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Hepatoprotective activity of *Hydrolea zeylanica* leaf extract on liver damage caused by carbon tetrachloride in rats

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Abstract : The hepatoprotective activity of *Hydrolea zeylanica* leaf methanolic extract(HZLME) (*Hydrophyllaceae*) at doses of 250 mg/kg and 500 mg/kg were evaluated by carbon tetrachloride (CCl_4) intoxication in rats. The toxic group which received CCl_4 (0.3 ml/kg) dissolved in 1:1 ratio in olive oil by subcutaneous (s.c.) alone exhibited significant increase in serum alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and total bilirubin (TB) levels. It also caused significant ($P<0.001$) decrease in protein levels. The groups received pre-treatment of HZLME at a dose of 500 mg/kg b.w. p.o. had controlled the AST, ALT, ALP and total bilirubin levels and the effects were comparable with standard drug (silymarin 100 mg/kg b.w.p.o.). The total protein (TP) and albumin (ALB) levels were significantly increased in the animals received pre-treatment of the extract at the higher dose level. The animals received pre-treatment of the extract shown decreased necrotic zones and hepatocellular degeneration when compared to the liver exposed to CCl_4 intoxication alone. Thus the histopathological studies also supported the protective effect of the extract.
Key words : CCL_4, *Hydrolea zeylanica*,methanol, extract, hepatoprotective, silymarin.

Introduction

Tissue damage is caused by excessive production of free radicles[1]. Liver is known to be the major organ involved in the detoxification of xenobiotics, and is thus the main target of tissue injury produced by these chemicals and their metabolites. Reactive oxygen species produce deleterious effect on membrane lipids of the cellular components thereby producing peroxidation of lipids which leads to cell death[2]. Liver is also responsible for regulating homeostasis in the body and is involved with almost all the biochemical pathways related to growth, nutrient supply, maintaining immunity and reproduction[3]. Hence prevention of hepatotoxic damage is of great concern. Phenolic acids and flavonoids possess diverse biological activities including antioxidant and hepatoprotective properties. Recently it has been considered that polyphenolic compounds are great antioxidants and proved to be more effective than Vitamin C, E and Carotenoids[4]. Several flavonoids such as catechin, apigenin, quercetin, naringenin, rutin, and venoruton are also reported to ameliorate hepatotoxic effects[5].CCL_4 is one of the most common hepatotoxin used for experimental induction of liver injury in animal studies[6, 7]. Impoverishment of modern system of medicine in terms of a reliable liver protective drug switched

on the exploration of traditional systems of medicine including Ayurveda, Siddha, Unani etc.for a probable answer to hepatotoxicity[8,9] .Numerous medicinal plants are being researched for an effective hepatoprotective remedy. A number of medicinal preparations in the Indian system of medicine (Ayurveda) have been used as effective hepatoprotective. In view of this several medicinal preparations and a number of medicinal plants mentioned in Ayurveda for treatment of liver disorders are being investigated[10] .

Hydrolea zeylanica (L.)Vahl.family *Hydrophyllaceae* is found throughout India in moist and swampy places[11] .It is an annual herb with procumbent and branching stems up to 30 cm long. The leaves are dark green, narrow and pointed at the tip and are arranged alternately on the swollen, spongy stems. The stems growing above water are firmer and sturdier[12]. It is also known as Koliary and used for antiseptic[13]and antidiabetic[14]activities. In our previous study, we performed pharmacognostical and phytochemical Analysis[15], anthelmintic[16], antiulcer[17], and wound healing[18], activity of *Hydrolea zeylanica.*

The present investigation was undertaken to screen hepatoprotective activity of *Hydrolea zeylanica* leaf extracts in rats, as the plant is traditionally used for skin diseases and wound healing[19-23].

Materials and Methods

Plant Material

The leaves of *H. zeylanica* (L) belonging to the family *Hydrophyllaceae* were collected from local area of Chittoor district Andhra Pradesh (India). The plant was identified and authenticated by Dr. K. Madhava Chetty, Plant Taxonomist (IAAT: 357), Department of Botany, Sri Venkateswara University Tirupati, Andhra Pradesh, India. The plant bearing voucher No. 1012 (10/12/2014) was deposited at Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Drugs and Chemicals

All the chemicals were analytical grade. CCl4 was obtained from Pharmacognoy lab, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad. India. Silymarin was obtained from Allied Chemicals & Pharmaceuticals (P) Ltd. New Delhi. It is a poly herbal formulation which produces hepatoprotective activity against CCl4.

