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Research Article

The Hepato-Renal Protective Effect of *Nelumbo nucifera* Gaertn Seeds against Carbon Tetra Chloride Toxicity in Rats

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ABSTRACT

Nelumbo nucifera Gaertn seeds (Lotus seeds) has been used in Traditional medicine since long ago. Lotus seeds shows notable antioxidant activity attributed to presence of alkaloids, Saponins, Phenolics and anti-ageing enzyme, which is believed to repair damaged protein. The purpose of study was to determine hepatorenal protective effect of aqueous extract of Nelumbo nucifera Gaertn (Lotus) seed on toxicity induced by Carbon tetrachloride. Female Wistor rats 200-250g were divided into three groups (n=6). Group I remained untreated whereas Group II & III received 0.8ml/kg of CCl_4 subcutaneously twice a week for 21 days. In addition, 200mg/kg of aqueous Lotus seed extract was given to Group III rats orally via gavage daily for 21 days. 0n 22nd day animals were sacrificed and blood and tissue samples were collected for biochemical and histopathological analysis.

AST levels ($p>0.05$), ALT levels, bilirubin levels.Urea, BUN, Creatinine levels ($p< 0.05$) were increased in group II (CCl_4 treated rats) as compared to untreated. The N. nucifera seed extract reduced AST levels ($p>0.05$), ALT levels, bilirubin levels, Urea, BUN, Creatinine levels ($p<0.05$) in group III (CCl_4 + N. nucifera Gaertn seed extract treated) rats as compared with group II showing protective effect of Lotus seeds. Elevation of body weight in group I and group II while reduction in group III was recorded. The histology features show mild enlargement and lobular inflammation in group II and group III and no pathologic evidence was found in group I. It is concluded that Lotus seed has profound hepatorenal protective effects.

Key words: *Aqueous extract, Nelumbo nucifera, Liver enzymes, Lotus seed.*

INTRODUCTION

Nelumbo nucifera Gaertn is an aquatic plant, comes under the family called as Nelumbonaceae. It has many local ethnic names such as Indian lotus, Kanwal, Chinese water Lilly, sacred lotus and 'behh' (sindhi) and valued in many countries specifically China, India and Egypt[12]. It is grown in lower Punjab and upper Sindh regions of Pakistan. All parts of lotus plant are edible and used in different forms.

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Rhizomes can be used as vegetable as it exhibits manifold nutritional and medicinal properties, whereas seeds are used raw, roasted or dried[32].

N. nucifera Gaertn has been used as alternative medicine since ancient times in Asian countries. Every part of *N. nucifera* plant including, seeds, flowers, leaves, rhizomes and stamens have been used for millennium in Chinese Medicine[37]. *N. nucifera* is used for pharmacological -purposes due to its anti-oxidant, anti-cancerous, anti-obesity, hypoglycemic, hepatoprotective properties attributed to its abundant of bioactive constitutes[30].

A protein repair enzyme, L-isoaspartyl methyltransferase is found in all organisms from humans to Bacteria that restores amino acids from D-state to its functional L-state and deficiency of which shows low survivability. Lotus seeds contain this anti-oxidant enzyme and can retain its ability at 50'C[34].

Exceptional longevity of Lotus seed is significantly known with world's record for enduring seed viability reported as 1300 years for a seed from China[16]. Moreover, the mature seeds of *N. nucifera* contains 25% protein, 8-10% moisture, 65% carbohydrate, 3.7% crude fat, 3-4% crude fiber, 4% ash. Lotus seed contain numerous minerals where, Calcium in high quantity and other with substantial amount including, copper, zinc, manganese, iron, selenium, sodium, potassium, phosphorous, and magnesium[18].

Hepatocellular carcinoma is next big issue in Pakistan[21]. Hepatitis is commonly reported in Pakistan in one of 10 reported diseases. According to World health Organization, Pakistan is 2nd on the list after Egypt in Hepatitis C virus cases. Additionally, Liver cancer is sixth common cancer in Pakistan[13,14]. Moreover, Kidney diseases are common cause of death every year as Pakistan ranks eight in Kidney diseases all over the world.

