**International Journal of Current Trends in Pharmaceutical Research**

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**Research Article** **Open Access**

# Evaluation of Hepatoprotective and *In-Vitro* Antioxidant Activities of "*Nelumbo Nucifera*" Flowers in Wistar Albino Rats

**Hussain Basha M*, Nagesh Kuruba, Nageswaraiah B, Nagaraju B**

*Sri Lakshmi Venkateswara College of Pharmcy, Proddatur, Kadapa, Andhra Pradesh, India.*

**A B S T R A C T**

With the advances in the field of science and technology, came the mostly used drugs of the day, the synthetic drugs. Though effective they possess side-effects and sometimes life threatening adverse effects. Hence, during the past several decades there has been a global trend for the revival of interest in the traditional system of medicine. Simultaneously, the need for basic scientific investigations of medicinal plants using indigenous medical systems has become more interesting and relevant. In the absence of reliable liver protection drugs in modern medicine, in our review of literature, we found that a plant "*Nelumbo nucifera*" contains alkaloids and flavonoids as the major chemical constituents and thus has the hepatoprotective and antioxidant potential. Taking the above information into consideration this study is undertaken to evaluate the hepato protective and antioxidant activities of "*Nelumbonucifera*" flowers in wistar albino rats. To evaluate this hepato protective activity need to prepare the ethanolic and aqueous flower extracts of *Nelumbo nucifera* (EENN and AENN respectively) and for evaluation of the in vitro antioxidant activity of ethanolic and aqueous extracts of *Nelumbonucifera* flowers in rats by different methods.

**Keywords:** Extended release, Eudragit polymers, Wet Granulation, Korsemeyer-peppas, diffusion, Erosion.

**A R T I C L E I N F O**

**CONTENTS**



**Article History: Received** 16 October 2014, **Accepted** 18 December 2014, **Available Online** 15 January 2015

***Corresponding Author**
Hussain Basha M
Sri Lakshmi Venkateswara College of Pharmcy, Proddatur, Kadapa, A.P, India
Manuscript ID: IJCTPR2347

**PAPER-QR CODE**

**Citation:** Hussain Basha M, et al. Evaluation of Hepatoprotective and *In Vitro* Antioxidant Activities of "*Nelumbo Nucifera*" Flowers in Wistar Albino Rats. *Int. J. Curnt. Tren. Pharm, Res.*, 2015, 3(1): 739-747.

## 1. Introduction

The use of medicinal plants with therapeutic properties is as old as human civilization. Various written records about medicinal plants that date back at least 5000 years support the use of mineral, plant and animal parts as source of drugs since antiquity. Texts of ancient Egyptians, Sumerians, and Greeks written 3100 BC precisely described the natural remedies in their herbal texts [3]. With the advances in the field of science and technology, came the mostly used drugs of the day, the synthetic drugs. Though effective they possess side-effects and sometimes life threatening adverse effects. Hence, during the past several decades there has been a global trend for the revival of interest in the traditional system of medicine. Simultaneously, the need for basic scientific investigations of medicinal plants using indigenous medical systems has become more interesting and relevant (Singaravel Sengottuvelu *et al.,* 2008) [2].

Liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy production and reproduction. Because of its unique metabolism and relationship to the gastrointestinal tract, the liver is an important target of the toxicity of drugs, xenobiotics and oxidative stress (Harmut et al., 2002). More than 900 drugs, toxins and herbs have been reported to cause liver injury and drugs account for 20% - 40% of all instances of fulminant liver failure. In the absence of reliable liver protection drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief. Attempts are being made globally to get scientific evidences for these traditionally reported herbal drugs. This scenario proves a severe necessity to carry out research works related to hepatotoxicity [3].

The use of natural remedies for the treatment of liver diseases has a long history, starting with the ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines. The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver diseases by carefully synergizing the strengths of the traditional systems of medicine with that of the modern concept of evidence-based medicinal evaluation, standardization of herbal products and randomized placebo controlled clinical trials to support clinical efficacy (Thyagarajan et al., 2008). Inspite of tremendous advances in modern medicine, no effective drugs are available that stimulate liver function and offer protection to the liver from the damage or help to regenerate hepatic cells (Chattopadhyay et al., 2003) [4].

Oxidative stress has been identified to be the major cause of hepatotoxicity which provides that plants with anti-oxidant chemical constituents would be useful in this regard. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them (Grover et al., 2002). Thus we have focused our attention on plants containing flavonoids and tannins as major chemical constituents since they have been proved of their anti-oxidant potential [5]. Taking the above information into consideration this study is undertaken to evaluate the hepatoprotective and antioxidant activities of "*Nelumbonucifera*" flowers in wistar albino rats.