Preparation of the Extracts

The leaves of *Hydrolea zeylanica* (L.) were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was then subjected to successive extraction by hot percolation method using petroleum ether, chloroform, methanol, and distilled water in a soxhlet extractor[24].The different extracts obtained were evaporated using a rotary evaporator to obtained semisolid mass. The extracts thus obtained were subjected to phytochemical screening and the *H. zeylanica* leaf methanolic extract (HZLME) was used for further studies.

Ethical Committee Approval

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) No.-IAEC/AUCOP/2016/01, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad, Telangana, India. The experimental animals were treated as per the guideline of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Phytochemical Analysis

The qualitative chemical tests carried out for the identification of the different phytoconstituents present in the powered crude drugs by standard procedures. They are usually tested for the presence of alkaloids, flavonoids, phenols and phenolic compounds, tannins, glycosides, triterpenes, steroids, saponins etc.[25,26].

Acute Oral Toxicity

Acute oral toxicity study was performed as per OECD- 423 guidelines category IV (acute toxic class method,). Albino rats (n = 3) of either sex selected by random sampling technique were employed in this study. The animals were kept fasting for 4 h with free access to water only. HZLME was administered orally with maximum dose of 2000 mg /kg body weight by gastric intubation. The mortality was observed for three days. If mortality was observed in 2 out of 3 animals or 3 out of 3 animals then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed then the procedure was repeated for further higher dose such as 3000 mg/kg of body weight[27-28].

Hepatoprotective activity

Animal Model

Healthy Wistar Albino Rats weighing about (180-250gm) of either sex was used for the studies. The animals were housed in large polypropylene cages in a temperature controlled room (25˚C ±3˚C), relative humidity (50±20 %) and provided with standardized pellet feed and clean drinking water *ad libitum*.

Experimental Design:CCl4 induced-hepatotoxic activity

The animals were randomly divided into 5 groups of 6 animals per group[29] and treated as follows.

1. **Group A** received 1 ml of 30 % PEG orally as a control group,
2. **Group B** received 1 mL/kg body weight of CCl4 subcutaneously for 7 days as a toxic group,
3. **Group C** received Silymarin (100mg/kg, p.o.) and CCl_4 (1mL/kg, s.c.) of body weight for 7 days,
4. **Group D** received HZLME (250mg/kg, p.o.) and CCl_4 (1mL/kg, s.c.) for 7 days,
5. **Group E** received HZLME (500mg/kg, p.o.) and CCl_4 (1mL/kg, s.c.) for 7 days

All rats were sacrificed by cervical dislocation 24 h after the last treatment. Just before sacrifice, blood was collected from the retro-orbital sinus plexus under mild ether anesthesia. Collected blood was allowed to clot and serum was separated at 3500 rpm for 15 min for carrying out further biochemical investigations. One part of liver was dissected out and used for biochemical and histopathological studies[30-33].

Measurement of serum biochemical parameters

The activities of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin were determined using the Hitachi 912 clinical chemistry automatic analyzer (Roche Diagnostic GmbH, Mannheim).

Histopathology

Animals from control and treated groups were used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver.The liver tissue was dissected out and fixed in 10% formalin solution and then dehydrated in ethanol (50-100%), cleared in xylene and embedded in paraffin wax. Afterwards thick sections (5–6 mm) were made and then stained with hematoxylin and eosin dye for photomicroscopic observation. Scoring on scale of 1-4 was done for the liver sections under microscope as given below[34, 35].The results are given in figure-1.

- 0 = Normal liver histology.
- 1 = Tiny and short septa of connective tissue without influence on the structure of hepatic lobules.
- 2 = Large septa of connective tissue, flowing together and penetrating into the parenchyma. Tendency to develop nodules.
- 3 = Nodular transformation of the liver architecture with loss of structure of hepatic lobules.
- 4 = Excessive formation and deposition of connective tissue with subdivision of the regenerating lobules and with development of scars.

Statistical analysis

The statistical significance were determined by using one way ANOVA followed by Dunnett's multiple comparison test by using Graph p Instat software. The values were represented as Mean ± SEM, (n=6). Less than 0.05 value of P was considered to be statistically significant. *P<0.5 **P<0.01 and ***P<0.001, when compared with control and toxicant group as applicable.

