ISSN No:-2456-2165

Bioefficacy Effect of *Nelumbium nucifera* on CCl_4 Induced Hepatotoxicity in Albino Rats

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Abstract:- Historically, since the dawn of civilization, medicinal plants are employed to treat a variety of physical disabilities in humans. India has a long history of using traditional medical practices and a traditional healthcare system. Treatment costs in poor nations are greatly influenced by traditional medicine. Consuming medicinal plants served as the primary form of treatment in ancient times, protecting and curing a number of illnesses until the advent of synthetic medications in the nineteenth century. The failure of a specific organ or even death can result from the toxicity of different organs. Since liver damage from numerous important causes, such as hepatotoxicity, is one of the harmful and hazardous toxic effects of the liver. This may result in serious effects in animals too. The disease that causes this harmful effect has been found, however the severity is low and the need is great. 40% of medicines prescribed are made from herbs, and the most popular pharmaceutical preparations in the world are made from natural ingredients. Approximately 80% of people in the globe utilize herbal remedies. This is evidence of the comeback in popularity of herbal therapy. Medicinal plants, herbs, roots, and fruits are the foundation of herbal medicine, sometimes referred to as phytomedicine. Fruits have been utilized for ages in traditional medicine, along with herbs, roots, stems, rhizomes, and bark. Medicinal fruits are also referred to as functional foods or nutraceuticals due to their purported health benefits. They fulfill both a dietary/nutritional and a medicinal purpose. Not only for humans even for some animals also have herbal medicines been preferred for their well being. In the same way, our article is based on the effect of *Nelumbium nucifera* on carbon tetrachloride which may induce the hepatotoxicity in Albino rats. Lets discuss the beneficial aspects of *Nelumbium* and the various effects of hepatotoxicity and how can be it treated elaborately.

Keywords:- *Hepatotoxicity, CCl_4, Nelumbium nucifera.*

I. INTRODUCTION

The oldest type of medicine that mankind is aware of is herbal medicine. The World Health Organisation reports that it is the type of medicine that is most commonly practiced worldwide. About 75–80% of the population in developing countries still primarily uses plant-based medicines for primary healthcare. The use of herbal remedies is two to three times greater than the use of conventional drugs globally, according to the World Health Organisation (WHO). The extraordinary herbs and flowers found in nature serve as a medicine chest for many modern drugs.

Herbal medications are widely recognised as the most important subset of complementary and alternative medicine. To encourage their correct use and assess their potential as the main ingredient in the creation of new pharmaceuticals, it is crucial to study medicinal plants. The chemical components of the plant may or may not have therapeutic benefits. Ayurveda, Unani, Siddha, Yoga and naturopathy are all branches of the Indian medical system, mostly based on medicinal plants that have been cultivated for a long time. Due to the negative side effects of contemporary synthetic medications, herbal remedies are becoming more and more important in the treatment of many disorders (Alok Bhardwaj & K. P. Modi, 2016).

Plants, animals, and minerals are natural resources used to treat human diseases. However, the foundation of contemporary medicine has always been the old wisdom, and it will continue to be a significant source of future treatment. The history of medicine has almost predated the development of human civilization. According to Lahlou 2013, the vast majority of new drugs are created from natural products (secondary metabolites) and compounds obtained from natural sources.

The most important source of fresh leads for pharmaceutical discovery has always been plants. It is now believed that roughly 25% of the compounds in our current pharmaceuticals come from plants. Currently, at least 120 different chemical compounds originating from plants are used as major medications in one or more Nations worldwide. A number of the medications on the market right now are straightforward synthetic alterations of or replicas of the naturally occurring chemicals (Leslie Taylor, 2000).