## 2. Materials and Methods

### 2.1 Collection of plant material

The *Nelumbonucifera* flowers used for the present study were collected near Shri Vishnu College of Pharmcay, Bhimavaram, West Godavari district, Andhra Pradesh. The flowers were shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction.

### 2.2 Preparation of Ethanolic Extract

The powdered drug was dried and packed well in Soxhlet apparatus and extracted with 500 ml of ethanol for 72 hrs. The extract was concentrated and dried using Rotary vaccum evaporator. It was kept in a desiccator until used.

### Preparation of Aqueous Extract

The aqueous extract was prepared by maceration; 1000 g of powder was soaked in about 1 L of distilled water for 7 days. The extract was decanted; remaining material was re soaked in about in the distilled water twice. The companied extract was dried completely by using Rotary vaccum evaporator.

### Preparation of Chloroform Extract

The powdered drug was dried and packed well in Soxhlet apparatus and extracted with 500 ml of chloroform for 72 hrs. The extract was concentrated and dried using Rotary vaccum evaporator. It was kept in a desiccator until used.

### 2.3 Qualitative phytochemical screening

The following tests were carried out on the standardized herbal extracts to detect various phytoconstituents present in the extracts of N*elumbonucifera.* Such as Tests for carbohydrates and glycosides, Molisch's test, Legal's test Borntrager's test,Test for alkaloids, Test for saponins, Test for proteins and free amino acids, Test for flavonoids etc,.

**Equipment:** Autoanalyzer

**Animal Model Used For Investigation (Albino Wister Rats):**

| | | |
|---|---|---|
| Class | : | Mammalia |
| Family | : | Muridae |
| Order | : | Rodent |
| Genus | : | Rattus |
| Scientific name | : | Rattusnorvegicus |

### 2.4 Hepatoprotective Activity

#### 2.4.1 Paracetamol induced-hepatotoxicity

The rats were selected and divided into 7 groups each containing 6 animals. Silymarin and flower extract was dissolved in water with 2% gum acacia suspension. The treatment protocol was planned in such a way that the flower extract in preventive aspect of paracetmol induced hepatotoxicity. The dose of PCM to induce the hepatic damage was selected as 2 gm/kg body weight for 3 days.

The dose of silymarin used was 100mg/kg body weight. The doses of *Nelumbonucifera*aqueous and alcoholic extracts were 200 mg/kg and 400 mg/kg.

Group I - Control: 2% gum acacia (1ml/kg,p.o.) once daily for 3 days.

Group II - Toxicant (paracetamol 2 g/kg, *p.o.*) once daily for 3 days.

Group III- Served as Standard (Silymarin 100 mg/kg, *p.o.*) + after 30 min paracetmol (2 gm/kg, p.o.) for 3 days

Group IV- Ethanol extract of *Nelumbonucifera* **(**200 mg/kg, *p.o.*)+ After 30 min paracetmol (2 gm/kg, p.o.) for 3 days.

Group V - Ethanol extract of *Nelumbonucifera***(**400 mg/kg, *p.o.*)+ After 30 min paracetmol (2 gm/kg, p.o.) for 3 days.

Group VI- Aqueous extract of *Nelumbonucifera***(**200 mg/kg, *p.o.*)+ After 30 min paracetmol (2 gm/kg, p.o.) for 3 days

Group VII - Aqueous extract of *Nelumbonucifera*(400mg/kg, *p.o.*)+ After 30 min paracetmol (2 gm/kg, p.o.) for 3 days

On 0 and 4$^{th}$ day blood sample was collected from all animals by retro orbital puncture method. Serum was separated by centrifugation (3000 rpm for 15 min) and is subjected for estimation of biochemical parameters (SGOT, SGPT, ALP and TB).

**2.4.2 CCl4 induced-hepatotoxicity**

The rats were selected and divided into 7 groups each containing 6 animals. Silymarin and flower extract was dissolved in water with 2% gum acacia suspension. The treatment protocol was planned in such a way that the flower extract in preventive aspect of CCl4 induced hepatotoxicity. The dose of CCl4 to induce the hepatic damage was selected as 1 ml/kg body weight for 14 days. The dose of silymarin used was 100mg/kg body weight. The doses of *Nelumbonucifera*aqueous and alcoholic extracts were 200 mg/kg and 400 mg/kg.

Group I- Control: 2% gum acacia (1ml/kg.) once daily for 7 days.

Group II - Toxicant (CCl4 1 ml/kg, s.c.) once daily for 7 days.

Group III -Served as Standard (Silymarin 100 mg/kg + after 30 min CCl4 (1 ml/kg, s.c.)once daily for 7 days.

Group IV- Ethanol extracts of*Nelumbonucifera* **(**200 mg/kg.)+ After 30 min CCl4 (1 ml/kg, s.c.) once daily for 7 days.

