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A Study on Antioxidant and Hepatoprotective Activity of *Hylocereus undatus* Fruits in Experimental Rats

Pandya Prutha Hitendraprasad[1], Aniruddha Banerjee[1], Karunakar Hegde[1]*
Srinivas College of Pharmacy, Valachil, Farangipete Post, Mangalore, Karnataka, India– 574 143

ABSTRACT

A study on antioxidant and hepatoprotective activity of hydroalcoholic extract of *Hylocereus undatus* fruits against acetaminophen and ethanol induced hepatotoxicity was conducted in experimental rats. Two doses 250mg/kg and 500mg/kg, p.o. of the extract were subjected for the evaluation of hepatoprotective potential against acetaminophen (2g/kg) and ethanol (2ml/100g) induced liver injury. Silymarin (25mg/kg) was used as a standard drug. The parameters like SGPT, SGOT, ALP, total bilirubin, direct bilirubin and endogenous enzymes were estimated to assess the liver functions. Both the lower (250mg/kg) and higher dose (500mg/kg) of *Hylocereus undatus* fruit extract showed dose dependent significant decrease in SGPT, SGOT, ALP, total bilirubin and direct bilirubin levels when compared with toxic control. Both the doses showed decrease in LPO and increase in GSH levels. The present study concluded that *Hylocereus undatus* fruit was found to be effective against hepatotoxicity induced by acetaminophen and ethanol. Further studies are needed to isolate and characterize the active principles and to find out the mechanism responsible for its hepatoprotective activity.

Keywords: Acetaminophen, Ethanol, Hepatoprotective, *Hylocereus undatus* fruit

*Corresponding Author Email: khegde_sh2003@yahoo.co.in
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INTRODUCTION

The liver is the largest organ in the body and the chief site for intense metabolism and excretion. So it plays an important role in the maintenance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction[1]. This is also the target organ for chemically induced injuries. It is also 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[2].

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects[3]. 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. In South Asia, numbers of medicinal plants and their formulations are used in ethno medical 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, 4].

Hylocereus undatus (white dragon fruit) is typically the most cultivated vine cactus belonging to the Cactaceae family of the subfamily Cactoideae of the tribe Cacteae[5], originating natively from Mexico and central and south America[6]. Commonly, it is well known under the name of "dragon fruit" or "pitaya". Dragon fruit is rich source of nutrients and minerals such as vitamin B1, vitamin B2, vitamin B3 and vitamin C, protein, fat, carbohydrate, crude fiber, flavonoid, thiamin, niacin, pyridoxine, kobalamin, glucose, phenolic, betacyanins, polyphenol, carotene, phosphorus, iron and Phytoalbumin[7]. It is also rich in phytoalbumin which are highly valued for their antioxidant properties[8]. The fruits are used as hypocholestrolemic, anti-microbial, antioxidant, in constipation, anti-cancer, to boost immune system, in diabetes, to maintain cholesterol level, to promote healthy hair and skin, to prevent anaemia, to improves appetite, vision and brain function[9]. On the basis of its usage in folk medicine for liver disorders and the presence of array of bioactive phytoconstituents *Hylocereus undatus* fruit is selected for the present study. However, the literature survey reveals no scientific data on hepatoprotective effect of *Hylocereus undatus* fruit.

Hence the current investigation is an attempt to study the hepatoprotective and antioxidant activities of hydroalcoholic extract of *Hylocereus undatus* fruit in acetaminophen and ethanol induced hepatotoxicity in experimental rats.

MATERIALS AND METHOD

Plant Material

The *Hylocereus undatus* Fruit (Dragon Fruit) was collected from local market of Mangaluru district. The fruits were identified and authenticated by a Taxonomist.

Preparation of Extracts

The collected fruit was washed thoroughly with distil water, chopped, air dried for a week and pulverized in electric grinder. The powder was extracted by cold maceration technique with hydro-alcohol (50:50) for 24h. The extract was filtered by using Whatman filter paper and concentrated by evaporation on water bath. The extract was preserved in airtight containers and kept at 4-5°C until further use.

Dose of the Extract

The dose was selected from the previous research work which is 250mg/kg and 500mg/kg[10].

