Original Research Article

An Assessment of Hepatoprotective Activity of *Catharanthus roseus* onCCL4 induced Rat Modelwith Safety Profile Analysis

Comment [A.1]: The letter L in CCl_4 should be in small letter and there should be hyphen (-) between CCl_4 and induced
4 in CCl_4should be in subscript

Abstract

Comment [A.2]: In the abstract, methods are not fully represented

Using herbs and herbal medications to preserve health and prevent, treat, or cure illness is the art or practice of herbal remedies. Herbal remedies are also known as herbal medicine. Research was conducted on rats to investigate the lipid profiles of *Catharanthus roseus* extract. Furthermore, when it comes to the kidney function test, there are outcomes that are statistically significant ($p<0.05$) in groups 5, 6, and 7. Regarding urea, however, there is no result that can be considered statistically significant ($p<0.05$). When the liver function test is performed, it is shown that there are statistically significant ($p<0.05$) results in the levels of SGOT in group 6, with the dosage being 600 mg/kg. On the other hand, there are no findings that are statistically significant in the case of SGOT. After conducting a lipid profile function test, it has been determined that there are statistically significant findings ($p<0.05$) in relation to the levels of triglycerides, LDL, and HDL. Group 5 showed statistically significant findings ($p<0.05$) in relation to HDL, while group 6 exhibited significant findings ($p<0.05$) in relation to LDL and triglyceride studies. Insights like this could help people with liver disorders.

Keywords: *Catharanthus roseus*; SGPT; Herbal medicine; Triglyceride, phytopharmacology; cholesterol.

Introduction

Among all the human organs, the liver serves the most purposes, and it is also the biggest glandular organ. Multiple times daily, the complete blood supply of an individual goes through the liver. When it comes to metabolism, the liver is crucial for humans [1]. Worldwide, liver illnesses are a leading cause of death and disability in both humans and other animals, with

hepatotoxicity from pharmaceuticals being the leading cause [2]. An increase in reactive oxygen species (ROS) activity (OH, H2O2, O2) can damage cells, especially those in the liver, when people drink a lot, are addicted to drugs, are exposed to some harmful chemicals, or have a virus or parasite. [2]. L-glutathione, which is made up of L-cysteine, glycine, and L-glutamate, is often taken by mouth with ascorbic acid because it fights free radicals and dissolves easily in water (1 molecular weight). Their cleansing and anti-oxidant qualities, in addition to boosting immunity, are highly valued [3]. The third. But they may make you sick to your stomach, bloat, have diarrhoea, have trouble breathing because of constriction of the airways, or even develop skin responses like dermatitis. The prevalence of chronic liver disease (CLD) has risen by 31% in the United States among adults aged 45–64 years, affecting an estimated 1.5 billion individuals globally [4]. The unique chemical compounds derived from medical plants may have therapeutic effects, say experts in the field of medicinal plants. Therefore, researchers are always looking for new herbal remedies or alternative treatments derived from plants to cure a wide range of illnesses. [5]. In contrast to phytotherapy, which is the scientific study of medicinal plants, herbalism involves its practical use. Plants have been a source of medicine for thousands of years due to their wide assortment of chemicals having medicinal qualities [6]. Many different chemical components, including phenols, alkaloids, terpenoids, saponins, glycosides, tannins, flavonoids, resins, polysaccharides, plant lipids, essential oils, and many more, allow these medicinal plants to exert a broad variety of pharmacological and therapeutic effects [7–9]. One possible therapeutic impact of plant genetic modification is to alter the concentration of the plant's chemical components. One application of reverse genetics is the enhancement of secondary metabolite biosynthesis, including alkaloid production [10].

Catharanthus roseus, sometimes referred to as Vinca rosea or Periwinkle, commonly occurs in the North Karnataka area of India. This subshrub is found throughout the whole country of India. These regions include: Brazil, the Cook Islands, the Dominican Republic, the UK, Jamaica, Mozambique, Pakistan, Taiwan, Thailand, and the West Indies [11]. The phytochemical analysis detected the existence of alkaloids, flavonoids, tannins, saponins, terpenoids, carbohydrates, ferric chloride, and cardiac glycosides [12–13]. This plant has several beneficial properties, such as antioxidant, antidiarrheal, anticancer, antidiabetic, anti-bacterial, hypotensive, and wound healing activities [14, 15].

Comment [A.3]: Be consistent in writing your formulas, the 2 should be in subscript (i.e H_2O_2, O_2)

Comment [A.4]: Remove the full stop

Comment [A.5]: Recast

Comment [A.6]: Avoid using pronouns in the first or second person. For example, it is generally not advisable to use terms like "I," "we," "my," "you", "your" or "our" when writing a research paper. The same idea holds true for many other academic writing genres, including research papers, lab reports, literature reviews, and rhetorical analyses.

The objective of our research is to examine the anti-hyperlipidemic effects of *Catharanthus roseus* in a rat model.

