Journal of Complementary and Alternative Medical Research

Volume 25, Issue 10, Page 24-31, 2024; Article no.JOCAMR.123287
ISSN: 2456-6276

Hepatoprotective Activity of *Bixa orellana* Plant Extract against Hepatotoxicity in Rodent

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jocamr/2024/v25i10576

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/123287

Original Research Article

Received: 14/07/2024
Accepted: 16/09/2024
Published: 19/09/2024

ABSTRACT

Introduction: Hepatotoxicity, or liver damage, can result from exposure to toxic substances such as medications, chemicals, alcohol, and environmental pollutants, affecting approximately 1.5 billion people worldwide. Although synthetic drugs offer therapeutic benefits, they often come with side effects. Medicinal plants may offer a viable alternative. This study examines *Bixa Orellana*, a plant known for its antioxidant properties, to evaluate its potential protective effects on the liver.
Methods: The study used 45 Swiss Albino rats, divided into 9 groups. Hepatotoxicity was induced using carbon tetrachloride (CCl4). Group 1 was negative control group where only normal foods

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Cite as: *Rashid, Sadiya Binte, Sadia Adnin Oyshi, Bushratul Jannat, Md Shakil, Angelina Samantha Halder, and Zulqarnain Haider. 2024. "Hepatoprotective Activity of Bixa Orellana Plant Extract Against Hepatotoxicity in Rodent". Journal of Complementary and Alternative Medical Research 25 (10):24-31. https://doi.org/10.9734/jocamr/2024/v25i10576.*

were given. Groups 2 to 6 were exposed to CCl4, while Groups 7 through 9 received low, medium, and high doses of *Bixa Orellana* extract, respectively. The treatments lasted for 42 days, after which the rats were sacrificed for analysis.

Results: Liver function tests (SGPT and SGOT) showed significant improvements, particularly with medium (600mg/kg) and high (1200mg/kg) doses of the extract. Kidney function tests revealed promising results for creatinine levels with low (300mg/kg) and medium (600mg/kg) doses, although urea levels did not change significantly. The lipid profile analysis indicated substantial benefits at high (1200mg/kg) doses, with improvements in HDL, LDL, and cholesterol levels, though triglyceride levels did not show significant changes.

Conclusion: The findings suggest that *Bixa Orellana* has considerable hepatoprotective effects. Further studies are needed to explore its potential as a therapeutic agent for liver protection and to develop detailed reports on its efficacy.

Keywords: Bixa Orellana plant; hepatotoxicity; liver damage.

1. INTRODUCTION

The liver, the largest and most intricate internal organ in the human body, makes up approximately 2-3% of an adult's body weight. Chronic Liver Diseases (CLD) affect an estimated 1.5 billion people globally, with a notable 31% increase in the United States among individuals aged 45-64 years [1]. The liver is especially prone to cellular damage from increased Reactive Oxygen Species (ROS) such as OH, H_2O_2, and O_2, which can result from excessive alcohol consumption, drug abuse, exposure to certain toxins, or viral and parasitic infections [2]. L-glutathione, composed of L-cysteine, glycine, and L-glutamate, is a low molecular weight, water-soluble tripeptide known for its role as a free-radical scavenger and is often taken as an oral supplement alongside ascorbic acid. This combination is valued for its detoxifying, antioxidant properties and its ability to bolster the immune system [3-6]. However, it may cause digestive issues, stomach cramps, bloating, diarrhea, breathing difficulties due to bronchoconstriction, and allergic reactions such as dermatitis.

Plants are a vital source of new drug discoveries and synthesis due to their rich diversity of naturally occurring therapeutic chemicals. These plant-derived compounds can serve as safer, more effective alternatives to current medicines or be further researched for their therapeutic potential. Scientists in the field of medicinal plants believe that unique chemical compounds produced by these plants may offer significant therapeutic benefits. Thus, there is a continuous search for alternative or plant-based herbal medications to treat various ailments. Medicinal plants are known for their wide range of pharmacological and therapeutic effects, attributable to their numerous chemical constituents such as phenols, alkaloids, terpenoids, saponins, glycosides, tannins, flavonoids, resins, polysaccharides, plant lipids, and essential oils [7-9]. The desired therapeutic effect can be achieved by manipulating the concentration of these chemical components, potentially through genetic manipulation of the plants. For example, reverse genetics can enhance the biosynthesis of secondary metabolites like alkaloids [10-11]. Hepatoprotective properties have been observed in Acacia mellifera, Adansonia digitata L, and Cannabis sativa L [12-14].

