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Hepato Reno Protective Effect Of Hylocereus Undatus On Liver And Kidney Biomarkers In Ccl_4 Intoxicated Rats

R. Sampath[1] and R. Udayakumar[2]*

[1,2*]PG and Research Department of Biochemistry, Government Arts College (Autonomous) (Affiliated to Bharathidasan University, Tiruchirappalli), Kumbakonam 612 002 Tamilnadu, India.

***Corresponding Author:-** R. Udayakumar

*PG and Research Department of Biochemistry, Government Arts College (Autonomous) (Affiliated to Bharathidasan University, Tiruchirappalli), Kumbakonam 612 002 Tamilnadu, India.

Abstract

The present study was aimed to evaluate the therapeutic effects of Hylocereus undatus fruit on liver and kidney markers in Carbon tetrachloride (CCl_4) intoxicated rats. The present experiment was designed as group I- control, group II -CCl_4 2ml/kg, group III -CCl_4 2ml/kg + Silymarin 100mg/kg, group IV- CCl_4 2ml/kg + H. undatus 200 mg/kg. The results indicate that group II possessed significantly ($P < 0.05$) increased level of liver and kidney markers like ALT, AST, ALP, bilirubin, urea and creatinine when compared with group I while Group III and Group IV were non-significant when compare with group I. The protein, albumin and globulin were indicate that group II (CCl_4 induction only) significantly ($P < 0.05$) decreased in the liver marker as compare with control group while Group III and Group IV were non-significant compared with group I. The histopathologic evaluation suggested that H. undatus decreased hepatic necrosis induced by CCl_4. This provides scientific support for the use of medicinal plant as H. undatus in the treatment of liver and kidney disorders.

Keywords: Hylocereus undatus, CCl_4, liver, kidney, biochemical parameters, histopathology.

INTRODUCTION

The liver and kidney have a key role in body hemostasis. In normal conditions, these vital organs remove toxins and free radicals and prevent the accumulation of noxious substances threatening body organs (Mohamed et al., 2021). Liver is one the most important and largest organ of the body which plays an important role inthe formation of hormones, serum proteins, clotting factors, cholesterol, bile, enzymes, detoxification of the toxic substances (certain drugs, alcohol), maintain homeostasis and metabolism of the various substances (carbohydrates, proteins and lipids). The kidney play an important role in human physiology, maintaining fluid homeostasis, regulating blood pressure, erythrocyte production and bone density, regulating hormonal balance, and filtering and removing nitrogenous and other waste products (Tienda et al., 2022). Deterioration of liver and kidney causes severe health problems (Stickel and Schuppan, 2007). A number of chemical toxicants have also been reported for toxicity in animals and humans. Carbon tetrachloride (CCl_4) is one of the toxic agent (Danladi et al., 2013). The level of tissue injury is related to the amount of dose and period of exposure to carbon tetrachloride (Abdel-Moneim et al., 2015). Its mechanism of toxicity is based on lipid membrane peroxidation and generation of trichlomethyl radical ($\bullet CCl_3$), causing severe cell injury (Tan et al., 2016).

There is a growing interest of natural products in human diet, both due to the possible negative effects of synthetic food additives on human health and the increased consumer perception of this problem in recent years. Many investigators have given the reports about plants, that having phenolic compounds such as tannins, flavonoids, procyanidins, anthocyanins and phenolic acids have liver, heart and nephroprotective activities (Oliboni et al., 2011). In the absence of reliable hepatoprotective drugs in conventional medicine (Baravalia et al., 2011), which in turn has protective activity at the kidney level, it is necessary to look

towards medicinal plants where those with hepato-renoprotective properties have been sought over the years (Qadir and Ahmad, 2017), So in this study the hepatoprotective and nephroprotective activity of ethanolic extracts of Hylocereus undatus on CCl_4-induced toxicity in rats was evaluated.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 180-200gms were used in this study. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºC and 12 hrs light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water ad libitum. They were acclimatized to the environment for 1 week prior to experimental use. All the animal experimental protocols were approved (Approval number: CPCSEA/265/2022) by the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Collection of plant materials

The fruits of Hylocereus undatus were collected from Fruit shop, Thanjavur, Tamil Nadu, India. The collected fruits were washed in water, cleaned well to remove all traces of insects, dust and other kinds of impurities. The fruits were peeled, cut into pieces and collected the flesh and dried under shade for two weeks. The dried flesh was pounded into powdered form and properly stored in sealed sterilized container for further study.

Preparation of extract

The dragon fruits (Hylocereus undatus) flesh were cleaned, dried, coarsely and extracted with ethanol using maceration technique for 24 h at room temperature. The extract were filtered by filter paper Whatman No. 42 (125mm). The extract was evaporated and concentrated under reduced pressure using rotary evaporator with the water bath set at 45°C. The crude extracts were used for experimental study.

