American Lotus or Water chinquapin or ***Nelumbo lutea*** (synonyms *N. pentapetala, Nelumbium luteum*) found in eastern &southern North America [6,7]. (Fig: 1)

Figure 1: (a) *Nelumbo nucifera* Asian species, (b) The American Yellow lotus, (c) seedpod above the petals, (d) 1 day lotus

Plant Details

Taxonomic Classification[6]

Kingdom: Plantae – Plants;

Sub Kingdom: Tracheobionta – Vascular Plants;

Super Division: Spermatophyta – Seed Plants;

Division: Magnoliophyta – Flowering Plants;

Class: Magnoliopsida;

Subclass: Magnoliidae;

Super order: Protaenae;

Order: Proteales;

Family: Nymphaeaceae– Lotus Family;

Genus: Nelumbo Adans – Lotus;

Species: *Nelumbo nucifera* Gaertn. – Sacred lotus.

Synonyms[8]

English – Sacred lotus;

Hindi – Kanwal, Kamal;

Sanskrit – Ambuja;

Tamil - Ambal, Thamarai, Padma, Pankaja, Kamala;

Bengal – Padma;

Gujarat –Suriyakamal;

Malayalam – Tamara;

French –Nelumbo;

German – Indische lotosblume;

Persian – Nilufer

Morphology

a) Leaves- large, 2 types-aerial as well as floating orbicular 20-90cm in diameter, petioles of the aerial

leaves are erect and stout white those of the floating ones are not strong enough.

b) Fruits- An aggregate of indehiscent nut-lets having remarkable power of dormancy.

c) Seeds- composed of 3 parts: integuments, plumule & cotyledons proved to have longevity retaining the power of germination even after 150 years of confinement in glass-topped box.

d) Flowers- solitary, large, 10-25cm in diameter, white pink or pinkish white fragrant peduncles arising from the nodes of the rhizomes.

e) Rhizomes- 60-140cm long, 0.5-2.5cm in diameter, yellowish white to yellowish brown in colour, smooth longitudinally striated with brown patches, nodes & internodes are present[6].

The Nelumbo nucifera in India is known as the *Kamala* or *Padma* having two varieties: one is the white lotus or *Pundarika* or *Sveta Kamala*; the other one in pink or reddish pink petals or *Rakta Kamala*[9]. The whole flower is known as *Padmini*, the rhizomes as *Kamalkand*, the leaves as *Sambartikai,* the peduncle as *Mrinal* or *Visa*, the stamens as *Kirijalika,* the torus as upon padma as *Makaranda* or *Padma-Madhu*[10]. It is particularly noted for its 1,300-year seed longevity and exceptional water repellency, known as the lotus effect. The latter property is due to the nanoscopic closely packed protuberances of its self-cleaning leaf surface, which have been adapted for the manufacture of a self-cleaning industrial paint, Lotusan. Ming, 2013 sequenced the genome of the china antique variety of the sacred lotus having a contig of N50 of 38.8kbp and a scaffold of N50 of 3.4 Mbp, and covers 86.5% of the estimated 929 Mbp total genome size. Several pharmacological activities of *Nelumbo nucifera* have been studied by methanolic, enthanol & hydroalcoholic extract and have been found to be possessing hepatoprotective[11], immunomodulatory[12], anti-analgesic[13], anti-fertility[14], antioxidant[15,20], psychopharmacological[21], anti-pyretic[22], anti-cancer[9,23,29], antiviral[30-31], hypoglycaemic, anti-diarrhoeal, anti-fungal, anti-bacterial, anti-inflammatory and diuretic.[26,32,38]

Phytochemical Properties

Besides these activities nutritional, physiochemical and presence of trace elements was also reported in different studies (Table1-6). In popular traditional medicine it has been used in the treatment of tissue inflammation, skin disease, cancer, leprosy and as a poison antidote[9,23]. These pharmacological activities are due to the presence of different classes of phytochemical constituents such as alkaloids, steroids, triterpenoids, flavonoids, glycosides and polyphenols present in different parts of the *Nelumbo nucifera* plant.