Bixa Orellana, is a tree from the Bixaceae family, native to Central America [15]. It contains various compounds, including bixin norbixin, isobixin, beta-carotene, cryptoxanthin, lutein, zeaxanthin, orellin, bixein, bixol, crocetin, ishwarane, ellagic acid, salicylic acid, threonine, tomentosic acid, tryptophan, and phenylalanine [16-17]. Recently, natural compounds from plants have drawn significant interest due to their diverse pharmacological properties, including antioxidant and hepatoprotective activities [18]. The *Bixa Orellana* plant contains various compounds that may protect the liver. Notably, bixin, a carotenoid pigment in the seeds, has been shown to have antioxidant properties that help shield the liver from damage. Other compounds such as norbixin also play a role in the plant's liver-protective effects [19]. This study aims to explore the hepatoprotective potential of *Bixa Orellana* in a Carbon tetrachloride (CCl_4) induced experimental rat model, as well as assess any potential adverse effects on the liver, in the pursuit of a safer, more affordable, and more effective drug.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extract Preparation

Fruits of *Bixa Orellana* were procured from a local market in Dhaka and authenticated by the Department of Pharmacy at the University of Dhaka. The fresh fruits were air-dried and meticulously crushed into a fine powder. This powder was then subjected to extraction with 50% ethanol over a span of 15 days, with filtration conducted every three days. The resulting extract was concentrated using a rotary evaporator under low temperature and pressure conditions. The final crude residue was prepared for subsequent pharmacological testing.

2.2 Drugs and Chemicals

Carbon tetrachloride (CCl_4), 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.

2.3 Experimental Animal Procurement, Nursing, and Grouping

45 male rats, each weighing between 120 and 150 grams, were obtained from Jahangirnagar University in Savar, Dhaka. They were housed individually in a climate-controlled environment (temperature 25±3°C, relative humidity 55±5%, and a 12-hour light/dark cycle) at the Institute of Nutrition & Food Science (INFS) at the University of Dhaka. The rats were provided with standard food and clean water. They were kept in this environment for at least one week before the start of the research to allow for acclimatization.

2.4 Animal Model Sample Size Detection

The 45 rats were randomly divided into 9 groups, with each group consisting of 5 rats. The random assignment was intended to enhance the validity of the study. Daily observations were conducted during the mating season. Both positive and negative control groups were included in the study.

2.5 Dose Selection and Route of Administration for Respective Study

Carbon tetrachloride (CCl4) is commonly used in laboratories to model various liver diseases, both acute and chronic. The metabolite trichloromethyl free radical (CCl3), produced by the CYP2E1 isozyme from CCl4, interacts with cellular lipids and proteins to form trichloromethyl peroxy radical, which causes lipid peroxidation and lobular necrosis more rapidly than trichloromethyl free radical. A single oral dose of CCl4 mixed with olive oil in a 1:1 ratio (3 ml/kg of rat body weight) induced liver damage in all groups except the normal control group. *Bixa Orellana* extracts were administered orally in various doses to the animals with hepatic injury as a post-treatment.

2.6 Evaluation of Hepato–Protective Activity

For this experiment, 45 rats were randomly picked and equally divided into 9 groups (Table 1).

In our experimental design, Group 1 served as the negative control, receiving only regular food, establishing a baseline for comparison. Group 2 acted as the disease control group, where CCl_4 was administered to induce disease, without any subsequent treatment, to provide a comparative basis for the effects of the treatments of group 4, 5 and 6 in the "One-way ANOVA " test . Groups 4, 5, and 6 were treated with varying doses of the drug: low (300 mg/kg), medium (600 mg/kg), and high (1200 mg/kg), respectively.