Experimental design (Hussain et al., 2022)

To test the hepatoprotective potential of Dragon fruit extract against CCl_4 induced hepatotoxicity, research experiment was conducted for the period of 28 days. The rats were uniformly divided into 4 groups, Olive oil was used as a vehicle (1:1 ratio of carbon tetrachloride: olive oil) for carbon tetrachloride treated (2 ml/kg b.w) rats.

Group I: Normal control-Olive oil treatment (Orally gavage).
Group II: Negative control-Carbon tetrachloride treatment (Orally gavage)
Group III: Positive control-Silymarin treatment (100 mg/kg b.w) with Carbon tetrachloride treatment (Orally gavage)
Group IV: Dragon fruit extract (200 mg/kg b.w) treatment with Carbon tetrachloride treatment (Orally gavage)

After 24 h of last dose of carbon tetrachloride, rats had been sacrificed by decapitation of the cervical region. Blood samples were collected by cardiac puncture using a sharp syringe. Blood collected in the purple top bottle was mixed with EDTA by inverting the tube about 8–10 times. For the purpose of serum blood was collected into red top bottles, and the tubes were kept vertically for 30 min, and then centrifuged at 3000 rpm for 15 min. Supernatant (serum) was gently taken into eppendorf tubes and analysis of liver and kidney markers. Liver was stored in 10% formalin for the purpose of histopathology.

Biochemical analysis and histopathological study

Protein was estimated by the method of Lowry et al. (1951). Albumin was estimated by the method of Rodkey (1965). The serum total bilirubin was estimated by the method of Malloy and Evenlyn (1937). The serum AST and ALT was estimated by the method of Reitman and Frankel (1957). The serum alkaline

phosphatase activity was estimated by the method of Kind and King (1954). Urea was estimated by the method of Natelson (1957). Serum creatinine was estimated by alkaline picrate method (Boneses and Taussy, 1954). Histological study of liver was carried out by standard method (Ochei and Kolhatkar, 2000).

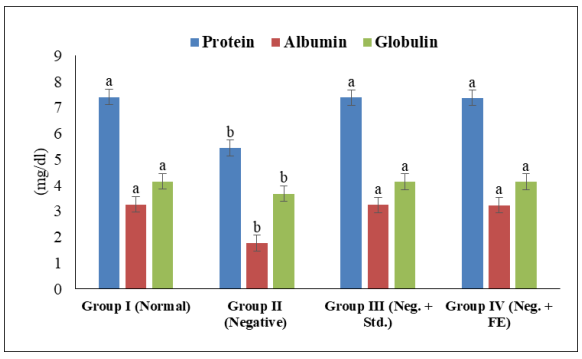
RESULTS AND DISCUSSIONS

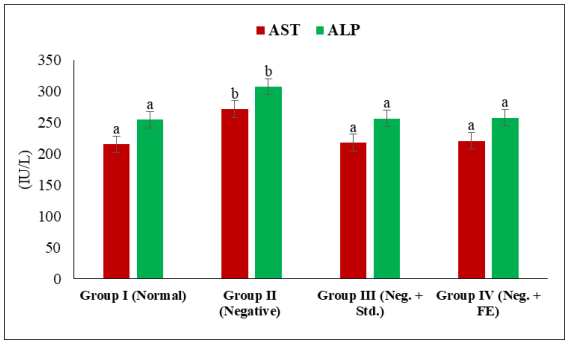
Liver damage is a major health problem and is a serious challenge to public health in the world (Pimpin et al., 2018). The existing synthetic liver treatment drugs create further complications hence; herbal medicines have more demands and widespread use in hepatic disorders since long time (Rotman and Sanyal, 2017). The effect of Dragon Fruit (Hylocereus undatus) on the values of protein, albumin, globulin, bilirubin, ALT, AST, ALP, urea and creatinine are presented in Table 1and Figure 1. The results indicate that group II (CCl_4 induction only) significantly ($P < 0.05$) increased the liver markers ALT, AST, ALP, bilirubin and kidney markers urea and creatinine as compared with group I while Group III and Group IV were non-significant compare with group I. The protein, albumin and globulin were indicate that group II (CCl_4 induction only) significantly ($P < 0.05$) decreased in the liver marker as compare with control group while Group III and Group IV were non-significant compare with Group I group I. Liver malfunction is recognized by increased blood levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) (Saputri et al 2018). Kidney damage was also characterized by a significant enhancement in serum level of creatinine and blood urea nitrogen as well as urinary excretion of proteins were reported (Hosseini et al 2020).