Group V - Ethanol extract of *Nelumbonucifera* **(**400 mg/kg,.)+ After 30 min paracetmol (1 ml/kg, s.c.) once daily for 7 days.

Group VI- Aqueous extract of *Nelumbonucifera* **(**200 mg/kg, .)+ after 30 min paracetmol (1 ml/kg, s.c.) once daily for 7 days.

Group VII - Aqueous extract of *Nelumbonucifera* **(**400mg/kg, )+ after 30 min paracetmol (1 ml/kg, s.c.) once daily for 7days.

On 0 and 15$^{th}$ day blood sample was collected from all animals by retro orbital puncture method. Serum was separated by centrifugation (3000 rpm for 15 min) and is subjected for estimation of biochemical parameters (SGOT, SGPT, ALP and BT).

**2.5 Biochemical parameters**

The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit using Semi Autoanalyser. These are estimated through Aspartate Amino Transferase (Ast) or SGOT Method, Alanine Amino Transferase (Alt) or Sgpt Method, Alkaline Phosphatase (Alp) and Total Bilirubin (Tb) Method.

**Reagents**

1. Tris –buffer 40mM solution :
   The reagent (484.4mg) was weighed, transferred to a volumetric flask and was made to 100ml with distilled water and the PH was adjusted to 7.0
2. Potassium chloride 300mM solution :
   The reagent (1.12g) was weighed, transferred to a volumetric flask and was made to 100 ml with distilled water
3. Ammonium ferrous sulphate 0.16mM solution :
   The reagent (31.37 mg) was weighed, transferred to a volumetric flask and was made to 100ml with distilled water.
4. Ascorbic acid 0.06mM solution :
   The reagent (5.3mg) was weighed, transferred to a volumetric flask and was made to 100ml with distilled water.
5. Thiobarbituric acid 0.8% solution :
   The reagent (0.8g) was weighed, transferred to a volumetric flask and was made to 100ml with distilled water etc,.

## 3. Results and Discussion

**3.1 Hepatoprotective Activity**

**3.1.1 Paracetamol Induced Toxicity:**

**Table 3.1:** Basal level of selected serum biochemical parameters in rats, Group-1 on day 0 (n=6)

| Parameters | R1 (120 g) | R2 (140 g) | R3 (150 g) | R4 (120 g) | R5 (130 g) | R6 (160 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 69 | 67 | 54 | 58 | 65 | 59 | 62±2.39 |
| SGPT(IU/L) | 47 | 51 | 49 | 42 | 37 | 39 | 44.16±2.32 |
| ALP(IU/L) | 138 | 134 | 155 | 152 | 159 | 147 | 147.5±4.01 |
| TB(mg/dl) | 0.62 | 0.52 | 1.0 | 0.9 | 0.46 | 0.72 | 0.70±0.09 |

**Table 3.2:** Basal level of selected serum biochemical parameters in rats, Group-2 on day 0 (n=6)

| Parameters | R1 (150 g) | R2 (130 g) | R3 (140 g) | R4 (120 g) | R5 (150 g) | R6 (170 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 61 | 65 | 64 | 67 | 63 | 60 | 63.3±1.05 |
| SGPT(IU/L) | 40 | 39 | 40 | 42 | 41 | 46 | 46.3±0.42 |
| ALP(IU/L) | 144 | 143 | 156 | 145 | 142 | 152 | 147.5±2.31 |
| TB(mg/dl) | 0.5 | 0.45 | 0.88 | 0.9 | 0.75 | 0.53 | 0.66±0.08 |

**Table 3.3:** Basal level of selected serum biochemical parameters in rats, Group-3 on day 0 (n=6)

| Parameters | R1 (160 g) | R2 (120 g) | R3 (150 g) | R4 (130 g) | R5 (140 g) | R6 (140 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 64 | 63 | 65 | 70 | 58 | 61 | 63.5±1.65 |
| SGPT(IU/L) | 39 | 46 | 47 | 41 | 44 | 42 | 43.1±1.25 |
| ALP(IU/L) | 151 | 154 | 136 | 159 | 144 | 150 | 149±3.29 |
| TB(mg/dl) | 0.42 | 0.55 | 0.66 | 0.52 | 0.81 | 0.4 | 0.56±0.06 |

**Table 3.4:** Basal level of selected serum biochemical parameters in rats, Group-4 on day 0 (n=6)

| Parameters | R1 (130 g) | R2 (140 g) | R3 (190 g) | R4 (160 g) | R5 (150 g) | R6 (170 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 66 | 69 | 72 | 52 | 65 | 68 | 65.3±2.85 |
| SGPT(IU/L) | 44 | 37 | 39 | 45 | 42 | 43 | 41.6±1.26 |
| ALP(IU/L) | 135 | 156 | 154 | 158 | 143 | 139 | 147.5±3.97 |
| TB(mg/dl) | 0.65 | 0.56 | 0.9 | 0.50 | 0.65 | 0.77 | 0.67±0.06 |