Results

Preliminary Phytochemical studies revealed the presence of phenolics compound and flavonoids were noticed in methanolic extract of *H. zeylanica* leaves. Therefore, there is possibility that methanolic extract of *H. zeylanica* leaves may possess hepatoprotective activity.The extract did not produce any toxic symptoms of mortality upto the dose level of 5000 mg/kg body weight in the treated animals, and hence $1/10^{th}$ (500mg/kg.) and $1/20^{th}$ (250) dose were selected for screening hepatoprotective property.

Hepatoprotective activity

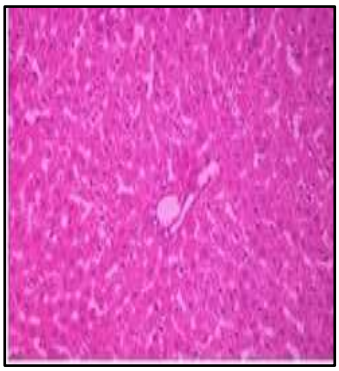
The results of Carbon tetrachloride inducedhepato-toxicity were shown in Table-1. In the Carbon tetrachloride control group, the significant acute hepato cellular damage, and biliary obstruction was indicated by the elevated level of ALT, AST, ALP and TBL But the group which received the test drug of methanolic extract at the dose of 250 and 500mg/kg body weight p.o showed a significant decrease in the elevated levels of ALT, AST, ALP, and TBL. These biochemical parameters are comparable with the standard silymarin hepatoprotective drug. Therefore, the silymarin and the methanolic extract restored the altered level of enzymes significantly.

Histopathological liver sections (fig. 1) of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein. Disarrangement of normal hepatic cells with necrosis and vacuolization are observed in Carbon tetrachloride intoxicated liver. The liver sections of the rat treated with 250,500 mg/kg bodyweight p.o of methanolic extract of *H. zeylanica* followed by carbon tetrachloride intoxication showed less vacuole formation and absence of necrosis and overall less visible changes observed were comparable with standard Silymarin, supplementing the protective effect of the test drug and the standard hepatoprotective drug.

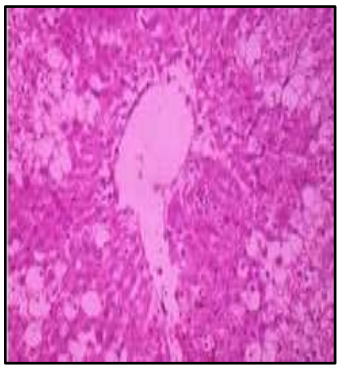
Table 1: Effect of *Hydrolea Zeylanica*on enzyme ALT, AST, ALP, Total bilirubin, direct bilirubin and Cholesterol levels in blood serum of CCl_4 induced hepatotoxicity

Groups	Treatment	ALT IU/L	AST IU/L	ALP IU/L	Total bilirubin mg/dl	Total protein g/dl	Albumin g/dl
Group A	Normal	17.19±0.08	09.38±4.25	36.34±4.37	0.17±0.04	7.28±0.16	3.89±0.14
Group B	CCl_4	163.02±5.87	152.5±45.3	127.78±6.86	0.98±0.53	6.59±0.25	2.47±0.25
Group C	Silymarin	33.52±8.24 ***	25.69±11.6 ***	39.60±2.16 ***	0.32±0.03** *	6.04±0.11 ***	3.86±0.12 ***
Group D	HZLME 250mg/kg	111.55±12.87	134.62±17.92	119.17±11.01**	0.69±0.08	5.74±0.24	1.82±0.16*
Group E	HZLME 500mg/kg	94.89±9.58 ***	112.66±17.1 **	68.79±9.08 ***	0.63±0.07*	6.65±0.69 ***	1.93±0.12 **

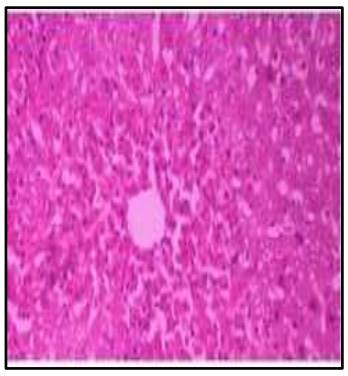
n = 6, Data expressed as Mean ± S.D, *P value < 0.05, **P value < 0.01, *** P value < 0.001 compared with toxic group.



