European Journal of Medicinal Plants
3(4): 500-507, 2013

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Protective Effect of Methanolic Extract of *Hylocereus polyrhizus* Fruits on Carbon Tetra Chloride-Induced Hepatotoxicity in Rat

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAR carried out the study design, data collection, data interpretation, manuscript preparation, statistical analysis and research grant collection. Authors AMTI, MAUC, MEU, MRH and MGMU participated in experiments, data collection, literature search and manuscript preparation. Author MMR has provided assistance in taxonomical identification and collections of voucher specimen's numbers for all the plants. Author MAR also supervised the study design, data interpretation and literature search. All authors read and approved the final version of the manuscript.

Research Article

Received 1st June 2013
Accepted 4th July 2013
Published 2nd August 2013

ABSTRACT

Aims: This research investigated the Protective effect of methanolic extract of *Hylocereus Polyrhizus* fruits on carbon tetra chloride-induced hepatotoxicity in Swiss-albino rat.
Place and Duration of Study: Department of Pharmacy, IIUC, Chittagong, Bangladesh and Functional Food Center, Korea Institute of Science and Technology (KIST), Gangneung Institute, Gangneung, Korea, between August 2011 to October, 2012.

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Methodology: Hepatoprotective potential was evaluated in carbon tetrachloride (CCl_4) induced liver injured animal model using male albino rats. Carbon tetrachloride significantly elevated the serum levels of biochemical markers like ALT, AST, ALP, bilirubin, total protein, total cholesterol, triglycerides.
Results: The methanolic extract of *Hylocereus polyrhizus* at a dose of 300 mg/kg body weight (p.o.) significantly protected the carbon tetrachloride (CCl_4) induced liver toxicity in albino rat model. The activity of extract was also comparable to that of silymarin, a known hepatoprotective drug.
Conclusion: The study suggests that oral intake of *Hylocereus polyrhizus* fruits extract enhances the defense status against liver injury.

Keywords: Hylocereus polyrhizus; hepatoprotective; liver toxicity; silymarin.

1. INTRODUCTION

Liver, one of the most important organs of body, plays a pivotal role in regulating various physiological processes. It is involved in several vital functions, such as metabolism, secretion, storage and excretion of many endogenous and exogenous compounds causing its injury or impairment. It has great capacity to detoxify toxic substances and synthesize useful material. It's typical position and functions make it not only the most essential organ but also prone to number of toxicant-targets leading to liver diseases [1].

Management of liver diseases is still a challenge to modern synthetic and allopathic medical practices. Presently, the use of herbal medicines for prevention and control of chronic liver diseases is in the focus for the physicians, pharmaceutical manufacturers and patients. The reasons for such shift toward the use of herbals include the cost of synthetic drugs, adverse drug reactions and the inefficacy of synthetic drugs to some extent [2]. In South Asia, numbers of medicinal plants and their formulations are used in ethnomedical practices and traditional system of medicine for serious liver diseases; most of them speed up the natural healing process of liver. Therefore the research for effective hepatoprotective drug is still continued [2].

Hylocereus polyrhizus (Dragon fruit) is also known as Pitaya, Red Pitahaya, Night blooming Cereus, Strawberry Pear, Belle of the Night and Conderella plant. It belongs to the Cactaceae family of the subfamily Cactoidea of the tribe Cactea. It is a magnificent plant with stunning and beautiful fruit of vibrant colors and shapes. Dragon fruit is believed to be native in central and Southern America and has been brought to Southeast Asian countries including Malaysia, Indonesia, Taiwan, Thailand, Sri Lanka, Bangladesh and Vietnam. Dragon plant has fleshy stems that grow up to 20 feet long when matured. Research has shown the cell proliferation-inhibiting, apoptosis-inducing, enzyme-inhibiting, antibacterial and antioxidant effects of *H. polyrhizus* [3-5]. Apart from these, flavonoids from this plant have various clinical properties, such as antiatherosclerotic, antiinflammatory, antitumour, antithrombogenic and antiosteoporoticand antiviral effects [3-4]. However, the hepatoprotective effect of *H. polyrhizus* extract is yet to be studied. Therefore, the current investigation is an attempt to study the hepatoprotective activity of the methanolic extract of *H. polyrhizus* fruits in carbon tetrachloride induced hepatotoxic model of albino rat.

