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Original Article

The therapeutic potential of Nelumbo Nucifera against oxidative damage induced by Carbon Tetrachloride in rats.

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Abstract

Background: Herbal treatment has paid much attention in the world over the past decades. Nelumbo nucifera is used all over the world as a medicinal plant. It is an aquatic perennial plant belonging to the Nelumbonaceae family. The protective effects of Nelumbo nucifera are due to the active ingredients present in it. The study was conducted to evaluate the protective effects of Nelumbo nucifera seeds against hepatotoxicity caused by carbon tetrachloride (CCl4) in rats.

Methodology: Eighteen Wistar Albino rats were allotted into three groups (n=6), control group, CCl4 treated group given CCl4 0.8 ml/kg body weight subcutaneously twice a week, CCl4 + N. Nucifera treated group given CCl4 0.8 ml/kg body weight 2 times a week subcutaneously and also given Nelumbo nucifera extract (200 mg/kg body weight) orally through gavage daily. After 28 days of the experiment, serum samples were taken for estimation of enzyme activities and liver tissue samples were collected for evaluation of antioxidant enzyme levels.

Results: It was observed that liver weight increased in the CCl4 treated group while liver weight decreased in CCl4 + N. Nucifera treated group. CCl4 administration significantly raises the transaminase levels such as Aspartate transaminase (AST) ($p<0.01$), Aspartate transaminase (ALT) ($p<0.05$), and Alkaline phosphatase (ALP) ($p<0.05$) whereas CCl4 + N. Nucifera treated group serum transaminases are reduced. Antioxidant activities such as Catalase ($p>0.05$), Glutathione (GSH) ($p>0.05$) and Superoxide dismutase (SOD) ($p=0.05$) decreased in the CCl4 treated group. Significant elevation of antioxidant enzymes; Catalase ($p<0.05$), GSH ($p<0.05$) and SOD ($p>0.05$) in CCl4 + N. Nucifera treated group. Serum Malondialdehyde (MDA) concentration ($p<0.05$) significantly raised in the CCl4 hepatotoxic group compared to the control group. A decreased MDA levels were observed in the CCl4 + N. Nucifera treated group, indicating its potential to counteract the harmful effects of CCl4.

Conclusion: This study suggests that Nelumbo nucifera seeds have overcome CCl4-induced hepatotoxicity by their antioxidant effects.

Keywords

Herbal Treatment, Nelumbo Nucifera, Carbon Tetrachloride, Oxidative Stress.

Introduction

Liver, a vital organ in the body, accounts approximately 2-3% of body weight. Liver is at risk of deterioration due to a variety of factors such as microorganisms, metabolic products, circulatory materials as well as toxins. Pathologic problems like liver triglycerides and fatty liver have a key role in the developmental of various metabolic disorders like hypertension, diabetes mellitus, dyslipidemia, insulin resistance and obesity indicates the contribution of non-alcoholic fatty liver disease (NAFLD) management in the health promotion[1]. Because drug metabolism is an important function of the liver, the liver is at higher risk of being damaged by drugs than other organs. The second major fatal cancer in the world is liver cancer[2]. Liver injury induced by the drug leads to hepatitis, liver fibrosis, failure and consequent death[3].

Herbs or their extracts have gained considerable worldwide concentration due to their considerable effectiveness in the treatment and prevention of certain diseases[4,5]. In Asian countries, herbs have been used since thousands of the year as a cure for the disease. A recent study documented that 65% of the European countries relies on the herbal remedies[6]. One thousand one hundred or more medicines have been identified to cause liver toxicity[7]. According to researchers, herbal medicines have significant protective effects due to the presence of compounds such as alkaloids, flavonoids, saccharides and phenylpropanoids[8].

