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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF LEAVES EXTRACT OF *CARISSA CARANDAS* LINN.

Sumsunnahar Shifa, Taslima Begum, Faruquzzaman and Pritesh Ranjan Dash *

Department of Pharmacy, Primeasia University, Dhaka - 1213, Bangladesh.

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Correspondence to Author:
Pritesh Ranjan Dash, Ph. D

Lecturer,
Department of Pharmacy,
Primeasia University, Dhaka - 1213,
Bangladesh.

E-mail: pritesh.ranjan@primeasia.edu.bd

ABSTRACT: *Carissa carandas Linn.* is an evergreen plant of the Apocynaceae family. It is native to India and also found in Bangladesh, Sri Lanka, Indonesia, Malaysia, Pakistan, and Myanmar. Almost every part of these plants has been investigated for various pharmacological activities. Among all parts, leaves are less investigated. The present study explores the hepatoprotective activity of 60 % ethanolic extract of leaves of *Carissa carandas Linn.* Extraction of fresh mature leaves by the process of maceration using Petroleum ether gave 0.84 gm crude extract and a yield of 1.68 %. Extraction by using 60 % Ethanol gave 6.9 gm and a yield of 13.8 %. This crude extract was investigated for the evaluation of a hepatoprotective activity. The carbon tetrachloride (CCl_4) induced method was used to study hepatoprotective activity. The extract showed significant dose-dependent hepatoprotective activity when compared with negative control group as the release of enzymes and bilirubin decreased with increasing dose of extract.

INTRODUCTION: Nowadays natural compound has become a great interest of exploration. World Health Organization estimated that about 80% of the population use herbal medicines because of less side effects and low cost [6]. Common name of *Carissa carandas Linn.* is koromcha. This evergreen shrub is found throughout Bangladesh [3-7]. It is a flowering shrub of the Apocynaceae family. It holds milky latex. The leaves are elliptical-oblong, glossy, and dark green on the dorsal and light green on the ventral side [9]. Any part of the plant, such as leaves, fruits, flowers, roots, seeds, barks, etc., can be a source of natural constituents [4].

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The pharmacological properties of koromcha include analgesic, antipyretic, anti-inflammatory, antibacterial, antifungal, hepatoprotective, antioxidant, anticancer, and acute hypotensive activities. Most of these activities were explored using roots, fruits and barks. Leaves are less explored [9]. Metabolism of certain biomolecules, detoxification, bile secretion, *etc.*, are the major function of the liver. Severe infections and autoimmune disorders may arise due to excess consumption of certain chemicals including carbon tetrachloride, alcohol, acetaminophen, antibiotics, peroxidized oil, *etc.* [1].

Carbon tetrachloride causes hepatotoxicity due to its conversion into reactive species or metabolites [5]. Recently herbal medicine has been increasingly used to treat liver disease. Around 600 herbal formulations are sold in the market as these agents claim to possess significant hepatoprotective activity [2]. This study aims at the exploration of hepatoprotective activity of leaves extract of

Carissa carandas Linn. in carbon tetrachloride-induced hepatotoxic model

MATERIALS AND METHODS:

Reagents and Chemicals: Silymarin, carbon tetrachloride, olive oil, chloroform, petroleum ether, ethanol. These chemicals were collected from reliable sources.

Preparation of Plant Material: After an extensive literature review, fresh mature leaves were collected from Cumilla district of Bangladesh in the month of September. Then it was authenticated by a taxonomist from Bangladesh National Herbarium, Bangladesh. The accession number was 46724. After collection, the leaves were winnowed from adhering dirt and shade dried for 7 days.

Extraction: After drying, the leaves were pounded into fine powder, 50 gm of which was dissolved into petroleum ether (40° to 60° C) for 24 h to remove chlorophyll. After utter removal of the solvent from the residue, it was again aspersed into 60 % ethanol for 7 days with fitful distortion and churning. After filtration with cotton and filter paper, the extract was obtained by the use of a rotary evaporator as well as gentle heat. The attained extract (13.8 %) was used to evaluate hepatoprotective activity.