**Table 3.5:** Basal level of selected serum biochemical parameters in rats, Group-5 on day 0 (n=6)

| Parameters | R1 (170 g) | R2 (160 g) | R3 (120 g) | R4 (130 g) | R5 (150 g) | R6 (130 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 65 | 66 | 56 | 69 | 60 | 75 | 65.1±2.73 |
| SGPT(IU/L) | 48 | 41 | 40 | 37 | 49 | 46 | 43.5±1.98 |
| ALP(IU/L) | 136 | 153 | 143 | 159 | 149 | 160 | 150±3.81 |
| TB(mg/dl) | 0.59 | 1.0 | 0.88 | 0.50 | 0.46 | 0.55 | 0.66±0.09 |

**Table 3.6:** Basal level of selected serum biochemical parameters in rats, Group-6 on day 0 (n=6)

| Parameters | R1 (190 g) | R2 (120 g) | R3 (140 g) | R4 (160 g) | R5 (180 g) | R6 (160 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 67 | 62 | 66 | 77 | 58 | 59 | 64.8±2.85 |
| SGPT(IU/L) | 37 | 49 | 48 | 43 | 39 | 41 | 42.8±1.97 |
| ALP(IU/L) | 145 | 147 | 156 | 143 | 153 | 140 | 147.3±2.49 |
| TB(mg/dl) | 0.23 | 0.16 | 0.46 | 0.40 | 0.68 | 0.99 | 0.66±0.13 |

**Table 3.7:** Basal level of selected serum biochemical parameters in rats, Group-7 on day 0 (n=6)

| Parameters | R1 (170 g) | R2 (190 g) | R3 (120 g) | R4 (180 g) | R5 (150 g) | R6 (130 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 57 | 64 | 52 | 63 | 66 | 59 | 60.1±2.12 |
| SGPT(IU/L) | 37 | 47 | 40 | 49 | 43 | 35 | 41.8±2.26 |
| ALP(IU/L) | 151 | 164 | 154 | 168 | 148 | 159 | 157.3±3.16 |
| TB(mg/dl) | 0.44 | 0.58 | 0.66 | 0.35 | 0.65 | 0.72 | 0.56±0.06 |

**Table 3.8:** Level of selected serum biochemical parameters in rats, Group-1treated with 2% gum acacia (1ml/kg) daily for 3 days

| Parameters | R1 (120 g) | R2 (140 g) | R3 (150 g) | R4 (120 g) | R5 (130 g) | R6 (160 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 59 | 65 | 75 | 69 | 72 | 67 | 67±2.29 |
| SGPT(IU/L) | 40 | 39 | 37 | 42 | 49 | 51 | 43±2.32 |
| ALP(IU/L) | 138 | 153 | 146 | 159 | 157 | 147 | 180±3.20 |
| TB(mg/dl) | 0.37 | 0.25 | 1.0 | 0.98 | 0.64 | 0.56 | 0.80±0.13 |

**Table 3.9:** Level of selected serum biochemical parameters in rats, Group-2treated with Paracetmol (2 gm/kg) daily for 3 days

| Parameters | R1 (150 g) | R2 (130 g) | R3 (140 g) | R4 (120 g) | R5 (150 g) | R6 (170 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 301.7 | 280 | 491.3 | 436 | 391.5 | 105 | 334.25±56.23 |
| SGPT(IU/L) | 81 | 102 | 89 | 83 | 97 | 95 | 91.16±3.37 |
| ALP(IU/L) | 458 | 413 | 655 | 493 | 525 | 576 | 520±35.31 |
| TB(mg/dl) | 2.7 | 3.5 | 3.6 | 2.9 | 2.5 | 3.2 | 3.06±0.18 |

**Table 3.10:** Level of selected serum biochemical parameters in rats, Group-3treated with Silymarin (100 gm/kg) + Paracetmol (2 gm/kg) daily for 3 days

| Parameters | R1 (160 g) | R2 (120 g) | R3 (150 g) | R4 (130 g) | R5 (140 g) | R6 (140 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 70.6 | 65.4 | 78.4 | 69.5 | 57.8 | 59.9 | 66.93±3.09 |
| SGPT(IU/L) | 57 | 59 | 49 | 42 | 35 | 45 | 47.83±3.73 |
| ALP(IU/L) | 204 | 152 | 237 | 173 | 165 | 180 | 185.16±12.55 |
| TB(mg/dl) | 0.64 | 0.79 | 0.98 | 0.89 | 0.25 | 0.65 | 0.70±0.11 |