Table 1: Nutritive Value of Lotus seeds[39]

Ash (%)	4.50
Moisture content (%)	10.50
Crude fat (%)	1.93
Protein (%)	10.60
Carbohydrate (%)	72.17
Crude fibre (%)	2.70
Energy (cal/100gm)	348.45

Table 2: Percent concentration of various elements of *Nelumbo nucifera* (seeds)[39]

Chromium	0.0042
Sodium	1.00
Potassium	28.5
Calcium	22.10
Magnesium	9.20
Copper	0.0463
Zinc	0.0840
Manganese	0.356
Iron	0.1990

Table 3: Chemical Evaluation of *Nelumbo nucifera* (seeds)[40]

Moisture %	7.26
Oil%	3.62

Table 4: Physicochemical values of the oil of *Nelumbo nucifera* (seeds)[40]

Saponification value	175.8
Iodine value	90
Free fatty acid (oleic acid)	1.86
Unsaponifiable matter	0.52
Acid value	3.70
Ester value	172.1

Table 5: Phytochemical analysis of *N.nucifera* seeds[41]

Biochemical composition	***Nelumbo nucifera* (methanol)**
Alkaloids	-
Carbohydrates	+++
Saponins	-
Proteins	+++
Phenolic compounds	++
Flavonoids	+
Tannins	-

Table 6: Antioxidant assay-DPPH free radical scavenging activity[41]

S. No	Sample (µL)	DMSO (µL)	DPPH (µL)	Nelumbo nucifera		
				M	H	E
1	20	38.0	2.960	40.415	86.22	23.58
2	40	36.0	2.960	50.115	88.39	37.78
3	60	34.0	2.960	57.04	88.67	26.69
4	80	32.0	2.960	67.89	89.34	29.79
5	100	30.0	2.960	85.68	91.16	25.77
6	120	28.0	2.960	90.53	91.24	37.84
7	140	26.0	2.960	84.98	91.6	35.67
8	160	24.0	2.960	90.99	92.98	39.67
9	180	22.0	2.960	92.14	96.06	36.56
10	200	20.0	2.960	92.84	96.36	42.23
11	control	Control	3	-	-	-

*('H' denotes hexane extract, 'M' denotes methanol extract, 'E' denotes ethyl acetate extract)

**[(-) denotes absent, (+) denotes mild, (++) denotes average, (+++) denotes large]

Pharmacological Properties

Hepatoprotective activity

It is the ability to prevent damage to the liver.

Hepatoprotective effects have been associated with plant extracts rich in antioxidants[42].

Seeds

An ethanolic extract of the seed of *N.nucifera* was studied for hepatoprotective effects in CCl4 and aflatoxin B1-induced hepatotoxicity models[17].

Cell death by CCl4 was significantly inhibited in a dose-dependent manner by the ethanolic extract at concentration between 10-500mg/ml.

The same extract reduced the genotoxicity of aflatoxin B, showing complete inhibition at a concentration of 250mg/plant. CCl4 in hepatic parenchyma cells and is metabolised to C·Cl3 by liver cytochrome P450-dependent monooxygenases[43].

One of the principal causes of CCl4- induced liver injury is lipid peroxidation induced and accelerated by free radical derivatives of CCl4[44-45].

Leaves

An ethanolic extract of leaves was studied for its hepatoprotective activity on Sprague–Dawley rats (200 ± 20 g) and KM mice (21 ± 2 g) of both sex against CCl4-induced liver toxicity by Huang. Hepatoprotective activity of lotus leaf extract (LLE) at doses of 300 and 500 mg/kg and *in vivo* antioxidant activity at 100 mg/kg that was comparable with that of a standard treatment comprising 100 mg/kg of silymarin, a standard hepatoprotective drug[46-47].

Antioxidant & free- radical scavenging activity

An Antioxidant is a substance which inhibits damages induced by oxidants or an antioxidant is a molecule that inhibits the oxidation of other molecules by termination of chain reactions and removal of free-radical intermediates.

These molecules are effective because they can give their own electron to form free radicals preventing the chain reaction[48]. Reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\circ -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (°OH) are generated as by products during aerobic metabolism in cells[49].

Seeds

Lotus seed and its seedpod extracts posses antioxidant, free-radical scavenging properties. Rai studied the antioxidant activity of hydro-alcoholic extract of lotus seeds using *in vitro* and *in vivo* models. Total phenolics in extract was 7.61% and exhibited strong free radical scavenging activity as evidenced by the low IC50 values (16.12 µg/ml) in 1,1-diphenyl-2-picryl hydrazyl (DPPH), which was comparable to rutin (IC50, 18.95 µg/ml)[50]. In nitric oxide method, the extract showed more activity (IC50, 84.86 µg/ml) than standard rutin (IC50, 152.17 µg/ml). No signs of acute toxicity were evident up to oral dose of 1,000 mg/kg body weight in Swiss Albino mice[50]. Lotus seed extracts (LSE) have also shown significant free-radical scavenging and protective effective against reactive nitrogen species, sodium nitroprusside (SNP), peroxynitrite ($ONOO^-$) induced cytotoxicity and DNA damage in macrophage RAW264.7 cell lines.