Green embryo's of mature *Nelumbo nucifera* Gaertn. Nelumbonaceae seeds, also known as plumula nelumbinis, have anti-inflammatory and antioxidant properties, and are widely used in traditional Chinese medicine. However, there have been few studies on the principal alkaloid components of P. nelumbinis and the anti-inflammatory activities of alkaloids. (Xie *et al*., 2023)

ISSN No:-2456-2165

➢ *Nelumbium nucifera:*

Nelumbium nucifera is also known as sacred lotus, Indian lotus, Indian bean or lotus alone. It was the national flower of India. *Nelumbium nucifera* is now classified as a member of the monogeneric families Nymphaeaceae. Nicotiana nucifera has a wide range of therapeutic applications. Traditional uses of leaves, rhizomes, seeds, and flowers include pharyngitis, chest pain, spermatorrhea, leucoderma, smallpox, dysentery, cough, hematemesis, epistaxis, hematuria, metrorrhagia, hyperlipidemia, fever, cholera, hepatopathy (and flea catopathy). Mukherjee et al., 2006).

Large aquatic herb *Nelumbo nucifera* Gaertn. (Family: Nymphaeaceae) has thick, creeping rhizomes that are yellowish white in colour. There are two varieties: one with white flowers is frequently referred to as "Pundarika" or "sveta kamala," while the other is known as "Rakta Kamala" and has pink or reddish pink blooms (Pulok K. Mukherjee *et al*., 1996).

Nelumbium nucifera has hepatoprotective effects that can stop liver damage (Lishuang LV et al., 2012). The largest gland in the body, the liver, acts as the engine of the body's metabolism. Maintaining, functioning and regulating homeostasis are important functions of the liver. It is involved in all metabolic pathways including reproduction, nutrient supply, disease prevention and growth (Sharma et al., 1991). The main functions of the liver are the metabolism of carbohydrates, proteins and fats, the removal of toxins, the secretion of bile and the storage of vitamins. These functions help keep the liver healthy and are important in maintaining overall health and well-being (Subramaniam and Pushpangadan, 1999). According to several studies (Larrey, 2000; Biour et al., 2004; Upadhyay et al., 2010; Porceddu et al., 2012), more than a thousand drugs and substances can damage the liver. Based on Pandit et al (2012), drug-induced liver injury may account for 50% of all cases of acute liver failure, 10% of acute hepatitis and 5% of hospitalizations. We've covered *Nelumbium nucifera* major impact on drug-induced hepatotoxicity in this article.

➢ *Carbon Tetrachloride*

CCl_4 is an essential component of industrial chemicals. It is used as a solvent to create other chemicals, as a dry-cleaning agent, and in laboratories to create a hepatotoxicity model. It causes harm to the lungs, liver, kidney, central nervous system, and gastrointestinal system. CCl_4 is used to imitate hepatotoxicity utilising a variety of techniques (Said *et al*., 2022).Significantly toxic polycyclic aromatic hydrocarbon (PAH) metabolites are toxins that affect the liver and build up ROS, which is what causes pro-inflammatory pathways to become active.

II. MATERIALS AND METHODS

The flower of *Nelumbium nucifera* were collected in the month of January in and around Trichy. Fresh petals of the flowers were powered after drying at room temperature.

➢ *Saline Extract*

About 2 g of salt extract was obtained from 15 g of dry flower. 15 g of dried petal powder was dissolved in 500 ml of saline and magnetically stirred at room temperature for 24 hours. The filtrate was freeze-dried and used for oral feeding.

➢ *Animals*

Fifteen locally bred adult albino rats (80-100 g each) were used in this study. Rats received commercial pelleted rat food and water. Albino rats were encased in a clean polypropylene cages.

➢ *Experimental Procedure*

Fifteen animals were equally divided into three groups of five animals each.

- Group I: Served as a control, which was fed only with pelleted diet.
- Group II: Albino rats were treated with CCl_4 at a dosage of 0.7 ml/kg body weight intraperitoneally, thrice a week for two weeks.
- Group III: Simultaneous treatment of *Nelumbium nucifera* (50 mg/Kg of body weight) given orally and CCl_4 injection (0.7 ml/Kg body weight intraperitoneally) thrice a week for two weeks.

