

Original Research Article

Evaluation of hepatoprotective activity of ethanolic extract of the seed of *Flacourtiajangomas* against CCl₄-induced liver damage in rat model

Abstract:

Background: Hepatotoxicity is a serious global concern caused by the increased use of non-pharmacological substances and drugs. There are numerous medications available to treat this condition, the most well-known of which being silymarin. Although few, silymarin has certain adverse effects, which is why many are looking for alternatives, and medicinal plants may be a viable option. According to studies, *Flacourtiajangomas* has the potential to scavenge free radicals among therapeutic plants. We carried out this research to determine the hepatoprotective effects of an ethanolic extract of *Flacourtiajangomas* seeds.

Method: The experiment used 45 rats divided into 9 groups of five each. 25 of them developed hepatotoxicity as a result of regular CCl₄ administration. One of the groups was the disease control group, while the other four were given their respective treatments. The treatment groups received 300, 600, and 1200 mg/kg of *Flacourtiajangomas* seed extract, respectively. After 28 days of treatment, the rats were sacrificed and blood samples were obtained to assess several parameters.

Results: Based on the result of the tests, all of the abnormal levels of serum glutamate oxidoacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), creatinine, urea, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride (TG) were restored to normal levels in a dose-dependent manner, but a high dose in the case of SGPT and creatinine, and a medium and high dose in the case of urea, HDL, LDL, and TG were required to significantly restore the abnormal level with 95% confidence intervals.

Discussion: This study provides evidence that *Flacourtiajangomas* seed extract has the ability to provide hepatoprotection by acting as a free radical scavenger, possibly due to the presence of components like flavonoids, phenolic acids, and tannins.

Conclusion: As the seed of *Flacourtiajangomas* has the potential to exhibit hepatoprotective activity, intensive research is needed to isolate the genuine therapeutic compounds utilizing hydrophilic and hydrophobic solvents and introduce novel compounds to the disease management system.

Keywords: *Flacourtiajangomas*, silymarin, hepatoprotective activity, hepatotoxicity, carbon tetra chloride

Introduction:

The liver performs a variety of functions and is a vital organ that provides support to almost every other organ in the body (1). Numerous endogenous and exogenous compounds depend on it for detoxification and excretion, thus any harm to it or disruption of its functioning could have a detrimental effect on one's health in a variety of ways (2). Hepatotoxicity is the term for injury or damage to the liver caused by exposure to nonpharmacological compounds such as carbon tetrachloride (CCl₄) or medications such as anti-tubercular treatments, general anesthetics, paracetamol, some anti-cancer drugs, etc. (1, 11). This is an uncommon but potentially fatal adverse medication event that can result in hepatocyte breakdown and death, mitochondrial inhibition, and altered lipid metabolism, all of which can raise oxidative stress (3, 12). Within the last ten to fifteen years, there has been a thirtyfold increase in the incidence of hepatotoxicity, which ranges from 1.27 to 40.6 occurrences per 100,000 patients worldwide (4).

There are numerous drug classes on the market to treat hepatotoxicity, such as thalidomide, colchicine, resveratrol, corticosteroid, interferon, silymarin, sulfoadenosylmethionine, etc. (13). Among these drugs, silymarin, an extract from the *Silybummarianum* plant, has been shown to have hepatoprotective activity through a number of mechanisms. These include boosting RNA polymerase I and ribosomal RNA synthesis, which in turn stimulates hepatocyte protein synthesis and leads to hepatic regeneration and stabilizing membrane permeability by preventing lipid peroxidation. Additionally, it increases the amount of glutathione produced by the liver, which strengthens antioxidant defenses and helps activate antioxidant enzymes like superoxide dismutase (5, 6, 7, 15, 16, 17). Silymarin also protects liver cells from toxic chemicals by inhibiting the activation of hepatic nuclear factor kappa B (NF-κB), which stops TNF-α, interferon-gamma, IL-2, and IL-4 from being produced (16). Headaches, skin problems, gastroenteritis, and cognitive events were the most often reported adverse responses; the toxicity and effectiveness are still being studied. Gastrointestinal symptoms were the most prominent adverse reaction (7, 8, 14).