Hepatoprotective Activity: Swiss albino mice were purchased from the Department of Pharmacy, Jahangir agar University. The mice were allowed to acclimatize in the laboratory for 7 days before starting the examination. The average weight of the mice was between 22-26 g. The mice were divided into six groups having six mice in each. The following treatments were given to the mice of the respective groups for the evaluation of biochemical parameters:

Group 1: The mice were given distilled water for 8 days (Normal control).

Group 2: The mice were given distilled water for 8 days and 1 ml/kg CCl_4 diluted with olive oil at 1: 1 ratio, intraperitoneally (i.p.) once on day 8 (Negative control).

Group 3: The mice were given Silymarin at 100 mg/kg body weight/d for 8 days (p.o.) and a single dose of CCl_4 (i.p.) on day 8 (Positive control).

Group 4: The mice were given extract of *Carissa carandas Linn.* at 100 mg/kg body weight/d for 8 days (p.o.) and a single dose of CCl_4 on day 8 (i.p.).

Group 5: The mice were given extract of *Carissa carandas Linn.* at 200 mg/kg body weight/d for 8 days (p.o.) and a single dose of CCl_4 on day 8 (i.p.).

Group 6: The mice were given extract of *Carissa carandas Linn.* at 400 mg/kg body weight/d for 8 days (p.o.) and a single dose of CCl_4 on day 8 (i.p.).

Biochemical Parameters Investigation: The animals were anesthetized using chloroform after 24 h of CCl_4 administration. After collecting 1 ml of blood by cardiac puncture, the blood was allowed to clot and then centrifuged at 2500 rpm for 10 min. The serum was separated and used for assay Aspartate transaminase (AST), Alkaline phosphatase (ALP), and bilirubin. The investigation of biochemical parameters was conducted in Exim Bank Hospital, Mirpur, Dhaka, Bangladesh.

RESULT AND DISCUSSION: Simple, inexpensive biochemical liver function test provides more useful information to monitor the damage or death of hepatocytes which may result in an elevation in the liver enzymes [8].

TABLE 1: EFFECT OF SILYMARIN AND *CARISSA CARANDAS LINN.* EXTRACT ON LIVER FUNCTION

Group	Liver enzymes		Bilirubin (mg/dl)
	AST(U/L)	ALP(U/L)	
Normal Control	89.667±2.603	63.333±1.202	0.267±0.0333
Toxic group	164.667±3.844^{a**}	449.333±3.383^{a**}	1.467±0.0333^{a**}
Silymarin	107.667±2.028^{b**}	294.33±2.208^{b**}	0.333±0.067^{b**}
100 mg/kg extract	140.333±2.028^{b**}	380.667±2.028^{b**}	0.733±0.0333^{b**}
200 mg/kg extract	124.333±3.283^{b**}	324±2.646^{b**}	0.567±0.0333^{b**}
400 mg/kg extract	103.667±3.180^{b**}	284.333±2.906^{b**}	0.367±0.0333^{b**}

The values are represented as mean ± S.E.M, (n=6). and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dunnett's test. P < 0.05*, 0.01** and 0.001*** were considered statistically significant, ns = not significant. a = when compared with normal control, b = when compared with ethanol control (negative control group).

AST, ALT and bilirubin were investigated in order to evaluate the hepatoprotective activity of *Carissa carandas linn.* compared to Silymarin as standard.

Here significant differences were obtained when the toxic group (ethanol control group) was compared with the normal control group and all other groups were compared with the toxic group. It has been found that ethanolic leaves extract of *Carissa carandas Linn.* showed significant dose-dependent activity when compared with the toxic group as the release of liver enzymes and bilirubin have been decreased with increasing dose of extract.

CONCLUSION: The ethanolic leaves extract of *Carissa carandas Linn.* showed significant dose-dependent hepatoprotective activity using CCl_4 induced hepatoxicity model. This extract can be further studied for hepatoprotective activity using different hepatotoxicity models.

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CONFLICTS OF INTEREST: Authors declare no conflict of interest.

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