Nelumbo nucifera (lotus) has been used as herbal medicine, functional food as well as vegetable over 2000 years[9]. It is an aquatic plant cultivated mostly in Asia and Africa. Flowers, leaves, rhizomes, seeds, almost all parts of the plant have beneficial protective effects. Seeds and rhizomes are the most widely used parts[10]. Studies have been investigated for the health benefits of using lotus seeds due to the presence of alkaloids. Lotus leaves are used for treatment of obesity in China[11]. Studies revealed that Nelumbo nucifera seeds have anti-amnesic[12], anti-tumor[13], anti-oxidant[14] and anti-inflammatory[15,16] and hepatoprotective[17] effects. It is believed that the protective effects of Nelumbo nucifera are attributed to its active ingredients such as alkaloids and flavonoids[18]. Nelumbo nucifera seeds contain protein 25%, carbohydrate 65%, ash 4%, crude fibre 3-4%, moisture 8-10% and 388 cal/100 g of energy. It contains minerals such as calcium, potassium, sodium, copper, phosphorus, magnesium, manganese, selenium, zinc and iron[19].

To develop new therapeutic agents require more effort as pharmacotherapeutic options for liver diseases are limited[20]. Therefore the aim of the present study is to investigate the protective effects of Nelumbo Nucifera seeds against oxidative damage induced by carbon tetrachloride in rats.

Methodology

Plant Material

Juna market in Karachi was selected to collect Nelumbo nucifera seeds. The dried seeds were ground into powder using a grinder. Then 200 grams of powder was soaked in 1000 ml D.H2O. It was well mixed and tightly covered with polythene paper. The resulting extract was then placed in shaking incubator at room temperature for 24 hrs. Whatman No 1 filter paper was used for extract filtration and filtrate so obtained was stored in a closed container at 2°C[21].

Experimental Animals

Male and female Albino wistar rats (n=18) weighing between 120-200 gms were obtained from the International Centre for Chemical and Biological Sciences, Karachi. The animals were housed in air-conditioned animal house at department of physiology, university of Karachi. They were placed in clean plastic cages with acclimatization with laboratory environment 1 week prior to start the experiment. Standard rodent diet and ad libitum water was given to rats. Body weights of the animals were recorded at specific time interval throughout the experiment.

Experimental Design

Rats were assigned into three different groups (n=6). Group I served as control group. Group II animals were administered CCl4 (0.8 ml/kg b.w) 2 times a week subcutaneously. Group III was given Nelumbo nucifera extract (200 mg/kg b.w) orally

daily along with CCl4 (0.8 ml/kg b.w) subcutaneously 2 times a week for 28 days.

Sample Collection

On the 29th day, animals were decapitated and blood samples were collected in heparin-coated gel tubes. Samples were then subjected to centrifugation for separation of serum & plasma at 3000 rpm for five minutes. Serum samples were then stored for further analysis at 20°C. Liver were excised out from the animals, washed with saline and weighed. Homogenization of liver samples was done to prepare liver homogenate.

Biochemical Assay

Liver function was estimated by measuring serum transaminases. Serum ALT and AST levels were determined according to Reitman and Frankel[22]. Tietz and associates method was used to analyze ALP serum activity[23]. Antioxidant enzyme activities such as catalase[24], glutathione reductase[25] and superoxide dismutase[26] were evaluated. The level of lipid peroxidation was estimated by the serum MDA concentration as per Okawa et al method[27].

Statistical analysis

Data was expressed as mean standard deviation and analyzed using SPSS version 16. One way analysis of variance (ANOVA) was used to compare means of control with experimental groups where $p<0.05$ was considered significant.

Results

In present study, it was observed that liver weight was higher in CCl4 treated and CCl4 + N. nucifera treated animals than control group (Table 1).

Table 1: Comparison of liver weights among experimental groups.