Researchers are presently looking for synthetic drug alternatives in medicinal plants because of their potential as an economical source, little to nonexistent adverse effects, and wide range of secondary byproducts with pharmacological action (9, 10). Furthermore, it is possible to genetically modify plants to increase their production of the desired secondary metabolites (18, 19). Many plants, including *Allium sativum*, *Nigella sativa*, *Capparis spinosa*, *Flacourtiajangomas*, *Launaeaprocumbens*, *Spondiasmombim*, and others, have been discovered by researchers to have hepatoprotective properties recently (20-25). *Flacourtiajangomas*, a plant belonging to the Salicaceae family, is also referred to as Paniala, coffee plum, Indian plum, or Painnagola. Although it originated in India, Bangladesh, and Myanmar, tropical areas of east Africa and south-eastern Asia are also home to the species. It's a little tree that grows to a height of 5 to 10 m with low branches, though it can occasionally reach 14 m (26-28). Bark, roots, leaves, fruits, and seeds are among the body parts that contain a variety of compounds, including lignans and flavanolignans, terpenoids, alkaloids, flavonoids and tannins, coumarins and

isocoumarins, and glucosides. These compounds have been shown to exhibit pharmacological activities such as hepatoprotective, anti-diarrheal, antioxidant, anti-diabetic, and anti-bacterial properties (23, 26, 29-31). *F. jangomas* is safe and doesn't have any hazardous effects compared to silymarin (33).

F. jangomas seeds have components that have good free radical scavenging properties, according to a recent study (32). Additionally, a study demonstrating strong hepatoprotective efficacy was conducted in vitro using a particular hepatotoxic model (23). Nevertheless, no research has been conducted to assess the hepatoprotective properties of *F. jangomas* seed using animal models. Therefore, the current study's objective is to assess the hepatoprotective properties of *F. jangomas* seeds against hepatotoxicity induced by CCl₄ by assessing biochemical parameters such as lipid profile and tests for liver and kidney functioning.

Materials and Methods:

Plant Collection and Extract preparation

F. jangomas seeds were procured from the local market in Dhaka and authorized by the University of Dhaka's Department of Pharmacy. Fresh seeds were air-dried, ground to a fine powder and then immersed in 50% ethanol for a duration of 15 days. Every three days, the extract was filtered. The extracted material was dried at low temperature and pressure using a rotary evaporator. Finally, the raw residue underwent the required pharmacological testing.

Drugs and Chemicals

The well-known hepatotoxic chemical carbon tetrachloride (CCl₄) was bought from the Sigma company in the US. Livasil 140 mg, a frequently used anti-oxidant marketed drug form of silymarin, was acquired from Incepta Pharmaceuticals Ltd.

Experimental Animal Procurement, Nursing and Grouping

45 male rats weighing between 120-150 grams were taken from Jahangirnagar University in Savar, Dhaka, which were randomly divided into 9 groups each group having 5 rats. The University of Dhaka's Institute of Nutrition & Food Science (INFS) maintained a climate-controlled environment for the test subjects, with a 12-hour light/dark cycle, a temperature of 25±3 °C, and a relative humidity of 55±5%. They were given access to clean water to drink and regular meals to eat. To allow for adaptation, the animals were housed in the environment for a minimum of one week prior to the study. The Institutional Animal Ethics Committee (IEAC) rules have been complied to in all experimental procedures. Both positive and negative control groups were included in our investigation.

Induction of hepatotoxicity

Carbon tetrachloride (CCl₄) is a common chemical used in laboratories to study a wide range of acute and chronic liver problems. The CYP2E1 isoenzyme produces the trichloromethyl free radical (CCl₃), a CCl₄ metabolite that reacts with proteins and lipids in cells to form trichloromethylperoxy radical. This free radical causes lipid peroxidation and lobular necrosis by attacking lipids on the endoplasmic reticulum membrane more quickly than the trichloromethyl free radical. To induce liver damage, each group—aside from the control group—received a single oral dosage of CCl₄ and olive oil at a 1:1 ration (3 ml/kg of rat body weight) every day on an empty stomach. Animals received different dosages (300, 600, and 1200 mg/kg) of *F. jangomas* seed extracts orally afterwards as treatment to hepatic injury.