Considering the Etiological factors that induce Liver damage are; production of Reactive Oxygen species, virus, alcohol, alteration in lipid and carbohydrate metabolism and xenobiotics[10]. Carbon tetrachloride (CCl_4) is extensively used as industrial solvent. It can cause hepatic injury by inducing oxidative stress. Hence widely used to develop as toxic Liver model in rats. It produces many types of reactive oxygen species and activate trichloromethyl free radical by cytochrome P450 system in Liver cells and consequently causes Liver injury by Lipid peroxidation[19]. Oxidative damage occurs due to disproportion of antioxidants and ROS. Therefore, responsible for many diseases[28]. Another mechanism suggests that CCl_4 breaks DNA strands and affect argyrophilic nucleolar organizer regions (AgNORs) and they scatter with unusual size and shape and leads to genotoxicity[15]. Research on hepatoprotective and nephroprotective herbs have shown potential to cure Liver and Kidney damage caused by many toxic substances. Lotus seed has profound hepatorenal protective effects due to its anti-inflammatory and anti-oxidant properties as reported in many studies[35,36]. The purpose of current study is to determine hepatorenal protective effect of aqueous extract of *N. nucifera* Gaertn seeds against damage caused by Carbon tetrachloride.

MATERIAL AND METHODS

Plant Collection

Dried Lotus seeds were collected from Local Market, Karachi in October 2018. The seeds had been verified from Department of Botany, University of Karachi.

Preparation of Extract

Dried seeds were crushed, powdered in an electric grinder and stored in air tight container. The Aqueous extract of *N. nucifera* seeds was prepared by soaking 200gm of powdered seeds in 1000 ml of distilled water. This was mixed well and covered tightly with polythene paper to prevent evaporation of solvent added. Then it was placed in shaking incubator for 24 hours at room temperature. The sample was then filtered through whatman No.1 filter paper. The extract was stored in a closed container at 2'C. 200mg/kg[24] of Extract was given orally to experimental groups[11].

Laboratory Animals

Female Wister rats, weigh between 200-250 grams were obtained from Dow University of Health and Sciences, Karachi. Animals were acclimatized in well-ventilated animal house in Department of Physiology, University of Karachi. They were kept in glass cages under standard laboratory conditions. Rats were provided with commercial rat chow and clean water *ad libitium.* Animal handling was per internationally recognized health Research extention act 1985 and ethical guidelines of institutional ERB.

Induction of Toxicity

CCl_4 was obtained from Standard Laboratory in University of Karachi and given in a dose of 0.8ml/kg body weight subcutaneously to experimental groups twice a week.

Experimental Design

Female age and body weight matched rats were divided into three groups (n=6) including, control group (group I), CCl_4 treated group (group II) and *N. nucifera* seeds extract + CCl_4 treated group. (groupIII). The study was conducted for 21 days.

Group 1: untreated rats

Group 2: 0.8 ml/kg of body weight CCl_4 administered subcutaneously twice a week for 21 days.

Group 3: 0.8 ml/kg of body weight CCl_4 administered subcutaneously together with Aqueous *N. nucifera* Gaertn seed extract orally through gavage daily for 21 days.

Sample Collection

All animals were sacrificed on day 22nd. Blood samples were collected from anaesthetized (Sodium Thiopentone) rats via Carotid artery in Heparin coated tubes and plasma was obtained by centrifugation at 3000rmp for 5 minutes. Then it was carefully stored at -70'C.

Biochemical Assay

plasma Aspartate aminotransferase (AST), Alanine aminotransferase (ALT)[27], total bilirubin[7], Urea[38], Creatinine were evaluated using commercially prepared Randox and AmiTech Kits.

Statistical Analysis

The results were expressed as mean ± S.E.M. Independent sample T-test was used to evaluate data and indicated significant figure ($p< 0.05$).

Histopathologic examinations

Formalin was used to fix Liver and Kidney tissues then embedded in paraffin blocks, segmented at 4µm and stained with Hematoxylin and eosin for microscopic examination. The degree of hepatic and nephrotic damage was evaluated from the histologic sections via gradings and scoring described by[8].

none (0) 0% damage, mild (+) 10-20% damage, moderate (++) 20-50% damage, severe (+++) >50% global damage

RESULTS

Effects of Carbon Tetrachloride and *Nelumbo nucifera* Seeds on Body, Liver and Kidney weight.