After 15 days, animals were sacrificed under mild chloroform anesthesia. Blood and liver samples were collected. The livers were preserved in 10% formaldehyde for histological analysis. Blood was collected from rats using the heart puncture method. Serum was separated from the collected blood, and the following parameters were studied: Serum Glutamate Oxaloacetate Transaminase (SGOT) by the Reitman and Farnkel method (1957).

- Serum Glutamate Pyruvate Transaminase (SGPT) using the Reitman and Farnkel method (1957).
- Serum Alkaline Phosphatase by King and Armstrong (1934).
- Serum Bilirubin by Malloy and Evelyn method (1937).

➢ *Histological Studies*

For histological analysis, the liver was fixed using Bouin’s solution. Classical paraffin sectioning and hematoxylin and eosin staining were used. Fixation, dehydration, clearing, impregnation, embedding, sectioning, staining, and mounting are some of the procedures used to prepare tissues for histological research (Bancroft and Stevans 1977).

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III. RESULT

The current investigation assessed the *Nelumbium nucifera* extract's hepatoprotective potential against CCl_4-induced hepatotoxicity in rats. Three groups of mice were used for the physical, biochemical, and histological studies: control (Group I), CCl_4-treated (Group II), and CCl_4 + extract-treated (Group III).

➢ *Effect of Body Weight and Liver Weight*

The body and liver weights of three different groups of animals are shown in Tables 1 and 2.

Table 1 Body Weight

Group	Weight in gram					Mean ± SD
	1	2	3	4	5	
Control	85	89	87	90	88	87.8 ± 1.72
CCl_4 treated	77	76	74	70	74	74.2 ± 2.4
CCl_4 treated + extract	85	80	81	82	83	82.2 ± 1.72

Table 2 Liver Weight

Group	Weight in gram					Mean ± SD
	1	2	3	4	5	
Control	4.00	4.20	4.18	4.48	4.70	4.29 ± 0.23
CCl_4 treated	3.86	3.08	3.40	3.82	3.86	3.60 ± 0.31*
CCl_4 treated + extract	4.36	3.5	4.28	4.4	4.4	4.19 ± 0.34**

Values are expressed as mean ± SD for five animals in each group.

*p-value< 0.05 Vs control

*p-value < 0.05 Vs CCl_4 treated

➢ *Effect on the biochemical parameters:*

- *Effect of Serum Transaminases (SGOT & SGPT)*

Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT), are the primary hepatic marker enzymes. The activities SGOT and SGPT, are presented in Tables 3 and 4, respectively.

Table 3 SGOT

Group	Enzyme activity in U/ml					Mean ± SD
	1	2	3	4	5	
Control	16	16.1	16.3	16.5	16.9	16.36 ± 0.32
CCl_4 treated	65.5	163.9	166.0	169.0	161.5	165.08 ± 2.62*
CCl_4 treated + extract	22	22	16	16	18	18.8 ± 2.71**

Table 4 SGPT

Group	Enzyme activity in U/ml					Mean ± SD
	1	2	3	4	5	
Control	15	13	16	14	17	15.07 ± 1.41
CCl_4 treated	55	63	58	62	58	59.2 ± 2.92*
CCl_4 treated + extract	19	13	15	21	11	15.8 ± 3.7**

Values are expressed as mean ± SD for five animals each group.

*p-value < 0.05 Vs control

*p-value < 0.05 Vs CCl_4 treated

- *Effect on Alkaline Phosphatase (ALP) and Serum Bilirubin*

The ALP activity and serum bilirubin level are shown in table 5 and 6

Table 5 Alkaline Phosphatase

Group	Enzyme activity in KA Units					Mean ± SD
	1	2	3	4	5	
Control	6.0	6.60	6.29	6.40	6.09	6.27 ± 0.21
CCl_4 treated	16.0	16.16	16.22	16.33	16.48	16.23 ± 0.16*
CCl_4 treated + extract	8.32	8.08	8.56	8.03	8.21	8.24 ± 0.18**

ISSN No:-2456-2165

Table 6 Bilirubin

Group	Concentration in mg%					Mean ± SD
	1	2	3	4	5	
Control	0.50	0.51	0.52	0.55	0.59	0.53 ± 0.03
CCl_4 treated	1.1	1.15	1.05	1.20	1.05	1.10 ± 0.05 *
CCl_4 treated + extract	0.89	0.81	0.82	0.80	0.86	0.83 ±0.03 **

Values are expressed as mean ± SD for five animals each group.