**Table 3.11:** Level of selected serum biochemical parameters in rats, Group-4treated with Paracetmol (2 gm/kg) + EENN (200 MG/KG) daily for 3 days

| Parameters | R1 (130 g) | R2 (140 g) | R3 (190 g) | R4 (160 g) | R5 (150 g) | R6 (170 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 71.5 | 45.1 | 79.5 | 69.4 | 73.7 | 54.9 | 65.68±5.30 |
| SGPT(IU/L) | 60 | 13 | 21 | 52 | 50 | 62 | 43±8.49 |
| ALP(IU/L) | 230 | 220 | 280 | 290 | 215 | 270 | 250.8±13.44 |
| TB(mg/dl) | 0.55 | 0.65 | 0.99 | 0.52 | 0.24 | 0.60 | 0.59±0.10 |

**Table 3.12:** Level of selected serum biochemical parameters in rats, Group-5treated with Paracetmol (2 gm/kg) + EENN (400 MG/KG) daily for 3 days

| Parameters | R1 (170 g) | R2 (160 g) | R3 (120 g) | R4 (130 g) | R5 (150 g) | R6 (130 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 57.6 | 66.4 | 48.4 | 39.9 | 59.3 | 65.3 | 56.15±4.18 |
| SGPT(IU/L) | 52 | 88 | 36 | 45 | 49 | 41 | 51.83±7.60 |
| ALP(IU/L) | 322 | 242 | 289 | 230 | 220 | 320 | 270.5±18.66 |
| TB(mg/dl) | 0.98 | 0.82 | 0.57 | 0.59 | 0.65 | 0.52 | 0.68±0.07 |

**Table 3.13:** Level of selected serum biochemical parameters in rats, Group-6treated with Paracetmol (2 gm/kg) + AENN (200 MG/KG) daily for 3 days

| Parameters | R1 (190 g) | R2 (120 g) | R3(140 g) | R4 (160 g) | R5 (180 g) | R6 (160 g) | Average±SEM |
|---|---|---|---|---|---|---|---|
| SGOT(IU/L) | 73.1 | 52.3 | 77.3 | 69 | 55.4 | 68.9 | 66±4.06 |
| SGPT(IU/L) | 62 | 49 | 59 | 58 | 47 | 45 | 53.3±2.93 |
| ALP(IU/L) | 252 | 374 | 390 | 226 | 326 | 341 | 318.16±26.92 |
| TB(mg/dl) | 0.46 | 0.59 | 0.29 | 0.53 | 0.88 | 0.65 | 0.56±0.08 |