Evaluation of Hepato-Protective Activity

For this experiment, 45 rats were selected at random and were evenly divided into nine groups (Table 1).

Table 1: Application of treatment efficacy

Group number	Group specification	Treatment species	Dose treatment species (mg/kg)	Abbreviation of Groups
1	Negative control	Physiological saline	10 ml/kg	N
2	CCl ₄ control	N/A	N/A	CCl ₄
3	CCl ₄ + Silymarin	Silymarin	80	CCl ₄ + S ₈₀
4	CCl ₄ + <i>Flacourtiajangomas</i>	<i>Flacourtiajangomas</i>	300	CCl ₄ + FJ ₃₀₀
5	CCl ₄ + <i>Flacourtiajangomas</i>	<i>Flacourtiajangomas</i>	600	CCl ₄ + FJ ₆₀₀
6	CCl ₄ + <i>Flacourtiajangomas</i>	<i>Flacourtiajangomas</i>	1200	CCl ₄ + FJ ₁₂₀₀
7	<i>Flacourtiajangomas</i>	<i>Flacourtiajangomas</i>	300	FJ ₃₀₀
8	<i>Flacourtiajangomas</i>	<i>Flacourtiajangomas</i>	600	FJ ₆₀₀
9	<i>Flacourtiajangomas</i>	<i>Flacourtiajangomas</i>	1200	FJ ₁₂₀₀

Out of the 9 groups, group 1 was the negative control group. It was fed physiological saline and a standard diet to create an optimal, neutral environment. In group 2, liver damage was caused using CCl₄, and no medication was administered. This group was compared to groups 4, 5, and 6, where seed extract was used as a treatment, with the potency of the plant extract being determined using a "One-way ANOVA test." In group 3, hepatic damage developed, and a

commercial drug was administered to see if a conventional medication would be effective in treating the condition. After liver damage was induced in groups 4, 5, and 6, seed extract was administered at low, medium, and high doses (300, 600, and 1200 mg/kg) as a form of treatment. In groups 7, 8, and 9, no disease was developed; however, the drug was administered at doses of 300, 600, and 1200 mg/kg to see if any side effects were noticed in healthy rats by monitoring changes in blood parameters. This is primarily carried out for toxicological research.

After 28 days of treatment, all of the rats were euthanized, and a cardiac puncture was performed to obtain blood. Then, in order to determine the impact of CCl₄, silymarin, and plant extract on the pathological condition of rats, various parameters were measured in each group, including serum glutamate oxidoacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), creatinine, urea, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride (TG).

Statistical Analysis:

The Microsoft Excel program was used to capture and analyze all of our discoveries (raw data) in terms of numerical parameters on a spreadsheet. Descriptive statistics were applied to the collected data, and the results were presented as mean SD. We interpreted inter-group heterogeneity in terms of many biological parameters using the "One-way Anova test" feature of the SPSS 16 software to assess statistical significance. The statistical significance of the occurrences is established by the fact that the 'p' value was less than 0.05 ($p < 0.05$).

Results:

The effect of *F. jangomas* extract on liver function tests is listed in Table 2. When compared to the negative control group, the CCl₄ treated group's SGPT and SGOT levels were higher. With increasing doses of *F. jangomas* seed extract, the SGPT and SGOT levels declined correspondingly; however, the SGPT level only decreased significantly ($p < 0.05$) in case of a high dose.

Table 2: Rat liver function test following medication administration and *F. Jangomas* extract.