*p-value < 0.05 Vs control

*p-value < 0.05 Vs CCl_4 treated

➢ *Histological Studies*

Histological studies were conducted using liver samples from control (Group I), CCl_4-treated (Group II), CCl_4-treated and *Nelumbium nucifera* extract-treated mice (Group III). Normal hepatic cells with well-preserved cytoplasm, pronounced nuclei, nucleoli, and central veins were visible in liver slices from control animals (Group I). More hydropic alterations surrounding the central veins, cellular degeneration, fatty changes, extensive hepatocellular necrosis, Kupffer cell hyperplasia, central lobular necrosis, and steatosis were visible in the sections of the animals treated with CCl_4 (Group II – 1, 2 and 3). Animals treated with extracts significantly recovered from injury (Group III).

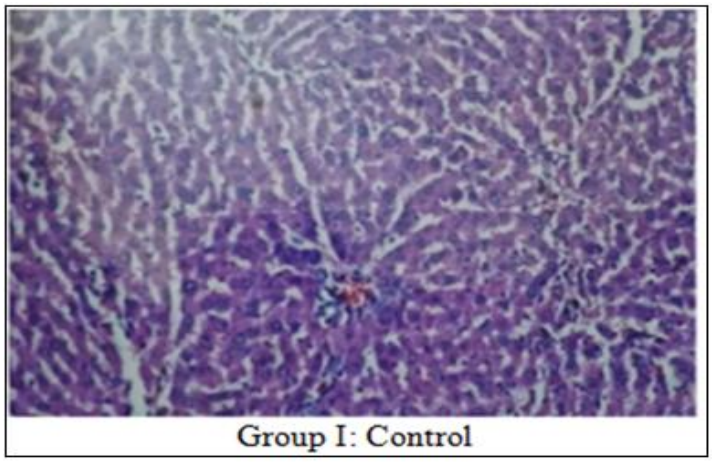

Fig 1 Liver Samples from Control

Legend: Liver sections showed normal hepatic cells with well-preserved cytoplasm, prominent nuclei, nucleoli, and central veins