Variable	Control (n=6)	CCl_4[1] (n=6)	CCl_4 + N. nucifera[1,2] (n=6)
		Mean ± SD	
Liver Weight (g)	4.75±0.911	7.31±1.00$^{\nu}$	7.483±0.626$^{\nu,n,\beta}$

[1]Compared with control, [2]Compared with CCl4 group.
$^{\alpha}p<0.05$, $^{\beta}p<0.01$, $^{\nu}p<0.001$, $^{\delta}p=0.05$, $^{n}p>0.05$.

Transaminases such as AST, ALT and ALP concentrations were assessed and compared (Table 2). Compared to Control, significant increases in AST ($p<0.01$), ALT ($p<0.05$) and ALP ($p<0.05$) levels in CCl4 treated group. In contrast, AST, ALT and ALP levels were decreased in CCl4 + N. nucifera group compared to CCl4 treated, but this change was observed non-significant ($p>0.05$).

Table 2: Liver enzymes; AST, ALT and ALP levels in experimental groups.

Variable	Control (n=6)	CCl_4[1] (n=6)	CCl_4 + N. nucifera[1,2] (n=6)
		Mean ± SD	
AST (U/L)	10.536± 6.51	28.62±8.09$^{\beta}$	16.612±14.355^{n,n,n}
ALT (U/L)	3.34±2.11	7.77±4.54$^{\alpha}$	4.966±2.978^{n,n,n}
ALP (U/L)	11.91±11.16	31.98±10.67$^{\alpha}$	28.115±11.88$^{\alpha,n,n}$

AST-Aspartate Transaminase; ALT-Aspartate Transaminase; ALP-Alkaline Phosphatase.
[1]Compared with control, [2]Compared with CCl4 group.
$^{\alpha}p<0.05$, $^{\beta}p<0.01$, $^{\nu}p<0.001$, $^{\delta}p=0.05$, $^{n}p>0.05$.

The activity of antioxidant enzymes was also analyzed in the present study (Table 3). Compared to control, the concentration of Catalase ($P>0.05$), GSH ($P>0.05$) and SOD ($p=0.05$) decreased significantly in CCl4 treated rats. It has been observed that N. nucifera administration significantly increased the levels of catalase ($p<0.05$)

and GSH ($p<0.05$) while SOD concentration increased ($p>0.05$) non-significantly compared to CCl4 treated group. CCl4 + N. nucifera group showed an increase in the SOD concentration as compared to CCl4 treated.

Table 3: Comparison of Catalase, GSH and SOD levels among experimental groups.

Variable	Control (n=6)	CCl_4 [1] (n=6)	CCl4 + N. nucifera[1,2] (n=6)
	Mean ± SD		
Catalase (µmol/g tissue)	135.63±2.90	132.55 ± 6.46^{n}	$140.93\pm3.73^{\alpha,\alpha,n}$
GSH (unit/g tissue)	89.12±18.64	71.58 ± 22.88^{n}	$196.83\pm78.54^{\alpha,\alpha,n}$
SOD (unit/g tissue)	20.23±6.14	$13.33\pm3.91^{\delta}$	$16.71\pm4.63^{n,n,n}$

GSH-Glutathione; SOD-Superoxide dismutase
[1]Compared with control, [2]Compared with CCl4 group.
$^{\alpha}p<0.05$, $^{\beta}p<0.01$, $^{\nu}p<0.001$, $^{\delta}p=0.05$, $^{n}p>0.05$.

The hepatic MDA level in the experimental groups was evaluated and compared (Table 4). There were significantly elevated MDA levels ($p<0.05$) in CCl4 treated group when compared to control. Decreased MDA concentration in CCl4 + N. nucifera group as compared with CCl4 treated group was observed.

Table 4: Comparison of MDA levels among experimental groups.

Variable	Control (n=6)	CCl4[1] (n=6)	CCl4 + N. nucifera[1,2] (n=6)
	Mean ± SD		
MDA (µmol/g tissue)	2.310±0.788	$6.191\pm2.08^{\alpha}$	$4.480\pm0.846^{\beta,n,n}$

MDA- Malondialdehyde.
[1]Compared with control, [2]Compared with CCl4 group.
$^{\alpha}p<0.05$, $^{\beta}p<0.01$, $^{\nu}p<0.001$, $^{\delta}p=0.05$, $^{n}p>0.05$.