2. MATERIALS AND METHODS

2.1 Plant Material

Hylocereus polyrhizus fruits were collected from a superstore named AGORA in Chittagong on 14th July 2011. The fruits were taxonomically identified and confirmed by Dr. Sheikh Bokhtear Uddin, Professor of Botany Department, University of Chittagong. A voucher specimen of the plant has been preserved (Accession number HCUD-02) in IIUC herbarium for future reference.

2.2 Preparation of Extracts

The collected fruits were washed thoroughly with distill water, chopped, air dried for a week and pulverized in electric grinder (Miyako 3 in One blender, Miyako, China). The powder (500 g) obtained was successively extracted in methanol (55-60ºC) for 10 days with a 2 days interval. The filtrated supernatant was evaporated to dry using a rotary evaporator (RE200, BB Sterling, UK) under reduced pressure. The crude extract (22.5 g, blackish green semisolid, yield 4.5%) was preserved at 4ºC until further use.

2.3 Experimental Animals

Six-week-old male albino rats weighing 100-120 g were obtained from the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). The animals were acclimatized under standard laboratory conditions (relative humidity 55-65%, room temperature 23.0 ± 2.0 ºC and 12 h light: dark cycle). The animals were fed standard diet and water ad libitum. Animal maintenance and experimentation were carried out according to the rules and regulations of the Institutional Animal Ethics Committee of International Islamic University Chittagong (Ref. 033/2011/animal).

2.4 Determination of Hepatoprotective Activity

The hepatoprotective activity of the extract was determined using carbon tetrachloride induced hepatotoxic rat model [6-7]. After seven days of acclimatization, the rats were divided into four groups five in each and the treatment was carried out for 7 days. Group I served as control and received only saline (1 ml/kg, i.p.). Group II-IV received CCl_4 (CCl_4: liquid paraffin 1:2; 1 ml/kg i.p.) once in every 72 h where Group-II served as CCl_4 control group. Group III (CCl_4+ sylmarin) was standard control group and Group IV (CCl_4+ extract) served as test control group. Test and standard groups received extract (300 mg/kg daily, p.o.) and silymarin (25 mg/kg daily, p.o.) respectively along with CCl_4. After 24 h of the last dose, blood was withdrawn from retro-orbital plexus under sodium phenobarbital anesthesia. Before collecting the blood, the syringe was rinsed with heparin to prevent hemolysis. The blood samples were then centrifuged at 2500 rpm for 10 min to separate serum which was used for the assay of the biochemical markers of liver damage viz. serum alanine transaminase (ALT) [8], serum aspartate transaminase (AST) [9], alkaline phosphatase (ALP) [10], bilirubin [11], total protein [12] and lipid profile like cholesterol [13], low density lipoprotein (LDL) [14], high density lipoprotein (HDL) [15], triglyceride (TG) [16] by using commercially available kits.

2.5 Acute Toxicity Test

Wistar albino rats maintained under standard laboratory condition were used for acute toxicity study. A total of five animals received a single oral dose (0.5, 1.0, 2.0 and 3.0 g/kg BW) of the extract. Animals were kept over-night fasting prior to administration. After administration of the extract, food was withheld for further 3 to 4 h. Animals were observed individually once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days for delayed toxicity. Once daily cage side observation including changes in skin and fur, eyes and mucous membrane, respiratory and circulatory rate, autonomic and CNS changes were observed [17]. The effective therapeutic dose was taken 300 mg/kg BW as one tenth of the median lethal dose (LD_{50} >3.0 g/kg) [18].