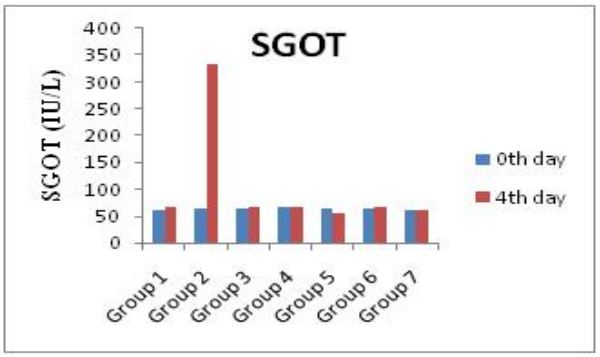


**Fig.3.1.**Effect of EENN and AENN on SGOT in Paracetmol hepatotoxicity

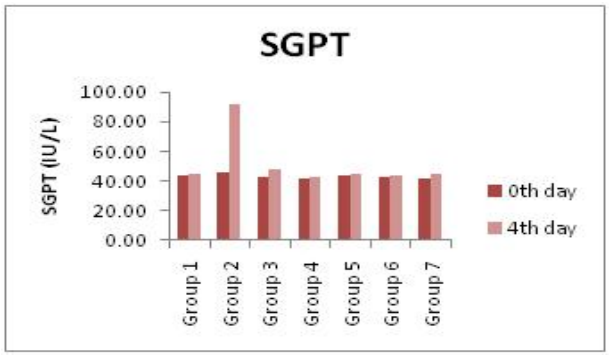


**Figure 3.2:** Effect of AENN on SGPT in Paracetamol Induced hepatotoxicity

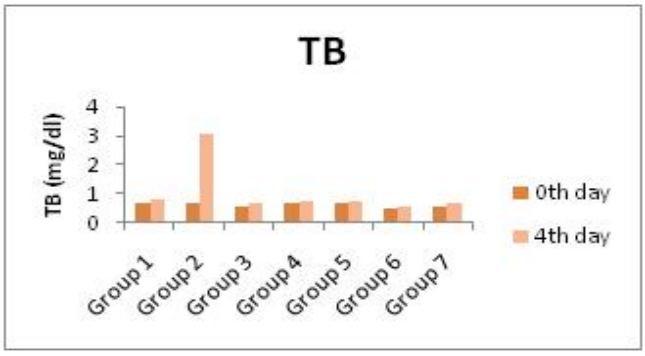


**Fig.3.3.**Effect of EENN and AENN on TB in Paracetamol induced Hepatotoxicity

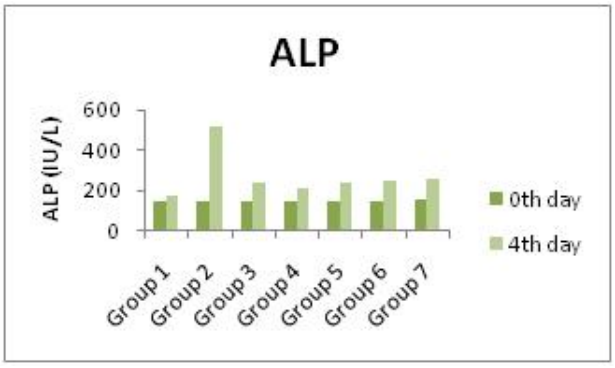


**Fig.3.4.**Effect of EENN and AENN on ALP in Paracetamol induced Hepatotoxicity

**Table 3.14**: Influence of *Nelumbonucifera*ethanolic and aqueous extract on selected serum biochemical parameters in Paracetmol induced hepatotoxicity in rats.

| Treatmet | SGOT(IU/L) | | SGPT(IU/L) | | ALP(IU/L) | | TB(IU/L) | |
|---|---|---|---|---|---|---|---|---|
| | **0 day** | **4 day** | **0 day** | **4 day** | **0 day** | **4 day** | **0 day** | **4 day** |
| Gum acacia (1ml/kg) | 62±2.39 | 67.8±2.29 | 44.16±2.32 | 45±2.32 | 147.5±4.01 | 180±3.20 | 0.70±0.09 | 0.80±0.13 |
| PCM (1gm/kg) | 63.3±1.05 | 334.25±56.23* | 46.3±0.42 | 91.16±3.37* | 147±2.31 | 520±35.31* | 0.66±0.08 | 3.06±0.1* |
| SIL (100mg/kg) | 63.5±1.65 | 66.93±3.09* | 43.1±1.25 | 47.83±3.7* | 149±3.2 | 240.16±12.55* | 0.56±0.06 | 0.70±0.11* |
| EENN (200 mg/kg)+ PCM | 65.3±2.85 | 65.68±5.30* | 41.6±1.26 | 43±4.49* | 147.5±3.97 | 210.80±13.44* | 0.67±0.06 | 0.77±0.10* |
| EENN (400 mg/kg)+ PCM | 65.1±2.73 | 56.15±4.18* | 43.5±1.98 | 44.83±3.60* | 150±3.81 | 239.50±18.66* | 0.66±0.09 | 0.78±0.07* |
| AENN (200 mg/kg)+ PCM | 64.8±2.85 | 66±4.06* | 42.8±1.97 | 43.3±2.93* | 147.3±2.49 | 248.16±16.92* | 0.48±0.13 | 0.56±0.08* |
| AENN (400 mg/kg)+ PCM | 60.1±2.12 | 59.95±4.26* | 41.8±2.26 | 44.83±3.05* | 157.3±3.16 | 256.5±21.07* | 0.56±0.06 | 0.67±0.06* |

**Table 3.15**: Average Percentage Change in Selected Serum Biochemical Parameters in Paracetmol Induced Hepatotoxicity in Rats

| Treatment | SGOT(IU/L) | SGPT(IU/L) | ALP(IU/L) | TB(IU/L) |
|---|---|---|---|---|
| Gum acacia (1ml/kg) | 9.35±1.17 | 2.04±2.33 | 22.03±3.21 | 3.89±0.126 |
| PCM (1gm/kg) | 428.04±56.44* | 126.20±8.26* | 253.74±35.43* | 363.6±0.177* |
| SIL (100mg/kg) | 5.40±3.13* | 8.13±3.74* | 61.18±12.59* | 25±0.104* |
| EENN (200 mg/kg)+ PCM | 0.58±5.29* | 3.36±8.25* | 42.91±13.81* | 1.14±0.090* |
| EENN (400mg/kg)+ PCM | 13.5±4.19* | 3.05±7.62* | 59.66±18.73* | 18.18±0.072* |
| AENN (200 mg/kg)+ PCM | 1.85±4.08* | 1.16±2.93* | 68.47±31.63* | 16.66±0.080* |
| AENN (400 mg/kg)+ PCM | 0.99±4.27* | 7.24±3.06* | 63.06±31.19* | 19.64±0.061* |