Group	SGPT (U/L)	SGOT (U/L)
N	38.24±2.91	45.54±5.32
CCl ₄	114.25±15.31	122.38±11.32
CCl ₄ + S ₈₀	61.5±9.29	69.24±7.58
CCl ₄ + FJ ₃₀₀	111.22±13.61	120.25±9.63
CCl ₄ + FJ ₆₀₀	109.201±10.22	118.21±7.93
CCl ₄ + FJ ₁₂₀₀	107.52±12.41*	115.25±10.24
FJ ₃₀₀	34.23±3.92	47.79±5.39
FJ ₆₀₀	39.21±4.51	44.3±5.21
FJ ₁₂₀₀	38.47±3.20	47.38±6.16

This table shows the standard deviation and average SGPT and SGOT values for 9 distinct groups, each with five rats. U/L is the unit of SGPT and SGOT. Following a 28-day course of treatment, the values were obtained from blood serum.

When compared to the control group, the administration of CCl₄ significantly raised the levels of kidney functioning test markers such as creatinine and urea (Table 3). *F. jangomas* seed extract and silymarin reduced the elevation of creatinine and urea levels. Significant (p<0.05) results were observed with a high dose of seed extract for creatinine and both medium and high doses for urea.

Table 3: Rat kidney function test following medication administration and *F. Jangomas* extract.

Group	Creatinine (mg/dL)	Urea (mg/dL)
N	0.57±0.12	37.21±3.16
CCl ₄	2.79±0.84	103.46±8.21
CCl ₄ + S ₈₀	1.27±0.79	61.75±6.29
CCl ₄ + FJ ₃₀₀	2.61±0.85	100.25±6.39
CCl ₄ + FJ ₆₀₀	2.39±0.29	95.91±7.2*
CCl ₄ + FJ ₁₂₀₀	2.01±0.82*	91.67±6.58*
FJ ₃₀₀	0.77±0.76	36.21±4.21
FJ ₆₀₀	0.85±0.63	32.46±7.21
FJ ₁₂₀₀	0.89±0.69	37.92±6.29

This table shows the standard deviation and average creatinine and urea values for 9 distinct groups, each with five rats. For creatinine and urea, the measurement is mg/dL. Following 28 days of treatment, the values were obtained from blood serum.

In comparison to the control group, the rats who received CCl₄ had higher levels of total cholesterol, LDL, and triglycerides, but their HDL levels were lower (Table 4). Although the elevated total cholesterol level was gradually lowered in a dose-dependent way, it was non-significant (p>0.05). However, both medium and high doses of seed extract significantly restored the abnormal levels of LDL, HDL, and TG (p<0.05).

Table 4: Rat lipid profile test following medication administration and *F. Jangomas* extract.

Group	Total cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglyceride (mg/dL)
N	104.26±5.21	86.29±4.23	47.39±4.73	58.62±2.94
CCl ₄	200.29±11.63	41.26±5.39	154.5±12.24	110.53±9.89
CCl ₄ + S ₈₀	146.41±8.29	63.39±5.12	81.7±9.98	75.25±12.31
CCl ₄ + FJ ₃₀₀	197.98±10.21	42.89±5.39	149.5±14.75	107.42±9.08
CCl ₄ + FJ ₆₀₀	193.24±9.82	45.73±6.91*	145.28±12.2*	101.25±7.63*
CCl ₄ + FJ ₁₂₀₀	191.57±12.21	49.73±5.87*	141.25±6.28*	95.86±8.8*
FJ ₃₀₀	106.27±7.51	83.21±2.2	49.2±3.73	59.95±4.4

FJ ₆₀₀	109.29±3.2	87.75±3.79	51.93±5.08	55.29±5.01
FJ ₁₂₀₀	105.5±5.23	82.2±5.08	48.63±4.53	59.73±6.68

This table shows the standard deviation and average values for total cholesterol, HDL, LDL, and triglycerides over 9 groups, each with five rats. The units for triglycerides, HDL, LDL, and total cholesterol are mg/dL. Following a 28-day course of treatment, the values were obtained from blood serum.

Discussion:

Our findings confirm the idea that the ethanolic extract of *Flacourtiajangomas* seeds has hepatoprotective effect against carbon tetrachloride-induced hepatic injury, suggesting that it does so via scavenging free radicals and gradually reducing liver cellular necrosis.