3. STATISTICAL ANALYSIS

All results were shown as average ± SEM. Data was statistically analyzed by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test using Statistical Package for Social Science (SPSS software, Version 18.0, IBM Corporation, NY). Values with $p<0.05$ were considered as statistically significant.

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Hepatoprotective effects

Biochemical parameters (AST, ALT, ALP, total protein, total bilirubin) and lipid profile were shown in Table 1. The level of AST, ALT, ALP, total protein and total bilirubin were restored towards the normal value by the extract and silymarin treated carbon tetrachloride intoxicated rats. Decrease of ALT, AST, ALP and Bilirubin by the test control was statistically significant compared to the reference drug Silymarin (Table 1). There was a significant increase in cholesterol, LDL and triglycerides whereas HDL level was decreased in CCl_4 control group compared to that of normal control group (Fig. 1). Treatment with plant extract and silymarin repaired the abnormal lipid profile towards the optimum level.

Table 1. Effect of *Hylocereus polyrhizus* fruit extract on serum biochemical parameters in CCl_4 induced liver damage in rats

Treated group	Biochemical parameters				
	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dl)	Total protein (mg/dL)
Normal control	21.66 ± 1.08[a]	24.33 ± 4.71[a]	18.67 ± 1.08[a]	0.5 ± 0.07[a]	5.80 ± 0.28[a]
CCl_4 control	97.67 ± 1.78[b]	275.67 ± 2.86[b]	934.00 ± 1.41[b]	1.13 ± 0.22[b]	7.50 ± 0.70[b]
Silymarin control	30.00 ± 1.41[c]	148.00 ± 5.34[c]	516.67 ± 2.48[c]	0.90 ± 0.07[c]	6.30 ± 0.14[c]
Test control	23.33 ± 1.08[d]	38.00 ± 3.74[d]	681.33 ± 4.71[d]	0.57 ± 0.10[d]	6.43 ± 0.21[c]

Data are shown as Mean ± SEM of five animals in each group. Values with superscript letters are significant ($p < 0.05$) to each other. Data were analyzed by one-way ANOVA followed by Dunnett's post hoc test (SPSS, Version 18.0) for multiple comparisons.

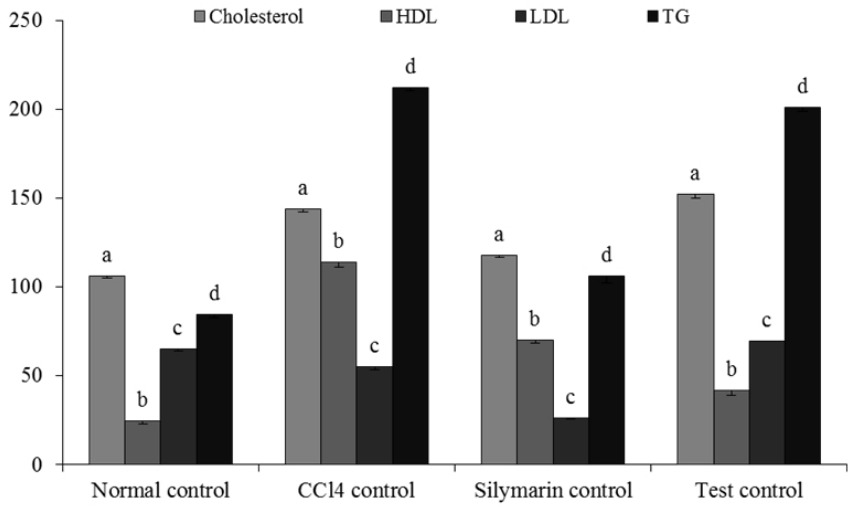

Fig. 1. Effect of *H. polyrhizus* fruit extract on lipid profile in CCl_4 induced liver damage in rats

Data are shown as Mean ± SEM of five animals in each group. Values with superscript letters are significant ($p < 0.05$) to each other. Data were analyzed by one-way ANOVA followed by Dunnett's post hoc test (SPSS, Version 18.0) for multiple comparisons.