Procyanidin and condensed tannin isolated from the seed of *N. nucifera* shows lipid auto-oxidation, lipoxygenase inhibition and free radical scavenging capability as compared to butylated hydroxytoulene (BHT) 0.1%. At a concentration of 62.5 mg/ml, procyanidin inhibited lipoxygenase activity by more than 90%, with an IC50 value of 21.6mg/ml[51]. The procyanidin extract of the LSP inhibits autoxidation of lard[52] and ameliorates age-related antioxidant deficits in older rats [53].

When hydroalcoholic extract of seed at100 and 200 mg/kg for 4 days was administered to Wister rats before CCl4 treatment cause significant dose dependent increase in the levels of SOD and catalase, in contrast to significant decrease in the level of thiobarbituric acid (TBA) comparable to the observed 100mg/kg dose to that of Vit E at 50mg/kg[54]. Ushimaru studied the antioxidative enzyme changes in seedlings of *N. nucifera*, which responds to oxygen deficiency by germination under water.

They reported that the activity of superoxide dismutase, dehydroascorbate reductase and glutathione reductase was lower in seedlings germinated under submerged condition in the darkness (SD) than those found in seedlings germinated in air and the darkness (AD)[55].

Rhizomes

Yang and co-workers have performed *in-vitro* studies of the antioxidant activity of methanol and acetone extracts of the *N. nucifera* rhizome using the DPPH assay[56].

The methanol and acetone extract showed highest DPPH scavenging activity, at 66.73mg/l and 133.3mg/l, respectively; the methanol extract exhibited a higher antioxidant activity coefficient than ascorbic acid. The rhizome knot also exhibited radical scavenging activity, measured spectophotometrically and by electron spins resonance[57].

Flowers

N. nucifera have the potential to scavenge DPPH free radicals and peroxynitrites ($ONOO^-$), and the inhibition of total ROS generation by kidney homogenates using 2v,7'-dicholodihydrofluorescein diacetate (DCHF-DA)[58].

Seven flavanoids were isolated from lotus stamens, most of them showed potent antioxidant activities. The glycosides and isorhamnetin rutinoside from its stamen also showed potent antioxidant activity in DPPH and ($ONOO^-$) assays[59].

The maximum scavenging activity on hydroxyl radicals (°OH) 40% could be achieved when lotus liquor was more than 500µg. Lotus liquor has a potent superoxide radical scavenging activity with value of 0.93unit/mg as SOD equivalents with an IC50 value of 1.07±0.04mg[62].

Leaves

The total phenolic (TP) content of lotus leaf extract (LLE) was determined using the Folin-Ciocalteau assay according to a previously described method[61]. An ethanolic extract of the leaves was studied against CCl4 induced liver toxicity in Sprague-Dawley rats (200±20g) and KM mice (21±2g) of both sexes.

LLE showed *in-vivo* antioxidant activity at 100mg/kg that was comparable with that of a standard treatment comprising 100mg/kg of silymarin, a hepatoprotective drug[47].

In a report it was shown that a dose-dependent protective effect against ROS-induced cytotoxicity was observed when Caco-2 cells were treated with 10mM H_2O_2 in combination with the methanol extract of the *N.nucifera* leaf (0.1-0.3mg/ml). The *N.nucifera* extract also exhibited concentration-dependent antioxidant activities against haemoglobin-induced linoleic acid peroxidation and Fenton reaction-mediated plasmid DNA oxidation[63].