Table 1: Effect of Dragon Fruit (**Hylocereus** undatus) on Liver and Kidney markers

Parameters	Group I (Normal)	Group II (Negative)	Group III (Neg. + Std.)	Group IV (Neg. + FE)
Protein (mg/dl)	7.41 ± 0.15^a	5.44 ± 0.16^b	7.39 ± 0.19^a	7.37 ± 0.13^a
Albumin (mg/dl)	3.26 ± 0.13^a	1.77 ± 0.03^b	3.24 ± 0.06^a	3.23 ± 0.10^a
Globulin (mg/dl)	4.15 ± 0.25^a	3.67 ± 0.17^b	4.14 ± 0.25^a	4.14 ± 0.24^a
Bilirubin (mg/dl)	0.81 ± 0.05^a	1.25 ± 0.07^b	0.82 ± 0.04^a	0.83 ± 0.05^a
ALT (IU/L)	72.04 ± 4.23^a	92.69 ± 5.60^b	73.62 ± 3.80^a	75.03 ± 6.47^a
AST (IU/L)	214.98 ± 8.39^a	271.53 ± 17.43^b	217.73 ± 13.34^a	220.80 ± 16.74^a
ALP (IU/L)	254.51 ± 9.78^a	307.54 ± 8.21^b	256.49 ± 13.16^a	257.66 ± 16.02^a
Creatinine (mg/dl)	0.73 ± 0.04^a	0.87 ± 0.02^b	0.74 ± 0.03^a	0.75 ± 0.03^a
Urea (mg/dl)	33.77 ± 3.31^a	59.39 ± 5.83^b	34.39 ± 2.75^a	34.51 ± 3.12^a

Values are expressed as Mean ± SD for six rats. Mean values within the Row followed by different letters (Superscript) are statistically significant ($P<0.05$) from each other group and same letter was statistically non-significant ($P>0.05$) were comparison by ANOVA, Duncan's multiple range test (DMRT), significant level **α** 0.05.





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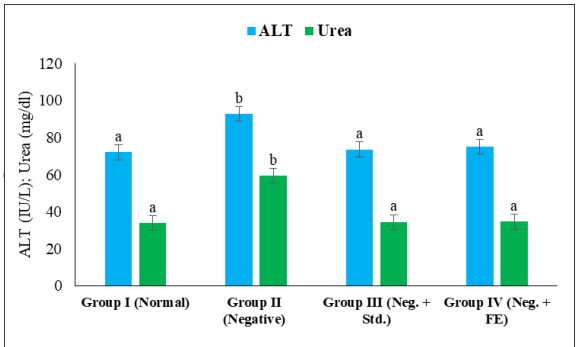


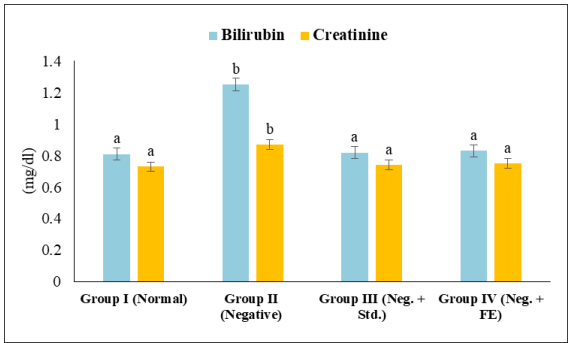


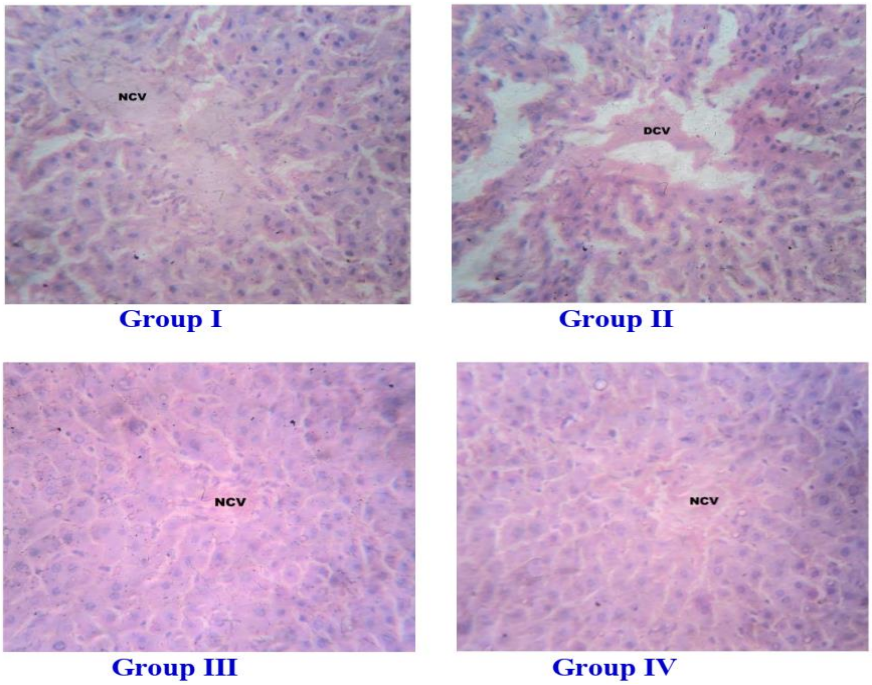
Figure 2: Effect of Dragon Fruit on Liver and Kidney markers in CCL4 induced hepato toxicity rats