Experimental Animals

Healthy Wistar albino rats of either sex weighing 150-200 g were used. Animals used in the study were procured from registered breeder. The animal care and handling was carried out according to CPCSEA guidelines. Animals were acclimatized to the animal quarantine for one week prior to the experiment under controlled conditions of temperature (23 ± 2°C) and were housed in sterile polypropylene cages containing paddy husk as bedding material with maximum of six animals in each cage. The rats were fed on standard food pellets and water ad libitum. The studies conducted were approved by the Institutional Animals Ethics Committee, Approval No.: SCP/IAEC/F150/P130/2018.

Determination of Hepatoprotective Activity

The hepatoprotective activity of the extract was determined using acetaminophen and alcohol induced hepatotoxic rat models. The Wistar albino rats (150-200 g) of either sex were randomly divided into five groups of six in each for both acetaminophen and alcohol induced hepatotoxic rat models. The different groups were assigned as follows.

Acetaminophen Induced Liver Toxicity

Group I: Negative control (vehicle; distilled water 1 ml/kg; p.o.)

Group II: Positive control (Acetaminophen 2g/kg; p.o.)

Group III: Acetaminophen + standard drug (Silymarin 25mg/kg; p.o.)

Group IV: Acetaminophen + *Hylocereus undatus* Fruit extract (250mg/kg; p.o.)

Group V: Acetaminophen + *Hylocereus undatus* Fruit extract (500mg/kg; p.o.)

All the treatment were given orally once daily for 7 days throughout the treatment. All the four groups except group I were intoxicated by oral administration of acetaminophen (2g/kg. b.w.) on 7th day of treatment. After 48h of acetaminophen intoxication, animals were anesthetized; blood was collected through retro orbital and analysed for various biochemical parameters. Animals were sacrificed by euthanasia; liver was dissected out and used for endogenous antioxidant analysis[11].

Alcohol Induced Liver Toxicity

Group I : Negative control (Vehicle; distilled water, 1ml/kg; p.o.)

Group II: Positive control (40% v/v ethanol 2ml/100g; p.o.)

Group III: Standard drug (Silymarin 25mg/kg + 40% v/v ethanol, 2ml/100g; p.o.)

Group IV: 40% v/v ethanol, 2ml/100g + *Hylocereus undatus* Fruit extract (250mg/kg; p.o.)

Group V: 40% v/v ethanol, 2ml/100g + *Hylocereus undatus* Fruit extract (500mg/kg; p.o.)

All the four groups except group I will be intoxicated by 40% v/v ethanol (2ml /100g body weight) daily for 21 days. All the treatment were given orally once daily for 21 days. After 24h of ethanol administration i.e on the 22nd day, animals were anesthetized; blood was collected through retro orbital and analyzed for various biochemical parameters. Animals were sacrificed by euthanasia; liver was dissected out and used for endogenous antioxidant analysis[12].

Serum collected from acetaminophen and alcohol induced model were used for the assay of the biochemical markers of liver damage viz. Serum glutamate pyruvate transaminase (SGPT)[13], Serum glutamate oxaloacetate transaminase (SGOT)[14], Serum alkaline phosphatase (ALP)[15], Serum direct bilirubin[16], Serum total bilirubin[17].

Determination of *In-vivo* antioxidant Activity

The in vivo antioxidant activity of extract was measured using liver tissue by Lipid peroxidation (LPO)[17] and Reduced glutathione (GSH)[18].

Statistical Analysis

All the data were expressed as mean ± SEM. The statistical significance between groups was compared using one way ANOVA, followed by Dunnett's multiple comparisons test.

RESULTS AND DISCUSSION

Evaluation of hepatoprotective activity of extract on acetaminophen induced hepatic damage in rats

Administration of Acetaminophen to rats caused significant liver damage, as evidenced by the altered serum biochemical parameters. Pre-treatment of rats with *Hylocereus undatus* fruit extract exhibited marked protection against acetaminophen induced hepatotoxicity. The effects produced by *Hylocereus undatus* fruit extract were comparable with that produced by the standard Silymarin. The results were further confirmed by evaluation of liver endogenous antioxidant enzymes of the rats**.** The effect of fruit extracts of *Hylocereus undatus* on various biochemical parameters (Table 1).