Table 1. Application of treatment efficacy

Group Number	Group Specification	Treatment species	Dose treatment species (mg/kg)
1	Negative control	Physiological saline	10 ml/kg
2	CCl_4	N/A	N/A
3	$CCl_4 + S_{80}$	Silymarin	80
4	$CCl_4 + BO_{300}$	*Bixa Orellana*	300
5	$CCl_4 + BO_{600}$	*Bixa Orellana*	600
6	$CCl_4 + BO_{1200}$	*Bixa Orellana*	1200
7	BO_{300}	*Bixa Orellana*	300
8	BO_{600}	*Bixa Orellana*	600
9	BO_{1200}	*Bixa Orellana*	1200

In contrast, in group 7, 8, and 9 were given the same low (300 mg/kg), medium (600 mg/kg), and high (1200 mg/kg) doses of the drug respectively without disease induction. This approach was designed to identify any potential side effects of the drug in the absence of the disease.

2.7 Statistical Analysis

All numerical data were recorded and analyzed using MS Excel. Descriptive statistics were applied to the collected data, and the results were reported as mean ± SD. To assess statistical significance, the "One-way ANOVA test" in SPSS 16 software was used to interpret inter-group variability for various biological factors. Results were considered statistically significant if the p-value was less than 0.05 ($p<0.05$).

3. RESULTS AND DISCUSSION

Assessing the hepatoprotective properties of plants typically involves various methods to quantify specific biochemical markers, as there is no single universal paradigm for accurately and qualitatively measuring hepatoprotective activity. The liver is more susceptible to toxic damage than other organs, making drug-induced liver injury a significant health concern, especially with the extensive use of certain medications. Research on the mechanisms of drug-induced liver injury is crucial for its treatment and prevention. Given the lack of reliable hepatoprotective drugs in contemporary medicine, herbal treatments for liver disorders have gained popularity. Numerous herbs notably as plant Silymarin demonstrated potential effectiveness in treating liver cirrhosis [20-21].

3.1 Liver Function Test

3.1.1 SGPT

Carbon tetrachloride (CCl4) increases serum glutamate pyruvate transaminase (SGPT) levels by damaging the liver. Its metabolism generates reactive free radicals that cause oxidative stress and harm liver cells. This damage disrupts cell membranes, allowing SGPT to leak into the blood, which signals liver cell injury and dysfunction [22]. In this study group 4,5 and 6 SGPT level was decreased in a dose dependent manner wherese in group 5 and 6 SGPT level declined is considered as statistically significant ($p<0.05$) when compared to diseased group 2.That means our plant can impart significant hepatoprotective activity.

3.1.2 SGOT

Here in case of SGOT test group 4,5 and 6 SGOT level was decreased in a dose dependent manner whereas in group 5 and 6 SGPT level declined is considered as statistically significant ($p<0.05$) when compared to diseased control group. That means our plant can give significant hepatoprotective activity.

Table 2. Liver function test

Group	SGPT	SGOT
Negative control	35.29 ± 4.27	46.28 ± 1.89
CCl_4	110.47 ± 11.60	110.10 ± 7.29
$CCl_4 + S_{10}$	52.57 ± 6.93	67.25 ± 8.85
$CCl_4 + BO_{300}$	105.67 ± 9.97	107.29 ± 8.89
$CCl_4 + BO_{600}$	101.23 ± 8.88*	103 ± 7.59
$CCl_4 + BO_{1200}$	97.75 ± 10.52*	98.52 ± 6.01
BO_{300}	37.81 ± 5.67	43.46 ± 2.87
BO_{600}	35.89 ± 7.86	47.37 ± 5.29
BO_{1200}	39.29 ± 8.75	42.74 ± 8.29

Table 3. Kidney function test

Goup	Creatinine	Urea
Negative control	0.57 ± 0.011	38.46 ± 8.24
CCl_4	2.86 ± 0.95	105.52 ± 13.23
$CCl_4 + S_{10}$	1.25 ± 0.87	52.24 ± 42.24
$CCl_4 + BO_{300}$	2.50 ± 0.93*	101.70 ± 18.58
$CCl_4 + BO_{600}$	2.23 ± 0.86*	96.29 ± 5.93
$CCl_4 + BO_{1200}$	1.89 ± 0.93	91.06 ± 4.20
BO_{300}	0.63 ± 0.13	39.75 ± 6.50
BO_{600}	0. 75 ± 0.45	35.20 ± 5.39
BO_{1200}	0.57 ± 0.48	38.20 ± 6.08