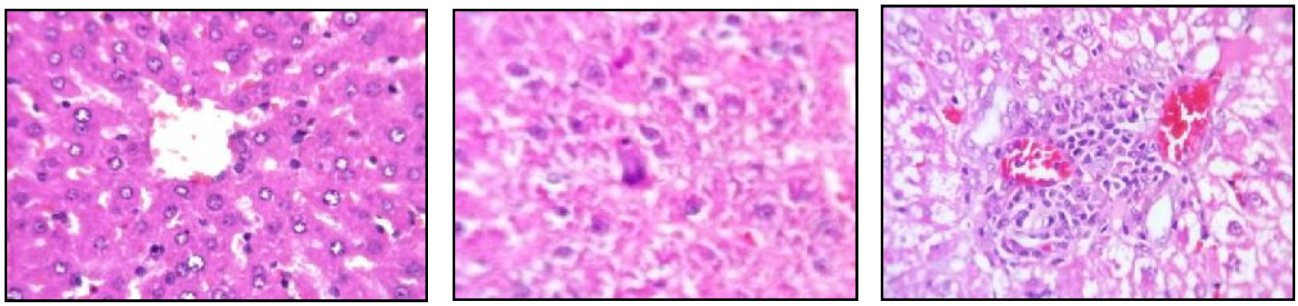

**Figure 3.5:** Liver tissues of control rats
**Figure 3.6:** Liver tissue of Paracetamoltreated rats
**Figure 3.7:** Liver tissues of EENN (200 mg/kg) + Paracetamol treated rats

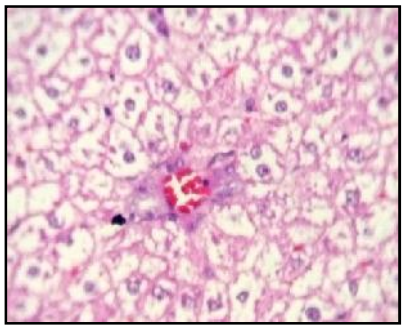
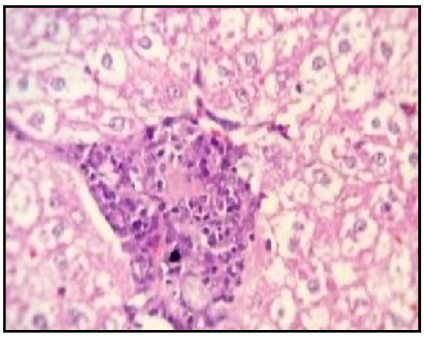
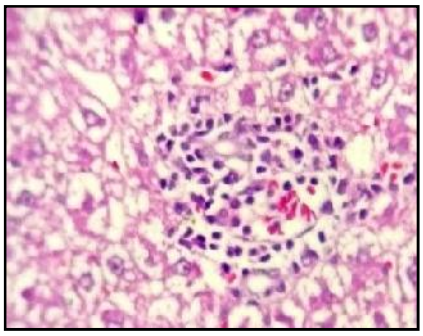

**Figure 3.8:** Liver tissues of EENN (200mg/kg) + Paracetamol treated rats
**Figure 3.9:** Liver tissues of EENN (400 mg/kg) + Paracetamol treated rats
**Figure 3.10:** Liver tissues of AENN (200 mg/kg) + Paracetamol treated rats

### 3.2 Antioxidant Activity:

**Table 3.16:** Percentage inhibition of DPPH by ethanolic and aqueous extracts of *Nelumbonucifera* / ascorbic acid invitro studies

| Extracts / AA | Quantity (µg) | | | | | |
|---|---|---|---|---|---|---|
| | 25 | 50 | 100 | 200 | 300 | 400 |
| EENN | 6.09±1.87 | 12.39±1.77 | 22.34±1.45 | 42.35±1.44 | 62.53±2.55 | 78.34±1.89 |
| AENN | 4.46±1.69 | 10.93±2.35 | 14.54±1.90 | 34.55±1.95 | 49.56±2.35 | 63.67±2.15 |
| Ascorbic acid | 5.05±1.34 | 11.45±2.08 | 20.05±2.66 | 39.11±1.37 | 56.15±2.17 | 75.26±2.6 |