Final body weight of control group is significantly increased ($p < 0.05$) as compared to initial weight. Whereas, there is reduction ($p <0.05$) in final body weight than initial weight of CCl_4 treated and CCl_4 + *N. nucifera* seeds treated group. There was gradual loss of weight in CCl_4 treated group but a slight reduction in weight was observed in CCl_4 + *N. nucifera* seeds treated group. Additionally, there is significant change ($p <0.05$) in relative Liver and Kidney weight of all three groups. (Table 1)

Effects of Carbon Tetrachloride and *Nelumbo nucifera* Seeds on Liver function tests.

In this study, AST levels is increased ($p > 0.05$) in CCl_4 treated as compared to control group. Whereas, AST levels are slightly decreased ($p > 0.05$) in *N. nucifera* seeds treated group when compared with CCl_4 treated. ALT levels are increased significantly ($p < 0.05$) in CCl_4 treated as compared to control group. Moreover, ALT levels decreased significantly ($p < 0.05$) in *N. nucifera* seeds treated group when compared with CCl_4 treated. Conversely, Bilirubin levels are increased ($p < 0.05$) in CCl_4 treated as compared to control group. Whereas, decreased ($p < 0.05$) in *N. nucifera* seeds

treated group when compared with CCl_4 treated. (Table2).

Effects of Carbon Tetrachloride and *Nelumbo nucifera* Seeds on Kidney function tests

Urea, blood urea Nitrogen (BUN) and Creatinine levels are significantly increased in ($p < 0.05$) in CCl_4 treated as compared to control group and decreased ($p < 0.05$) in *N. nucifera* seeds treated group when compared with CCl_4 treated group. (Table2).

DISCUSSION

Liver performs diverse functions such as metabolism macromolecules, detoxification and mediate immune responses[23]. It also regulates several physiochemical functions such as hydroxylation, hydrolysis, sulfation, oxidation, reduction, acetylation and conjugation[33]. Liver fibrosis is considered as a healing process which results due to chronic Liver diseases such as NASH (Non-Alcoholic steatohepatitis), Alcoholic Liver disease (ALD) or Viral hepatitis. Accumulation of excess extracellular matrix (ECM) protein and disturbed Homeostasis of ECM leads to fibrosis. Additionally, altered blood flow, disorganized architecture and oxidative stress may add to process of Fibrosis and consequently to Liver Cirrhosis[17]. Oxidative stress is a significant factor in pathophysiology of Liver injury from many conditions as ALD, NASH and Hepatitis C[39].

CCl_4 is extensively used in animal models for inducing Liver and Kidney toxicity. Production of trichloromethyl and trichloromethyl peroxy radicals play crucial role to generate oxidative stress in the course of CCl_4 induced toxicity[41].

CCl_4 is a strong toxic chemical that produces free radicals by activating cytochrome p450 and a trigger Kupffer cells to produce ROS that cause Liver damage[4]. The radicals form from CCl_4 react with oxygen and biomolecules such as proteins and Lipid and lead to lipid peroxidation and contribute to renal and hepatic injury[33].

Hepatic cells are sensitive to oxidative stress. Parenchyma and non-parenchyma both cells involve in production of ROS, which alter immune responses that invite inflammatory cells and activate hormones, chemokines and cytokines that leads to chronic Liver disease and Fibrosis[5]. Various herbs and herbal extracts have been investigated to protect Liver and Kidney from CCl_4 induced toxicity[3]. The antioxidants, bioactive substance and phytochemicals help to overcome toxicity by CCl_4. *N. nucifera* Gaertn seeds are rich in alkaloids, saponins and phenolics which are responsible for anti-oxidant property. Moreover, it increases action of anti-oxidant such as superoxide dismutase and catalases for free radical scavenging activity[25].

Altered membrane permeability allows hepatic cytosolic enzymes to move out from membrane blebs or mitochondrial damage into blood. The damage is due to metabolism or injury to hepatocytes[26]. The levels of AST and ALT were measured as these enzymes are indicator of Liver damage. As hepatocytes are damaged, the enzymes and Bilirubin elevated and moves into blood[9]. Another mechanism revealed that hepatic insufficiency cause increased in uptake of free bilirubin or hepatic beta-glucuronidase[1]. In present study, ALT, AST and total bilirubin levels were increased in CCl_4 treated group which coincides with the study by Khan *et al.,*[13], Khan *et al.,*[14]. As our study revealed that Aqueous extract of N. nucifera Gaertn seeds declined levels of ALT ($p<0.05$), AST ($p > 0.05$) and Bilirubin ($p < 0.05$) levels (Table 2).