Hepatotoxins like CCl₄ cause damage to liver cells by overproducing reactive oxygen species (ROS) and trichloromethyl free radicals during hepatic metabolism by cytochrome P-450. These free radicals then cause lipid peroxidation, covalent bonding of radicals with structural proteins, and ultimately, degradation of cellular membranes. Serum levels of important biomarkers, such as SGPT and SGOT, indicate the physiological activity of the liver. While SGOT is primarily bound in mitochondria, SGPT is the primary metabolic enzyme found in intracellular components. CCl₄ affects the liver's transport capacity and membrane permeability, which increases the amount of the vital liver biomarkers SGPT and SGOT that leak into the circulation (20, 34, 35, 36, 37). Our research showed that the disease control group's SGPT and SGOT levels increased significantly, supporting previous studies that found CCl₄ to be the cause of the hepatic damage (20). The *F. jangomas* seed's ethanolic extract reduced the levels of SGPT and SGOT in a dose-dependent manner, demonstrating the seed's hepatoprotective qualities by healing liver damage, raising serum antioxidant enzyme levels through oxidative stress detoxification, and reducing ROS production (37) While a study found that a methanolic extract of the fruit and leaves of *F. jangomas* exhibited hepatoprotective activity, our study found that the higher dose had a better capacity to lower SGOT and SGPT levels, which may have resulted from the use of different extraction medium (23). Nonetheless, the majority of research indicates that escalating the dosage enhances the ability to decrease SGPT and SGOT levels (20).

According to studies, use of CCl₄ can also result in nephrotoxicity by impairing glomerular function and producing free radicals, as seen by a rise in plasma creatinine and urea levels (38, 39). Similar to conventional silymarin, *F. jangomas* restored the CCl₄-induced changes in serum creatinine and urea in a dose-dependent manner, demonstrating the plant's possible hepatoprotective activity and confirming the hepatoprotective properties of the seed. Other investigations that were conducted on plants that had hepatoprotective action also indicated a decrease in serum creatinine and urea levels (40).

CCl₄ affects the lipid profile by increasing total cholesterol, LDL, and triglyceride levels while decreasing HDL levels through decreased protein synthesis and disturbance in phospholipid

metabolism, which may contribute to this abnormality (41). Following administration of *F. jangomas* ethanolic seed extract, levels of HDL were up and triglyceride, cholesterol, and LDL were lowered. Similar findings for other therapeutic plants were also shown in other investigations (41).

Flacourtiajangomas contains a wide range of phytochemical elements, including anthocyanin, alkaloids, β -carotene, flavonoids, tannin, saponin, and phenolic compounds, which are often found in fruits and have a variety of pharmacological effects (42). Seeds may also include these components. These ingredients exhibit antioxidant properties that protect against hepatotoxicity, according to several studies (43–49). Because phenolic groups serve as hydrogen donors, phenolic compounds—including tannins, phenolic acids, and flavonoids—have the ability to scavenge free radicals and limit the oxidation of proteins while also increasing the activity of antioxidant enzymes (32, 37). However, screening should have been done to determine the chemical contents contained in the seed and measurements should have been made to determine the quantity of components present in order to improve the comprehensiveness of this study. All things considered, *Flacourtiajangomas* may represent a viable, clinically relevant, natural remedy against side effects caused by drugs when prolonged or multiple drug therapy is necessary for a strong hepatoprotective effect.

Conclusion:

The present investigation of *F. jangomas* in rat models sheds light on its potential hepatoprotective properties. A potential hepatoprotective effect of the plant extract was established by a decrease in SGPT, SGOT, ALP, creatinine, TC, LDL, and TG and an increase in HDL level in rats treated with CCl_4 . To identify the responsible active chemicals and their potential mechanism of action, and to obtain extractions using different hydrophobic and hydrophilic solvents to assess the activity of different compounds so they may be used in different disease management systems, rigorous research is required.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s)

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