Table 1: Effect Hydroalcoholic extract *of Hylocereus undatus* fruit on Acetaminophen induced liver toxicity

Groups	Treatment	ALP (U/I)	SGOT (U/I)	SGPT (U/I)	TB (mg/dl)	DB (mg/dl)
Vehicle control	Dis. Water 1ml/ Kg	141.5±0.5	103.3±0.61	85.5±0.92	0.62±0.08	0.58±0.08
Toxic control	Acetaminophen 2g/Kg	364.2± 4.17[a]	345.8±6.38[a]	208.7±6.81[a]	2.47±0.19[a]	1.99±0.09[a]
Standard	Silymarin 25mg/Kg	145.5±4.46[***]	115.5±2.22[***]	94.0±1.65[***]	0.7±0.07[***]	0.65±0.06[***]
Low dose	*H. undatus* 250mg/Kg	210.0±6.45[*]	190.8±2.62[*]	140.5±2.11[*]	1.40±0.07[*]	1.12±0.05[*]
High dose	*H. undatus* 500mg/Kg	170.0±2.91[**]	150.8±2.64[**]	120.3±2.89[**]	0.96±0.14[**]	0.92±0.10[**]

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test. [a]p<0.001 when compared with vehicle treated control group.*p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Evaluation of liver endogenous antioxidant enzymes

It was observed that animals treated with Acetaminophen developed a hepatic damage, increase in LPO and decrease in GSH when compared to normal control. Animals treated with standard (Silymarin) showed extremely significant (P<0.001) increase in GSH and decrease in LPO. *Hylocereus undatus* (250mg/kg) treated animals showed significant (P<0.05) decrease in LPO and significant (P<0.05) increase in GSH. *Hylocereus undatus* (500mg/kg) treated animals showed moderately significant (P<0.01) decrease in LPO and moderately significant (P<0.01) increase in GSH (Table 2).

Table 2: Effect of Hydroalcoholic extract of *Hylocereus undatus* fruit on LPO and GSH in Acetaminophen induced liver toxicity

Groups	Treatment	LPO (Abs at 535 nm)	GSH (Abs at 412nm)
Normal Control	Distilled water 1 ml/Kg	3.5±0.42	28.83±0.6

Toxic control	Acetaminophen 2g/Kg	18.33±0.76[a]	20.0± 1.03[a]
Standard	Silymarin 25mg/Kg	5.5±0.55***	26.0±0.57***
Low dose	*H. undatus* 250mg/Kg	13.15±0.6*	22.6±0.96*
High dose	*H. undatus* 500mg/Kg	11.16±0.6**	24.79±0.48**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test, [a]p<0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01,***p<0.001 when compared with toxic control.

Evaluation of hepatoprotective activity of extract on ethanol induced hepatic damage in rats

In the present study, the hepatotoxicity was successfully produced by administration of ethanol 2ml/100g body weight for once daily and the hepatoprotective activity of *Hylocereus Undatus* fruit was determined from the serum parameters SGOT, SGPT, ALP, total bilirubin and direct bilirubin. The results were further confirmed by evaluation of liver endogenous antioxidant enzymes. The effects of hydroalcoholic extract of *Hylocereus undatus* fruit on various biochemical parameters are shown in Table 3.

Table 3: Effect of Hydroalcoholic extract of *Hylocereus undatus* fruit on in ethanol induced liver toxicity

Groups	Treatment	ALP(U/I)	SGOT(U/I)	SGPT(U/I)	TB(mg/dl)	DB(mg/dl)
Normal control	Dis. Water 1ml/kg	106.3± 0.67	55.3± 1.02	44.8± 1.14	0.62± 0.06	0.58±0.08
Toxic control	Ethanol 2ml/100 g	230.0± 2.35	124.7± 1.2	112.8±1.53	2.09± 0.07	1.90± 0.07
Standard	Silymarin 25mg/Kg	135.0± 2.6***	69.0±1.0***	54.7±1.3***	0.72±0.05***	0.62± 0.05***
Low dose	*H. undatus* 250 mg/Kg	202.2± 1.35*	98.4± 1.3*	89.3± 1.2*	1.49± 0.05*	1.05± 0.05*
High dose	*H. undatus* 500 mg/Kg	182.3± 1.1**	86.4± 1.1**	76.3± 0.8**	1.09±0.04**	0.96±0.04**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test. ap<0.001 when compared with vehicle treated control group.*p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Evaluation of liver endogenous antioxidant enzymes

Table.4 shows the effects of extracts of *Hylocereus undatus* on LPO and GSH concentrations in rat liver after challenging with ethanol. It was observed that animals treated with ethanol developed a hepatic damage observed as increase in LPO and decrease in GSH when compared to normal control. Animals treated with standard (Silymarin) showed extremely significant (P<0.001) increase in GSH and decrease in LPO. *Hylocereus undatus* extract (250mg/kg) treated animals showed moderately significant (P<0.05) decrease in LPO and moderately significant (P<0.05) increase in GSH as compared to toxic control. *Hylocereus undatus* extract (500mg/kg) treated animals showed significant (P<0.01) decrease in LPO and significant (P<0.01) increase in GSH.