The Urea, BUN, Creatinine are normally eliminated by the Kidney. Elevated levels of Urea, BUN, Creatinine ($p< 0.05$) in CCl_4 treated group shows that Kidney function is impaired as suggested in Safhi [31]. Whereas, Urea, BUN, Creatinine levels were significantly reduced ($p < 0.05$) when treated with extract of Lotus seeds (Table 2) that overcome Kidney impairment[29]. The demonstrated mechanism might be inhibition of renal expression of TGF-β1 and MCP-1 provoking fibrocytes recruitment to site of nephrotic injury[20].

Our results reinforced with[40], body weight was gained by control group ($p < 0.05$)

and on the contrary, CCl_4 treated group reduced its bodyweight. ($p < 0.05$). extract treated group showed increase in body weight compared to initial weight. Administration of CCl_4 reduced body weight but increased Kidney weight[2] and Liver weight[6] (Table 1). Organ weight is a typical measure of drug toxicity which often leads to morphological changes. Several factors influence animal organ weight such as sex, age, strain, environment and experimental surroundings[22]. The histological features depict Liver enlargement and Lobular inflammation in CCl_4 treated group and Lotus seed extract treated group whereas hemorrhage in CCl_4 treated group. (Table3). Additionally, it showed tubular cell swelling, congestion of Bowman and interstitial inflammation in group III and necrosis in group II (Table 4). The histopathological results coincide with biochemical analysis. Aqueous extract of *Nelumbo nucifera* Gaertn seed has high reducing power as suggested by Raajeswari and Meenakshi[24] that supports current study as its antioxidant property protects Hepatocytes and Nephrocytes.