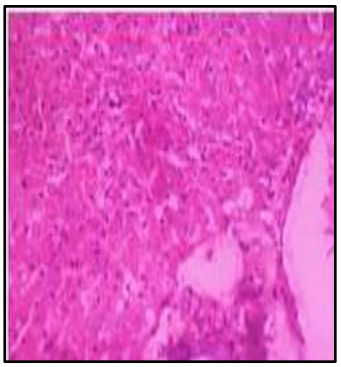
Liver architecture of Normal



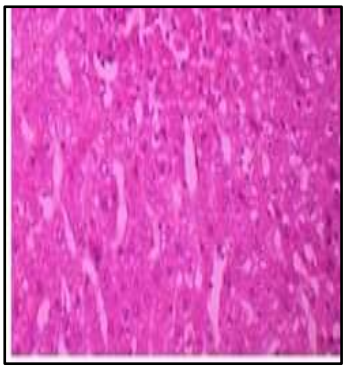
Liver with CCl_4 treatment



Liver with CCl4 treatment + Silymarin treatment



Liver with CCl_4 treatment + 250 mg/kg of HZLME



Liver with CCl_4 treatment + 500 mg/kg of HZLME

Figure 1: Images of Liver architecture in CCl_4 induced Hepatotoxicity in rats.

Discussion

The present studies were performed to determine the hepatoprotective activity against carbon tetrachloride in rats as hepatotoxin to report its claims in traditional practice against liver disorders. Carbon tetrachloride-induced (CCl_4) hepatic injury is most commonly used as an experimental method for the study of hepatoprotective effects of various medicinal plants extracts and drugs. The extent of hepatic damage is assessed by histological evaluation and the level of different bio-chemical parameters in circulation. Most reactive trichloro free radical formation, which attacks PUFAs (polyunsaturated fatty acids) of the endoplasmic reticulum, is mainly responsible for the hepatotoxicity of CCl_4[36].It generates hepatotoxicity by altering liver micro-somal membranes in experimental animals[37].

From the Table 1 it was evident that HZLME was able to reduce all the increase biochemical parameters due to the hepatotoxin intoxication. The levels of albumin and total proteins were reduced due to the CCl_4induced hepatotoxicity. The reduction is attributed to the initial damage produced and localized in the ER (endoplasmic reticulum) which results in the loss of P_{450} leading to its functional failure with a reduction in protein synthesis and accumulation of triglycerides leading to fatty liver. Stoppage of bile acids synthesis from cholesterol which is generated in liver from plasma lipids, leading to increase in level of cholesterol which also resulted due to CCl_4 intoxication. Suppression of level of cholesterol by the plant extracts suggest the bile acids synthesis inhibition was reversed. Reduction in the levels of AST and ALT towards the normal value is an indication of regeneration process. The albumin and protein levels were also raised suggesting the stabilization of ER (endoplasmic reticulum) leading to synthesis of protein. The protective effect exhibited by HZLME at dose level of 500 mg/kg was comparable with the standard drug silymarin.

The histological observation of the liver sections indicates that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the treated rats with HZLME and intoxicated with CCl_4; the normal cellular architecture was retained as compared to silymarin, there by confirming the protective

effect of the HZLME.In accordance with these results, it may be hypothesized that flavonoids, which are present in HZLME, could be considered responsible for the hepatoprotective activity.

Conclusion

The *Hydrolea zeylanica* leaf methanolic extract could effectively controlled the AST, ALT, ALP and TB levels and increased the protein levels in the protective studies. The histopathological studies also substantiate the activity of the drug. Therefore the study scientifically supports the traditional use of this drug for the treatment of liver disorders. Further studies can be carried out on this plant by isolating and characterizing pure compounds, which can yield potent phytotherapeutic agent.