Materials and methods

Plant Collection and Extract Preparation

Catharanthus roseus specimens were obtained from a local market in Dhaka. The University of Dhaka's Department of Pharmacy verified the authenticity of the item. The *Catharanthus roseus* plants were dried in the air and then crushed extensively. Next, we subjected the powders to a 15-day extraction process using a 50% ethanol solution. The extract underwent filtration every three days. The obtained substance was dehydrated using a rotary evaporator under reduced temperature and pressure conditions. Ultimately, the unrefined remains underwent the necessary pharmacological examination.

Drugs and Chemicals

Carbon tetrachloride (CCl4), a well-known hepatotoxicity causing chemical, was purchased from the Sigma firm in the United States. The typical anti-oxidant medication silymarin was purchased as Livasil 140 mg from Incepta Pharmaceuticals Ltd.

Experimental Animal Procurement, Nursing, and Grouping

A total of 100 male rats weighing between 120and 150 grams were obtained from Jahangirnagar University in Savar, Dhaka. Each of them was housed in a climate-controlled environment (temperature 25±3°C, relative humidity 55±5%, and a 12-h light/dark cycle) at the University of Dhaka's Institute of Nutrition& Food Science (INFS). They were given a conventional food and were permitted to drink clean water. All of the animals were maintained in this habitat for at least one week prior to the research for adaption. All experimental methods followed the recommendations of the Institutional Animals Ethics Committee (IEAC).

Animal Model Sample Size Detection

A total of 100 rats were allocated at random into10 groups of ten rats each. The rats were assigned to each group at random in all of the studies. We used ten rats in each group toincreasethe investigation's validity. During the mating season, we, on the other

Comment [A.7]: Letter "M" should be in caps

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hand,maintained a close eye on the rat every day. We included both positive and negative control groups in our study.

Dose Selection and Route of Administration for Respective Study

Many laboratory studies on acute and chronic liver illnesses make use of carbon tetrachloride (CCL4). The trichloromethyl free radical (CCL3), a metabolite of CCL4 produced by the CYP2E1 isozyme, reacts with proteins and cellular lipids to form the trichloromethyl peroxy radical, causing lipidomic lipid peroxidation and lobular necrosis. A single oral administration of CCl4 combined with olive oil as a vehicle in a 1:1 ratio (3 ml/kg of rat body weight) caused liver damage in all animal groups except for the usual control group. After receiving therapy for hepatic damage, the researchers administered extracts of the *Catharanthus roseus* plant to the animals. People took different doses of the extract orally. A researcher gathered flowers of the *Catharanthus roseus* species from a Dhaka bazaar. The Department of Pharmacy at the University of Dhaka verified the content. After being air-dried, the *Catharanthus roseus* was ground to a powder. Next, we conducted a 15-day extraction process in 50% ethanol using the powders. We removed the filtrate at three-day intervals. We dried the extracted material at low pressure and temperature using a rotary evaporator. We then performed the necessary pharmacological testing on the crude residue.

Evaluation of Hepato-Protective Activity

For this experiment, 100 rats were randomlypicked and equally divided into fourteen groups

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	A
3	$CCl_4 + S_{10}$	Silymarin	10	S_{10}
4	$CCl_4 + CR_{200}$	*Catharanthus*	200	CR_{200}

Comment [A.12]: Space

Comment [A.13]: Subscript: CCl_3, CCl_4

Comment [A.14]: Recast.
Avoid using pronouns in the first or second person.

Comment [A.15]: Space

Comment [A.16]: The rats were divided into fourteen groups but only ten groups were presented on the table 1 and 2.

However, under **Animal Model Sample Size Detection,** the design showed that 100 rats were allocated at random into 10 groups of ten rats each. Therefore, there is a need to harmonize.

Comment [A.17]: There should be a key at the bottom of table 1 and 2 that explain what N/A, N, A, CR, or AB, S stands for.

		roseus		
5	CCl_4 + CR_{400}	*Catharanthus roseus*	400	CR_{400}
6	CCl_4+*CR_{600}*	*Catharanthus roseus*	600	CR_{600}
7	S_{10}	Silymarin	10	S_{10}
8	CR_{200}	*Catharanthus roseus*	200	CR_{200}
9	CR_{400}	*Catharanthus roseus*	400	CR_{400}
10	CR_{600}	*Catharanthus roseus*	600	CR_{600}

Comment [A.18]: In table 1 CR was used as acronym for *Catharanthus roseus* whereas AB as acronym for *Catharanthus roseus*in table 2, reconcile the two, please.

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Biological Sample Collection.

In order to assess blood glucose levels, rats had their tail tips punctured to collect blood samples. On the other hand, blood was drawn from the animal as soon as its heart was punctured and transferred to a micro centrifuge tube after killing. Following 5 minutes of centrifugation at 5,000 rpm, the collected samples yielded the supernatant fluid. For biochemical testing, this fluid was then transferred to an additional micro centrifuge tube. Following sacrifice, the organs were quickly extracted and carefully cleansed in ice-cold saline in order to assess their function.