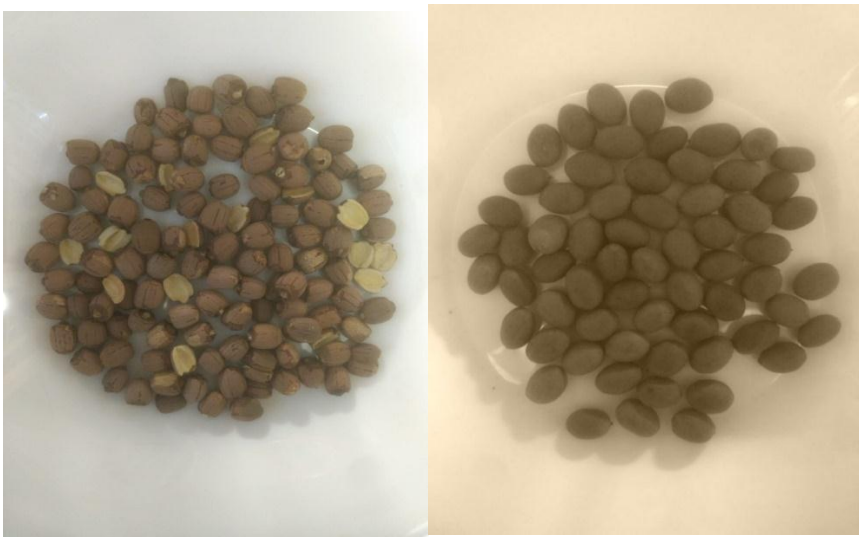

Fig 1: Dried Lotus seed

HISTO-PATHOLOGICAL FINDINGS

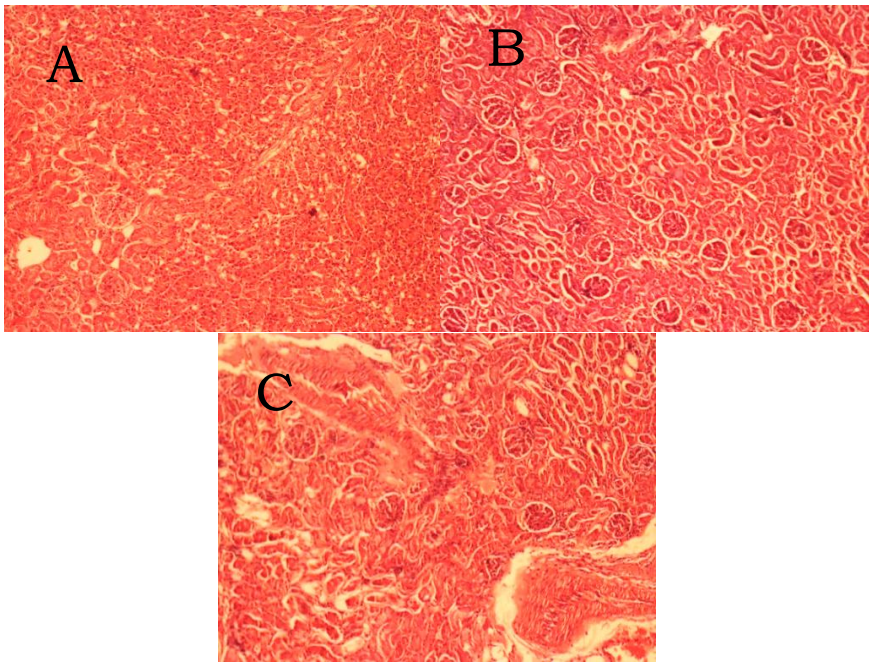

Fig. 2: Histological slides of Kidney at 4x magnification. A; control group. B; CCl_4 treated. C; CCl_4+ *N. nucifera* seeds treated

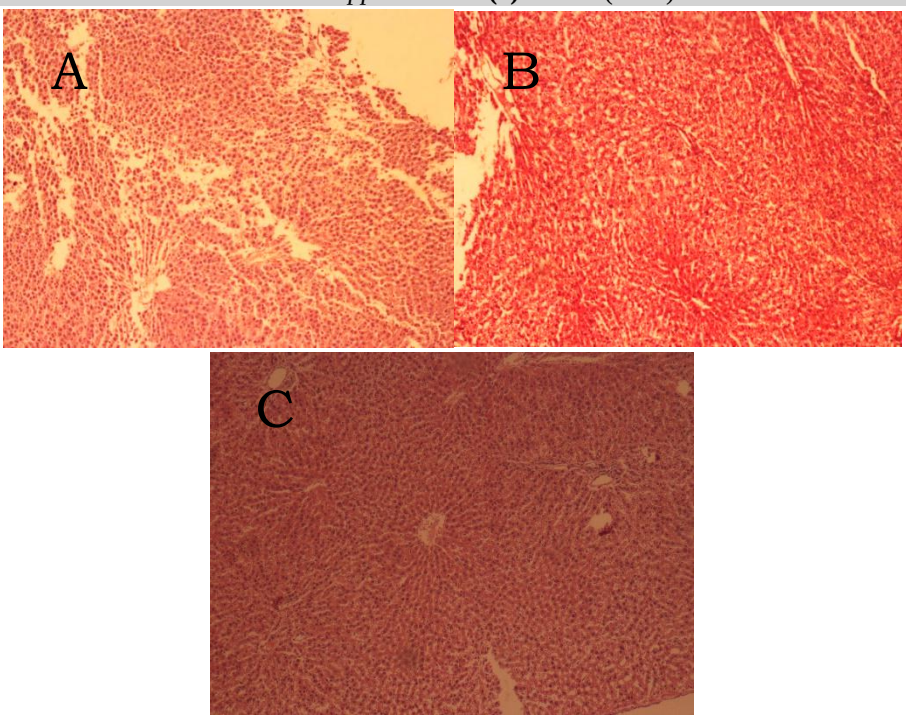

Fig. 3: Histological slides of Liver at 4x maginification. A; control group. B; CCl_4 treated. C; CCl_4 + N. nucifera seeds treated

Table 1: Comparison of body weight, Liver weight and Kidney weight in control, CCl_4 treated and CCl_4 + *N. nucifera* seeds treated group