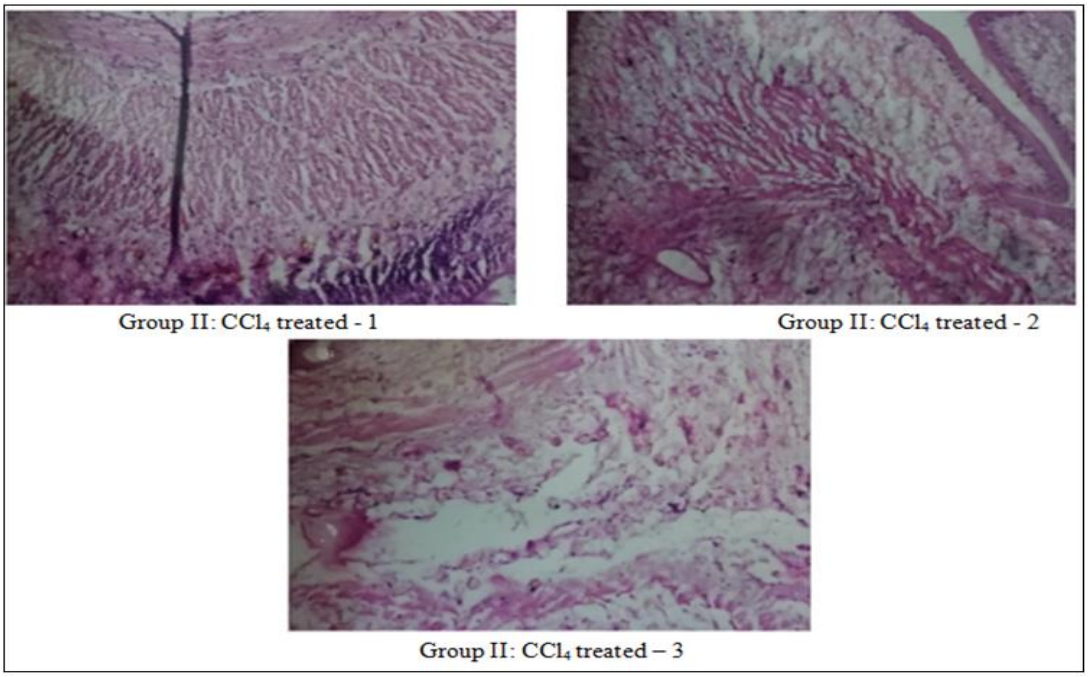

Fig 2 CCl_4-Treated

Legend: Group II: CCl_4 treated - 1 showing hydropic changes around the central veins and cellular degeneration.
Group II: CCl_4 treated - 2 showing fatty changes, widespread hepatocellular necrosis and Kupffer cell hyperplasia.
Group II: CCl_4 treated - 3 showing central lobular necrosis, and steatosis.

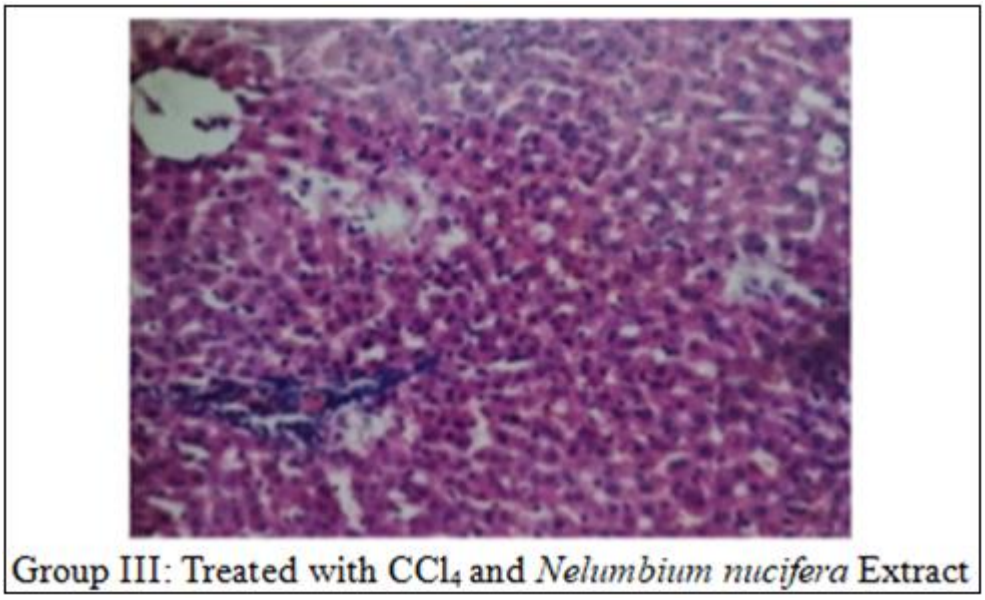

Fig 3 Significant Recovery from Damage

Legend: Significant recovery from damage

IV. DISCUSSION

➢ *Effect of Body and Liver Weight:*

Both liver weight and body weight were found to be significantly decreased in CCl_4 treated animals as compared to the control animals.

In CCl_4 + *Nelumbium nucifera* extract-treated animals, body weight and liver weight were also decreased compared to the control, but the values were not significant. However, when compared to CCl_4 treated animals, the liver and body weight values of the extract-treated animals were significant. High Fat Diet, increased serum levels of creatinine, gamma-glutamyl transferase (GGT), glutamic-oxaloacetic transaminase (GOT), and alkaline phosphatase (ALP), while decreasing serum levels of albumin and total protein (Abdelrahman *et al.*, 2023).