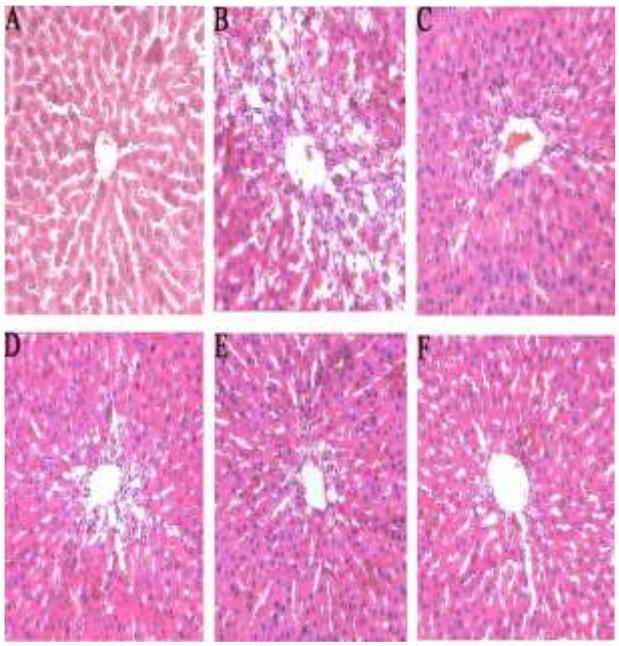

Figure 2: Sections of the livers of CCl4-treated rats showing the ventral vein (VV) and hepatic cells (haematoxylin–eosin staining, 200). (**A**) Control group, (**B**) CCl4/olive oil (1:1, 2 ml/kg), (**C**) CCl4 + silymarin (100 mg/kg), (**D**) CCl4 + LLE (100 mg/kg), (**E**) CCl4 + LLE (300 mg/kg), and (**F**) CCl4 + LLE (500 mg/kg)[64].

Histopathological observations of liver sections from the control group showed normal hepatic cells (Fig. 2A). In contrast, the most severe damage was reported in the CCl4 treated groups; the liver sections showed massive fatty changes, necrosis, ballooning degeneration, broad infiltration of lymphocytes, and the loss of cellular boundaries (Fig. 2B). The liver sections of the rats treated with LLE (Figs. 2D- F) exhibited a more or less normal lobular pattern almost comparable to the control (Fig. 2A) and the silymarin-treated group (Fig. 2C).

However, limited information is available with regard to the evaluation of its antioxidant activity on cells, specifically on human hepatocyte. Ethyl acetate fraction (EAF) from lotus leaves was prepared and its fraction composition determined. Further, antioxidant activities of EAF were measured using free radical scavenging activity, protection ability against DNA damage and cytoprotective effect on hydrogen peroxide-induced hepatic damage in cultured human hepatocytes.

The DPPH scavenging activity was calculated by the following equation:

$$Scavenging\ activity\ (\%) = \frac{A_{517}\ of\ control - A_{517} of\ sample}{A_{517}\ of\ control} \times 100$$

The IC50 value was defined as the concentration required for scavenging 50% of DPPH[65].

The ABTS+ radical scavenging activity was calculated by the following equation:

$$Scavenging\ activity\ (\%) = \frac{A_{414}\ of\ control - A_{414} of\ sample}{A_{414}\ of\ control} \times 100$$

The IC50 value was defined as the concentration required for scavenging 50% of ABTS+ radical[66].

Table 7: Antioxidant activities of EAF from lotus leaves[67]

Assays	EAF	Ascorbic Acid
DPPH (IC50, g/ml)	4.46±0.01a	3.04±0.01b
ABTS+ (IC50, g/mL)	5.35±0.02a	3.45±0.01b
Reducing power (A700)2)	0.224±0.003a	0.218±0.001b

[a,b]Different letters indicated a significant difference at the same assay (P ＜0.05).

1) The IC50 value was defined as the concentration required to scavenge 50% of DPPH or ABTS+ radical.

2) Reducing power was evaluated at 100μg/mL.

The result shower in this report stated that IC_{50} value of EAF against the $ABTS^{+}$ radical was 5.35 μg/mL and the IC_{50} value of ascorbic acid as a positive control was 3.45 μg/mL.

A high absorbance value at 700 nm indicates high reducing power, i.e. antioxidant compounds possess high hydrogen and/or electron donating ability the optical value of EAF and ascorbic acid at 700 nm was 0.224 and 0.218 respectively (Table 7).

CONCLUSION

Nelumbo nucifera and its other species have very significant advantage against the free-radicals and ROS species damage. The above review covers all the experimental data and facts proved and revealed till date which shows that all the parts of *Nelumbo nucifera* are greatly effective in imparting the antioxidant and hepatoprotective activities in animal modules.

It is of great value that its phenolic, hydroalcoholic & methanol extract are effective in various pharmacological activities such as anti-pyretic, hypoglycaemic, immunomodulatory, psychopharmacological, spermatorrhoea, haematemesis, antioxidant, antiviral, anti-diarrheal, anti-inflammatory, anti-cancer & hepatoprotective activities.

Now the research should enhance in this field to improvise methods of extraction, and making use of this natural, traditional & medical herb in curing various diseases without side effects and should be progressed for clinical trials at human levels.

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