	CONTROL (group I)	CCl_4 TREATED (group II)[1]	CCl_4 AND *NELUMBO NUCIFERA* SEED TREATED (group III)[2]
Initial body weight	150.6 ± 0.33	157.3 ± 0.66	178.3 ± 1.33
Final body weight	157.33 ± 5.36 α	138.6 ± 11.28 α	173.6 ± 3.71 α
Liver weight	3.65 ± 0.12	6.13 ± 0.87 α	5.14 ± 0.80 α
Relative Liver weight	2.32± 0.08	4.55± 0.81 α	2.95± 0.48 β
Left Kidney weight	0.40 ± 0.04	0.44 ± 0.12 β	0.51± 0.06 β
Right Kidney weight	0.40 ± 0.02	0.48 ± 0.10 β	0.54 ± 0.06 β
Relative Kidney weight (left)	0.25 ± 0.02	0.31 ± 0.03 α	0.29± 0.04 α
Relative Kidney weight (right)	0.24± 0.01	0.32± 0.02 α	0.31± 0.04 α

Values are mean ± S.E.M, n= 6, statistical significance represented with symbols α: P< 0.05 non-significant β: P > 0.05
1: group I compared with group II, 2: group II compared with group III

Table 2: Comparison of serum marker of Liver injury in control, CCl_4 treated and CCl_4 + *N. nucifera* seeds treated group

PARAMETER	CONTROL	CCl_4 TREATED (group II)[1]	CCl_4 AND *NELUMBO NUCIFERA* SEED TREATED (group III)[2]
AST (U/l)	43.66±14.80	60 ± 16.74 β	59 ± 15.69 β
ALT (U/l)	7.33 ± 2.4	20 ± 1.73α	12.16 ± 1.30 α
TB (Mg/dL)	0.23 ± 0.07	0.92± 0.90 α	0.26 ± 0.11 α
Urea (Mg/dL)	15.03 ± 8.28	47.27 ± 13.74 α	33.3 ± 8.05 α
BUN (Mg/dL)	7.02 ± 3.86	22.11 ± 6.44 α	15.55 ± 3.76 α
Creatinine (Mg/dL)	0.35 ± 0.09	0.71± 0.25 α	0.48 ± 0.26 α

Values are mean ± S.E.M, n= 6, statistical significance represented as symbol α: P< 0.05 non-significant β: P > 0.05 Liver *function tests*, ALT (Alanine Aminotransferase), AST (aspartate Aminotransferase), TB (total bilirubin), BUN (blood urea Nitrogen). 1: group I compared to group II, 2: group II compared to group III

Table 3: Histopathological features of Liver in control, CCl_4 treated and CCl_4 + *N. nucifera* seeds treated group

FEATURE	CONTROL	CCl_4 TREATED	CCl_4 AND *NELUMBO NUCIFERA* SEED TREATED
Enlargement	0	+	+
Necrotic Hepatocytes	0	0	0
Lobular Inflammation	0	+	+
Hepatocyte Ballooning	0	0	0
Hemorrhage	0	+	0
Macrovasicular Steatosis	0	0	0
Microvasicular Steatosis	0	0	0
Cholestatic Hepatitis	0	0	0
Fibrosis	0	0	0

Scoring: absent (0), mild (+), moderate (++), severe (+++) Absent 0% mild 10-20% moderate 30-50% severe > 50%

Table 4: Histopathological features of Kidney in control, CCl4 treated and CCl4 + *N.nucifera* seeds treated group

FEATURE	CONTROL	CCl_4 TREATED	CCl_4 AND *NELUMBO NUCIFERA* SEED TREATED
Glomerular Basement Membrane Thickning	0	+	0
Congestion of Bowman	0	+	+
Tubular Cell Swelling	0	+	+
Tubular Brushborder Loss	0	+	0
Necrosis of Epithelium	0	+	0
Collagen Deposition	0	0	0
Interstitial Inflammation	0	+	+

Scoring: absent (0), mild (+), moderate (++), severe (+++) Absent 0% mild 10-20% moderate 30-50% severe > 5

CONCLUSION

In the present study, it is evidenced that Lotus seeds prevent process of Lipid peroxidation and repair damaged proteins. *N. nucifera* Gaertn seed is beneficial to use in dietary Supplement as it can protect against Liver and Kidney injury.

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CONFLICT OF INTEREST

The author declares that she has no conflict of Interest.

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