Discussion

Xenobiotics are known as hepatotoxic agents that damage intracellular structures such as mitochondria and plasma membrane[28]. CCl4, a halogenated alkane, is one of the xenobiotics that contribute to hepatic damage through lipid per oxidation. It causes extensive damage to liver tissue such as fibrosis, fatty degeneration and impaired liver function. CCl4 induced liver injury involves transformation of carbon tetrachloride into trichloromethyl radicals that leads to reactive oxygen species (ROS) generation resulting in oxidative damage[29]. In this study, CCl4 treatment group showed decrease in body weights whereas and an increase in liver weight indicating the liver damage.

Elevation of serum liver enzymes is the prominent marker of liver damage. Accordingly, significant elevations of AST ($p<0.01$), ALT ($p<0.05$) and ALP ($p<0.05$) levels of CCl4 hepatotoxic group were found in our results. These results indicate cellular leakage, damage of membrane functional integrity and hepatocyte dysfunction as reported previously[30]. Animals received CCl4 + N. nucifera seeds showed decrease non-significantly in the serum concentrations of AST, ALT and ALP indicates the ability of N. nucifera to counteract the CCl4 effects. The protective effects of N. nucifera are attributed to the various alkaloids present in it.

Oxidative stress affects mitochondrial function by acting directly impairing oxidative phosphorylation. Reactive nitrogen species or reactive oxygen species can provoke mitochondrial membrane permeability transition and mitochondrial DNA deletions resulting in activation of caspases that leads to cell death. Studies reported that the liver diseases linked with oxidative stress are fatty liver, fibrosis, cirrhosis chronic hepatitis and carcinoma[31]. Our result showed a decline in the antioxidant enzymes such

as CAT (p>0.05), SOD (p=0.05) and GSH (p>0.05) in the CCl4 treatment group, which is consistent with previous studies[32]. Nelumbo nucifera administration enhanced the catalase (p<0.05), GSH (p<0.05) and SOD (p>0.05) concentration in our study that shows the antioxidant effects of Nelumbo nucifera against CCl4 induced liver injury. Similarly, the findings of Sohn et al, resemble with our observation in which liver and kidney peroxidize activity improved by administration of Nelumbo nuciera alcoholic extract[33].

Carbon tetrachloride transformed into CCl3 radical when binds with liver cytochrome P450 that result in initiation of lipid peroxidation[34]. The secondary metabolites of CCl4 react with proteins and lipids leading alteration of membrane permeability, mitochondria and plasma membrane results in cell damage. During lipid peroxidation secondary metabolites such as 4-hydroxynonenal, hexanal and MDA formed. In our results increased in the MDA levels in CCl4 administered experimental group shows the progressive lipid peroxidation caused by CCl4. Decreased MDA concentration in CCl4 + Nelumbo nucifera treated group indicates the potential of N.nucifera to overcome deleterious effects of CCl4. Studies documented that phytochemicals such as saponins, alkaloids, carbohydrates and polyphenolics found in the Nelumbo nucifera seeds attributed their antioxidant potential[10].

Specific doses of herbs used and their active ingredients need further research as a possible natural treatment to limit the progression of liver disease.

Conclusion

The findings of our study suggest that carbon tetrachloride produces hepatotoxicity as it elevates liver serum enzymes and lowers antioxidant concentration that results in oxidative stress. Nelumbo nucifera seeds have hepatoprotective potential by reducing liver enzymes and improve antioxidant levels thus counteracting the harmful effects of CCl4. Further studies are required to explore the protective effects of Nelumbo nucifera and their active components to limit the liver disease progression.

Conflicts of Interest

The authors have declared that no competing interests exist.

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