➢ *Effect on the Biochemical Parameters:*

- *Effect on Serum Transaminases (SGOT & SGPT) by Reitman and Farnkel Method (1957).*

SGOT activity was significantly increased in CCl_4 treated animals in comparison to that in the control. A very high SGOT value was observed in the CCl_4 group (three times per week).

A significant decrease in SGOT levels was noted in Group III animals owing to the administration of *Nelumbium nucifera* extract (50 mg/kg body weight).

Similar to SGOT activity, SGPT activity was found to be significantly increased in CCl_4 treated animals as compared to the control. Administration of the extract caused a significant decrease in SGPT levels in Group III animals.

- *Effect on Alkaline Phosphatase (ALP) and Serum Bilirubin*

When CCl_4 was administered to animals, the Alkaline Phosphatase activity was higher than it was in control animals. However, group III animals underwent concurrent CCl_4 and extract therapy, which resulted in a decrease in these two biochemical measures.

Our findings suggested that CCl_4 induced hepatotoxicity in rats can be prevented by using *Nelumbium nucifera* extract.

One of the most frequently utilized hepatotoxins in experimental investigations of liver disease is carbon tetrachloride (Johnston, 1998).

According to Srivastava *et al*., (1990), the active metabolites of CCl_4 - trichloro methyl radicals (CCl_3-) - were principally responsible for the substance's hepatotoxic effects. The membrane lipids of the endoplasmic reticulum, which are rich in poly unsaturated fatty acids, are subjected to peroxidative destruction when these activated radicals bond covalently to the macromolecules. As a result, lipid peroxidases are produced, which in turn results in compounds such malondialdehyde (MDA), which harm membranes. One of the main factors contributing to the liver toxicity of CCl_4 is the lipid peroxidative breakdown of hepatic cell membranes (Cortan *et al*., 1994; Kaplowitz *et al*., 1986).

Enzymes leak into the bloodstream and damage liver cell membranes. This study showed that all CCl_4-treated rats (group II) showed liver dysfunction as their serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and serum bilirubin (Table III IV, V and VI). These data confirm previous reports of CCl_4 hepatotoxicity (Shenoy et al., 2001, Buwa et al., 2001 and Subramoniam et al., 1998).

Co-treatment with *Nelumbium nucifera* and CCl_4 shows significant improvement in CCl_4-induced damage. This effect is particularly evident at a dose of *Nelumbium nucifera* (50 mg/kg body weight) because it caused a drastic decrease in transaminases (SGOT and SGPT) and ALP activity, which is associated with a decrease in bilirubin levels.

- *Histological Studies*

Histologically, treatment of *Nelumbium nucifera* together with CCl_4 also showed effects of hepatocytes from their damage induced by CCl_4.

Many compounds protect the liver against CCl_4 by reducing CCl3 free radical formation (Mailing et al., 1974) or by attenuating CCl_4-induced lipid peroxidation (Yasuda et al., 1980). It is possible that *Nelumbium nucifera* at a dose level (50 mg/kg body weight) can accelerate liver cell regeneration by reducing the leakage of GOT, GPT and ALP into the blood and lowering their serum levels. This is because the improved histology of the liver after treatment with *Nelumbium nucifera* extract was compared to that seen in animals treated with CCl_4. As the liver parenchyma heals and new liver cells form, the level of serum transaminases normalizes.

V. CONCLUSION

According to the results of our study, *Nelumbium nucifera* effectively protects albino rats against CCl_4-induced hepatotoxicity in vivo. *Nelumbium nucifera* has a remarkable ability to reduce the activity of transaminases (SGOT and SGPT) and ALP, which in turn lowers bilirubin levels. Therefore, more experimental and clinical studies are needed to elucidate the exact functional mechanism of *Nelumbium nucifera* to support its use as an effective therapeutic agent.

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