Table 4: Effect of extract of *Hylocereus undatus* fruit on LPO and GSH in ethanol induced liver toxicity

Groups	Treatment	LPO(Abs at 535 nm)	GSH(Abs at 412nm)
Normal Control	Distilled water 1ml/kg	1.7± 0.12	5.12± 0.12
Toxic control	Ethanol 2ml/100 g	5.8± 0.12[a]	2.03± 0.14[a]
Standard	Silymarin 25mg/kg	1.89± 0.13[***]	4.38± 0.12[***]
Low dose	*H. undatus* 250mg/kg	4.29± 0.13[*]	2.9±0.08[*]
High dose	*H. undatus* 500mg/kg	3.05± 0.08[**]	3.89±0.05[**]

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test, ap<0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

DISCUSSION

The present study was under taken to evaluate the hepatoprotective activity of Hylocereus undatus fruit extract by using two models i.e., acetaminophen and ethanol induced liver damage model. The parameters used for the assessment of hepatoprotective activity were serum enzyme estimations like SGPT, SGOT, ALP, total bilirubin, direct bilirubin, endogenous antioxidants like LPO and GSH level and histopathological studies.

Administration of acetaminophen elevates the serum levels of SGPT, SGOT, ALP, Total bilirubin and direct bilirubin significantly. This is due to its bio activation to a toxic electrophile, N- acetyl-p-benzoquine-imine. Acetaminophen is normally eliminated mainly as sulphate and glucuronide. Only 5% of the acetaminophen is converted into N- acetyl-p- benzoquinineimine. However, upon administration of toxic doses of acetaminophen, the sulfation and glucuronidation routes become saturated and hence, higher percentage of acetaminophen molecules are oxidized to highly reactive N-acetyl-p-benzoquineimine (NAPQI) by cytochrome-450 enzymes. A semi Quinone radical, obtained by one electron reduction of NAPQI, can covalently binds to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage[19].

In liver, ethanol gets metabolized into acetaldehyde in the presence of alcohol dehydrogenase enzyme. This acetaldehyde further metabolized to acetate by acetaldehyde dehydrogenase enzyme. These two enzymes cause the reduction of nicotinamide adenine dinucleotide (NAD) to NADH. That results an alteration in the ratio of NAD/NADH, which leads to steatosis or fatty liver[20]. This causes impairment of carbohydrate metabolism, lipid metabolism, gluconeogenesis and finally results in the diversion of metabolism to ketogenesis and fatty acid synthesis[21]. Long term exposure to alcohol causes the activation of kupffer cells that induce the generation of reactive

oxygen species and finally precipitate to oxidative stress, this in turn promotes hepatocyte necrosis, apoptosis, lipid peroxidation, inflammation and fibrosis[22].

Studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many plant extracts[23]. Various classes of phytoconstituents are well established to be antioxidant, viz., flavonoids[24], tannins, vit-E, vit-C, etc.

Preliminary phytochemical screening *of Hylocereus undatus* fruit extract revealed the presence of saponins, flavonoids and polyphenols[7,8]. This indicates that the high soluble phenolics as well as flavonoids present in the fruit extract could have the strong antioxidant[9] and free radical scavenging activity[9] in all used assay. Natural antioxidants from the plant extracts provide a measure of production of radical scavengers that slows the process of oxidative damage in liver, in turn showed hepatoprotective activity.

The present study revealed that the *Hylocereus undatus* fruit extract have proved its antioxidant effects of bioactive constituents for observed hepatoprotective activity.

CONCLUSION

In the present study the hydroalcoholic extract of *Hylocereus undatus* fruit found to have significant hepatoprotective activity and antioxidant activity in both acetaminophen and ethanol induced hepatic injury models. Our results showed that the hepatoprotective effects of fruit extract may be due to both an increase in the activity of the antioxidant defence system and an inhibition of lipid peroxidation. It reveals that the *Hylocereus undatus* fruit extract can be utilized for its hepatoprotective activity in hepatic disorders. Further studies are needed to isolate and characterize the active principles and to find out the mechanism responsible for its hepatoprotective activity.

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