Estimation of Biochemical Parameters

The blood glucose level was measured using a glucometer. Aside from the Humaluzer 3000, lipid profile, kidney, and liver function tests were performed. In addition, the gluconeogenic and glycolytic enzyme activity of kidney and liver samples was examined

Statistical analysis:

All of our findings (raw data) in terms of numerical parameters were recorded and analyzed on a broadsheet using the MS Excel application. The gathered data were subjected to descriptive statistics, with the findings reported as mean SD. To evaluate statistical significance, we used the SPSS 16 software's "One-way Anova test" to interpret inter-group heterogenicity in terms of several biological factors. The occurrences are considered statistically significant since the 'p' value was less than 0.05 ($p<0.5$).

Comment [A.20]: Insert "±"between mean and SD.

Results and discussion:

Table 2: Lipid profile of rat after administration of drug and *Catharanthus roseus* extract.

Comment [A.21]: Insert a key at the bottom of each table to describe S, CR or AB

Group no.	**Group status**	**Kidney Function test**		**Liver Function test**		**Lipid Profile Function Test**			
		Creatinine (mg/dl)	Urea	SGOT (u/l)	SGPT (u/l)	Cholesterol (mg/dl)	Triglisride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
1	Negative Control	0.6±0.02	28.29±3.49	40.35±3.70	35.33±2.40	96.24±6.19	46.44±2.52	38.47±3.37	69.24±4.50
2	CCl_4 Control	2.7±0.08	94.89±5.26	102.36±7.51	99.37±6.45	162.24±8.14	105.39±5.68	89.49±8.34	40.32±3.19
3	$CCl_4 + S_{10}$	1.4±0.06	68.26±4.59	64.51±4.29	68.22±7.15	119.23±6.15	72.26±3.96	65.60±6.30	57.15±4.69
4	$CCl_4 + AB_{200}$	2.4±0.08	94.26±3.86	100.29±5.26	98.10±5.23	159.29±4.60	102.89±2.33	88.28±3.46	41.44±2.29
5	$CCl_4 + AB_{400}$	2.2±0.09*	92.57±5.53	97.59±3.89	95.46±3.20	158.39±5.50	99.57±4.61	86.19±3.49	44.16±3.38*
6	CCl_4+AB_{600}	1.8±0.05*	90.97±4.23	42.40±4.19	91.07±5.22*	155.34±4.19	95.36±2.22*	83.83±3.71*	47.18±4.50*

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7	S_{10}	0.8±0.02*	31.29±4.7	40.23±1.04	37.24±3.19	99.10±5.11	48.46±2.70	40.30±0.86	41.69±2.84
8	AB_{200}	0.7±0.05	34.57±2.38	42.39±3.19	33.63±2.08	96.27±4.70	44.82±1.26	42.34±1.50	39.57±1.89
9	AB_{400}	0.6±0.07	29.49±0.83	39.59	38.17±3.50	95.29±3.09	43.95±2.96	39.69±1.28	42.79±2.58
10	AB_{600}	0.7±0.08	33.57±2.62	4.14	34.48±2.57	96.41±4.01	40.50±3.10	43.40±2.60	43.69±3.24

Note: Each value represents the mean ± SEM. (n=5). One- way ANOVA followed byDunnett's t test. ***P<0.001, **P<0.01, *P<0.05 compared with control.

Patients suffering from severe chronic kidney disease (CKD) as well as end-stage renal disease (ESRD) may experience cognitive dysfunction. The correlation between chronic kidney disease (CKD) and cognitive impairment is a serious public health issue [16]. This is due to the fact that the prevalence of CKD in the United States increased from 10% in 1988–1994 to 13% in 1999–2004, and it is possible that this trend may continue in the future. Furthermore, when it comes to the kidney function test, there are outcomes that are statistically significant ($p<0.05$) in groups 5, 6, and 7. But in the case of urea, there is no outcome that can be considered statistically significant. Liver function tests, often known as LFTs, are a useful screening tool that is an efficient method for identifying hepatic impairment. Given that the liver is responsible for a wide range of processes, it is impossible for a single test to offer an accurate and comprehensive estimation of the liver's function [17]. In the case of the liver function test, the findings of the SGPT level are statistically significant ($p<0.05$) in group 6, with the dosage being 600 mg/kg. On the other hand, there are no findings that are statistically significant ($p<0.05$ in the case of SGOT). Several investigations [18–20] came to the same conclusions about the same phenomenon. After conducting a lipid profile function test, it has been determined that there are statistically significant findings ($p<0.05$) in relation to the levels of triglycerides, LDL, and HDL. Group 5 showed statistically significant ($p<0.05$) outcomes in the case of HDL, whereas group 6 showed statistically significant results in the case of LDL and triglyceride. There were several studies [21–23] that came to the same conclusions.

Conclusion:

The ethanolic extract of *Catharanthus roseus* was shown to possess hepatoprotective effects, which were identified within the context of this investigation. By lowering the buildup of lipids and liver problems, this extract helps to reduce the negative effects that a diet high in fat may have on the body. In order to determine which component of the entire extract really provides the anti-hyperlipidemic action through a screening approach, more research is necessary.

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