The liver is a vital organ responsible for various metabolic functions and clearance and transformation of drugs and toxins from the blood and regulate immune responses (Yan et al., 2014). Kidney is also important homeostatic organ (Mitrakou, 2011). Thus both are exposed to toxic injury. More than thousand drugs of the current pharmaceutical era have been shown to cause hepato and renal toxicity with diverse clinical appearances (Björnsson, 2016). More over serum levels of many biochemical markers like alanine amino transaminase (ALT), aspartate amino transaminase (AST), serum glutamic oxalo acetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total cholesterol, and total bilirubin (TB) are evaluated along with serum electrolyte, urea, uric acid and creatinine imbalances are assessed (Olaniyan and Ateni, 2018). Different aspects of liver injury during hepatotoxicity may include hepatitis (Wang et al., 2013); granuloma (Kamal et al., 2012); vascular lesions (Rubbia-Brandt et al., 2010). The major effect of hepatotoxicity is jaundice which is caused due to bilirubin accumulation in the extra cellular fluid, causing weakness, severe fatigue, dark urine and light colored stool (Frenzel and Teschke, 2016). A number of chemical toxicants have also been reported for toxicity in animals and humans. Carbon tetrachloride (CCl_4) is one of the toxic agent (Danladi et al., 2013) The level of tissue injury is related to the amount of dose and period of exposure to carbon tetrachloride (Abdel-Moneim et al., 2015).

Hepatic and renal injury is related always with cellular necrosis and increases the markers (Contreras-Zentella and Hernandez-Munoz, 2016). During the present research the rats intoxicated with carbon tetrachloride (CCl_4) were treated with a standard antioxidant drug, Silymarine and Hylocereus undatus. Silymarine is a standard drug and frequently used as a hepatoprotective medicine, derived from a plant, Silybum marianum (Tajmohammadi et al., 2018). These results are nearly equivalent to the standard drug Silymarine. The current study is in agreement with that of Cordeiro and Kaliwal, (2013) who investigated the liver protective activity of the alcohol extract of Capparis sepiaria stem against CCl_4 intoxicated Albino rats. The kidney is a major homeostatic organ keeps the balance of body fluids by cleaning and secreting metabolites like urea, uric acid, creatinine, and minerals from the blood and eliminate the nitrogenous wastes together with water, as urine (Javaid et al., 2012).

Histopathological studies

The photomicrograph of the normal control showed a normal liver architectural design, but the carbon tetrachloride-treated rats showed degeneration of the central vein compared to the control. More so, the hepatocytes appeared a bit distorted and many microcysts within the stroma were found. The histopathological evaluation indicated that the extract did not have any adverse effects on the morphology of the tissues and these observations supported the biochemical results mentioned.



NCV: Normal central vein; DCV: Degeneration of central vein

Plate 1: Histopathology of Liver (10 × 40X) in control and experimental rats

Immediate rise in serum transaminases showed, CCl_4 induced severe toxicity (Jannu et al., 2012). The statement was confirmed by necrosis and infiltration of inflammatory cells during histopathological examination of microphotographs of liver sections (Jiang et al., 2016), The liver sections of rat treated with H. undatus after CCl_4 intoxication are revealed to have amended cellular membrane architecture or less damage to the hepatic cells as compared to rat treated with CCl_4. The improved histoarchitecture further verify the liver preventive potential of the H. undatus and support the results of biochemical parameters. Normalization of CCl_4 impaired liver and kidney architecture by therapeutic plants were described by many investigators like Nwaigwe et al., (2012) reported the regulation of hepatotoxicity by Olax viridis, Tephrosia calophylla and Curcuma longa respectively. This action could be attributed to the high content of phytochemical constituents which are potent antioxidants can protect liver and kidney cells from injury.

CONCLUSION

In conclusion, ethanolic extract of the fruits of H. undatus showed promising hepato-reno protective activity against CCl_4 induced toxicity in experimental rats. Protection of the liver and kidney were measured via serum liver markers AST, ALT, ALP, Protein, and bilirubin and kidney markers urea, creatinine and protein and histopathology of liver section. A moderate degree of protection was also offered on both liver and kidney functions. Hence this research recommends that the H. undatus fruit extract possess potential hepato-reno protective activity and may be used for therapeutic purpose.

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