4.2 Discussions

Carbon tetra chloride is a well-known model compound for inducing liver injury [19-20]. It is a potent hepatotoxicant and a single exposure to it can rapidly lead to severe centrizonal necrosis and steatosis [21-22]. Its biotransformation by the hepatic microsomal cytochrome P450 produces hepatotoxic metabolites in liver endoplasmic reticulum to the highly reactive trichloromethyl free radical. This free radical in turn reacts with oxygen to form trichloromethylperoxy radical which may attack lipids on the membrane of endoplasmic reticulum more readily than the trichloromethyl free radical [23]. Thetrichloromethylperoxy radical leads to elicit lipid peroxidation, the distribution of Ca homeostasis, elevation of hepatic enzymes and finally results in cell death [24]. This affects the functional integrity of the membranes of plasma, mitochondria and endoplasmic reticulum, resulting in the loss of calcium sequestration and homeostasis and consequently leading to liver damage. This hepatic injury is responsible for the leakage cellular enzymes into the blood. Increase of hepatic enzymes, therefore, mark the liver dysfunction/injury and lowering of enzyme level is a definite indication of hepatoprotective action of the drug.

The results of the present study showed that CCl_4 administration caused severe acute liver damage in rat, demonstrated by remarkable elevation of serum AST and ALT levels. Administration of *H. polyrhizus* fruits extract (300 mg/kg) significantly ($p< 0.05$) normalizes the elevated serum marker enzymes (ALT, AST, ALP) and bilirubin in the treatment group compared to the control group indicating the extract prevented CCl4-induced lethality, elevation of ALT and AST in a single dose. The decrease of ALT, AST, ALP and Bilirubin by the test control was also statistically significant ($p< 0.05$) compared to the reference drug Silymarin demonstrating the potential of the *H. polyrhizus* extract to preserve the hepatocellular tranquility. The enzyme AST predominantly found in mitochondria of hepatocyte cells. ALT is relatively more specific to liver, and thus is a dependable parameter

for detecting liver injury. Serum ALP and bilirubin are also associated with hepatocyte damage. Additionally, the ALT, AST and ALP and serum bilirubin level are largely used as most common biochemical markers for the assessment of liver injury [25,26]. Administration of CCl_4 caused a significant elevation of enzymes level such as AST, ALT, ALP and bilirubin level has been attributed to the damage structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages indicating development of hepatotoxicity [27,28]. The serum lipid profile such as total cholesterol, triglycerides and LDL were also significantly ($p< 0.05$) decreased compared to CCl_4-induced group indicating the amelioration in hepatic function by *H. polyrhizus* administration.

The observed effect of *H. polyrhizus* fruit extract could be due to the presence of phytochemical metabolites in the fruit extract. Lim et al. [29] reported the presence of at least seven phenolic compounds and huge amount of total tocopherol compounds in *H. polyrhizus* seed extract. Rebecca et al. [30] also documented the strong radical scavenging and reducing power contributed by 86.1g of total polyphenol in 0.5 g of dry dragon fruit (gallic acid equivalent). This is quite consistent with the phenolics and tocopherols content which have strong effect in reducing the oxidative stress enhances the cardiac and nephrological damage including hepatic injury. Importantly the fruit extract was found no toxicity or abnormality in acute toxicity test supports the safe use of the extract.

5. CONCLUSION

In vivo biochemical and lipid profile results concluded that methanolic extract of *H. polyrhizus* fruits have protective effect against CCl_4 induced hepatotoxicity in rats. However, further studies are suggested to isolate and identify the lead compounds involved with this function.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed to accomplish this animal experiment. All experiments have been examined and approved by the institutional ethics committee (Ref. 033/2011/animal)". "All authors also declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

ACKNOWLEDGEMENTS

The authors wish to thank International Islamic University Chittagong, Chittagong, Bangladesh for providing research facilities. The research was supported and granted by Department of Pharmacy, International Islamic University Chittagong, Bangladesh. Grant number: Pharm - P & D 29/05-2011.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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