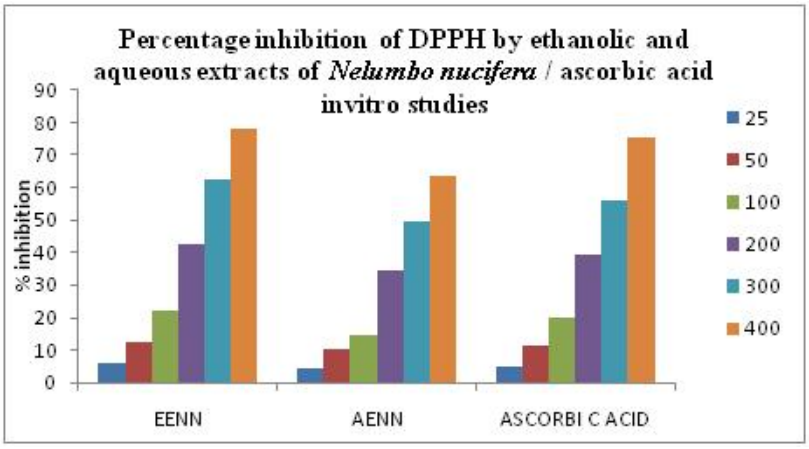


**Figure 3.11:** Percentage inhibition of DPPH by ethanolic and aqueous extracts of *Nelumbonucifera* / ascorbic acid invitro studies

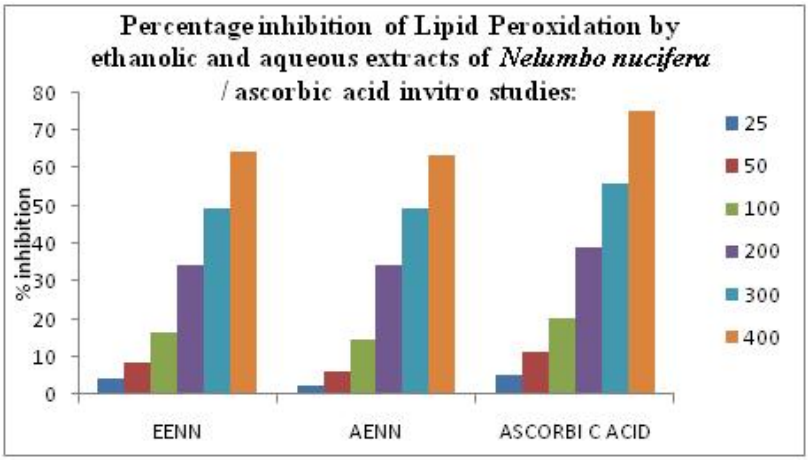


**Figure 3.12:** Percentage inhibition of Lipid Peroxidation by ethanolic and aqueous extracts of *Nelumbonucifera*/ ascorbic acid invitro studies

**Table: 3.17.** Percentage inhibition of Lipid Peroxidation by ethanolic and aqueous extracts of *Nelumbonucifera* / ascorbic acid invitro studies

| Extracts / AA | Quantity (µg) | | | | | |
|---|---|---|---|---|---|---|
| | 25 | 50 | 100 | 200 | 300 | 400 |
| EENN | 4.09±1.67 | 8.39±1.77 | 16.34±1.45 | 34.35±1.44 | 49.53±2.55 | 64.34±1.89 |
| AENN | 2.46±1.69 | 5.93±2.35 | 14.54±1.90 | 34.55±1.95 | 49.56±2.35 | 63.67±2.15 |
| Ascorbic acid | 5.05±1.34 | 11.45±2.08 | 20.05±2.66 | 39.11±1.37 | 56.15±2.17 | 75.26±2.6 |

## 4. Conclusion

The study was aimed to investigate the hepatoprotective activity of *Nelumbonucifera* flower extracts in paracetamol and $CCl_4$ induced hepatotoxicity in rats and invitro antioxidant activity by methods like hydroxyl free radical scavenging activity and lipid peoxidation. The acute toxicity studies were conducted as per OECD guidelines 423 for 2000mg/kg dose *Nelumbonucifera* extracts. It was found that the extracts even at 2000mg/kg dose did not show any mortality confirming its practically non-toxic nature. Treatment with *Nelumbonucifera* flower extracts also produce significant decrease in both $CCl_4$ and paracetamol induced rise in SGOT, SGPT, ALP and TB. The hepatoprotective activity may be due to the presence of strong antioxidants such as total phenolic compounds, tannins, and flavonoids and the potent diuretic such as saponins in the extract, which causes excretion of sodium, potassium, drug metabolites toxins, etc. thereby protecting the liver from toxic effects of paracetamol and $CCl_4$. The antioxidant activity of*Nelumbonucifera* was well established. The quantities of aqueous and ethanolic extracts needed for invitro inhibition of hydroxyl radical and lipid peroxidation were relatively similar to the known antioxidant ascorbic acid. In conclusion, the present study indicates the *Nelumbonucifera* extracts possess significant hepatoprotective activity similar to the standard drug silymarin.

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