Table 4. Lipid profile

Group	Total cholesterol	HDL	LDL	Triglyceride
Negative control	107.34 ± 6.24	88.24 ± 2.82	46.25 ± 4.25	55.39 ± 4.67
CCl_4	203.34 ± 14.21	47.90 ± 6.50	150.29 ± 6.36	107.54 ± 10.30
CCl_4 + S_{10}	151.23 ± 10.39	65.29 ± 5.55	77.79 ± 6.39	69.96 ± 8.62
CCl_4 + BO_{300}	200.04 ± 12.47	48.29 ± 0.29	147.50 ± 8.37	105.26 ± 8.67
CCl_4 + BO_{600}	196.93 ± 13.39	51.29 ± 5.29	143.90 ± 6.29	104.22 ± 7.62
CCl_4 + BO_{1200}	199.96 ±13.26*	54.48 ± 6.18*	140.19 ± 7.53*	101.39 ± 5.29
BO_{300}	103.52 ± 6.39	86.26 ± 3.38	48.19 ± 5.30	52.52 ± 5.19
BO_{600}	109.39 ± 8.24	84.52 ± 2.93	45.53 ± 6.12	54.39 ± 4.18
BO_{1200}	105.39 ± 7.29	85.80 ± 3.45	48.59 ± 5.73	54.18 ± 2.20

3.2 Kidney Function Test

3.2.1 Creatinine level analysis

The analysis of creatinine levels revealed a significant decrease ($p<0.05$) in both low and medium doses when compared to the diseased control group. This suggests that administration of these doses resulted in a notable decrease in creatinine levels. The increase in creatinine, a marker often associated with kidney function, indicates that the low and medium dosages had a significant impact on creatinine levels, differentiating them from the control group with disease.

3.2.2 Urea level analysis

In contrast, the analysis of urea levels showed no statistically significant effect across any of the dosages tested. This indicates that, regardless of the dosage administered, there were no meaningful changes in urea levels compared to the diseased control group. Consequently, urea levels remained stable and unaffected by the dosage variations in this study.

3.3 Lipid Profile Test

3.3.1 Total cholesterol level analysis

When analyzing total cholesterol levels, it was observed that there was a significant decrease only at high dosages in group 6 compared to group 2. Specifically, while total cholesterol levels in group 2 remained relatively stable, high doses administered to group 6 resulted in a marked reduction in total cholesterol, demonstrating the effectiveness of the high dosage treatment in lowering cholesterol levels.

3.3.2 HDL level analysis

Regarding HDL (high-density lipoprotein) levels, a notable increase was observed only at high doses in group 6 when compared to the disease control group. HDL, often referred to as "good" cholesterol, showed significant improvement under high dosage treatment conditions in group 6, indicating a beneficial effect of the treatment on HDL levels in contrast to the disease control group, which did not show such improvements.

3.3.3 LDL level analysis

For LDL (low-density lipoprotein) levels, significant reductions were observed exclusively at high doses in group 6. This decrease in LDL levels was statistically significant when compared to those in the diseased group 2. This finding highlights the impact of high-dose treatment in effectively lowering LDL levels, which is crucial for managing cholesterol-related health risks.

3.3.4 Triglyceride level analysis

In the study, the examination of triglyceride levels did not reveal any dosage that produced statistically significant differences when compared to the group with the disease. This means that, regardless of the specific levels or amounts of triglycerides administered or observed, there was no clear evidence suggesting that these triglyceride levels had a meaningful impact on the outcomes for the diseased group. Consequently, the data indicates that triglyceride dosage, in this context, did not have a significant effect on the disease-related measures or conditions being studied.

Similar findings have been observed in studies using plant extracts such as *Diplazium esculentum*, and *Tamarindus* indica. Further research could focus on isolating individual beneficial molecules, using advanced techniques like NMR and mass spectrometry, to develop novel therapeutic drugs for managing hepatoprotective activities.

4. CONCLUSION

The study shows that *Bixa Orellana* has significant hepatoprotective effects, especially at

medium and high doses, improving liver function markers and lipid profiles. It also positively impacts kidney function. These results highlight its potential as a natural liver-protective agent. Further research is needed to explore its mechanisms, long-term efficacy, and safety to confirm its potential as a treatment option [23-25].

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 writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental procedures adhered to the guidelines of the Institutional Animals Ethics Committee (IEAC).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/123287