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References

1. Halliwell B., Gutterridge J. M. C., Free Radicals in Biology and Medicine, Clarendon Press, Oxford University press, 1985, 260.
2. Ryter S.W., Kim H.P, Hoetzel A. Mechanisms of cell death in oxidative stress. Antioxid Redox Signal, 2007, 9: 49–89.
3. Hogade M.G., Patil K.S., Wadkar G.H. Hepatoprotective activity of *Morusalba* (Linn.) leaves extract against carbon tetrachloride induced hepatotoxicity in rats. Afr. J. Pharm. Pharmacol., 2010 4: 731–734.
4. Ghasemzadeh A., Ghasemzadeh N. Flavonoids and phenolic acids: role and biochemical activity in plants and human. J. Med. Plants Res., 2011 5: 6697–6703.
5. Tapas A. R., Sakarkar D. M., Kakde R. B. Flavonoids as nutraceuticals: a review. Trop. J. Pharm. Res., 2008, 7: 1089–1099.
6. Kuo DH, Kang WH, Shieh PC, Chen FA, Chang CD, Tsai ML. Protective effect of Pracparatummungo extract on carbon tetrachloride-induced hepatotoxicity in rats. Food Chem., 2010, 123(4): 1007-1012.
7. Lee HS, Kim HH, Ku SK. Hepatoprotective effects of *Artemisiae capillaris* herba and *Picrorrhiza* rhizoma combinations on carbon tetrachloride-induced subacute liver damage in rats. Nutr Res., 2008, 28(4): 270-277.
8. Kshirsagar AD, Mohite R, Aggrawal AS, Suralkar UR. Hepatoprotective medicinal plants of Ayurveda-A review. Asian J Pharm Clin Res., 2011, 4(3): 1-8.
9. Khare CP. Indian medicinal plants: An illustrated dictionary. New York: Springer Science Business Media, LLC, 2007.
10. Shaik A, Elumalai A, Eswaraiah MC, Usha. An updated review on hepatoprotective medicinal plants. J Drug Delivery &Ther., 2012, 2(2): 1-3.
11. Khare CP. Indian medicinal plants: An illustrated dictionary. New York: Springer Science Business Media, LLC, 2007.
12. WV De Thabrew. A Manual of Water Plants. Bloomington, Author House, 2014, 117.
13. Bhagyaleena P, Gopalan R. Aquatic medicinal plants in ponds of Palakkad, Kerala, India. J. Pharm. Biol. Sci. 2012, 2: 29–35.
14. Vijay SB, Kumaran KS, Kumar KL, Hemant HG, Mayur AC. Antidiabetic activity and isolation of bioactive compounds from *Hydrolea zeylanica*. Indones J Pharm., 2015, 26: 185-91.
15. Qureshi MS, Reddy AV, Kumar GS, Nousheen L. Pharmacognostic, physicochemical standardization and phytochemical analysis of leaves of *Hydrolea zeylanica* Vahl. (*Hydrophyllaceae*). Res J Pharmacogn Phytochem., 2017, 9: 1-7.
16. Qureshi MS, Kumar GS, Reddy AV. Exploring the *in-vitro* anthelmintic potential of *Hydrolea zeylanica* - An aquatic medicinal herb. Res J Pharm Technol., 2015, 8: 1645-8.
17. Qureshi MS, Reddy AV, Kumar GS. Protective effect of *Hydrolea zeylanica* Vahl. Leaf extract in ethanol and cold restraint stress induced ulcers in rats. Res J Pharm Techno., 2017, 10: 49-54.

18. Mohammad Shamim Qureshi, A. Venkateshwar Reddy, G. S. Kumar, Lubna Nousheen. Chemical composition and wound healing activity of methanolic leaf extract of *Hydrolea zeylanica* Vahl. by in vivo excision and incision models. International Journal of Green Pharmacy, 2017, 11(2): 114-120.
19. Panda A, Misra MK. Ethnomedicinal survey of some wetland plants of South Orissa and their conservation. Indian J TraditKnowl., 2011, 10: 296-303.
20. Borkar VS, Kumaran KS, Kumar KL, Gangurde HH, Chordiya MA. Ethno medicinal properties of *Echinochloacolona* and *Hydrolea zeylanica*: A review. World J Pharm Pharmc Sci., 2016, 5: 354-60.
21. Pullaiah T. Encyclopaedia of World Medicinal Plants, New Delhi, India, Regency Publication, 2006, 1116.
22. Haque M, Jahan S, Rahmatullah M. Ethnomedicinal uses of *Crinum asiaticum*: A review. World J Pharm Pharm Sci., 2014, 3: 119-28.
23. Gupta A, Pandey VN. Herbal remedies of aquatic macrophytes of Gorakhpur district, Uttar Pradesh (India). Int J Pharm Bio Sci., 2014, 5: 300-8.
24. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Netherlands, Springer, 2012, 78.
25. Kokate CK. Practical Pharmacognosy, Delhi, Vallabh Prakashan, 1997, 107-11.
26. Khandelwal KR. Practical Pharmacognosy, Pune, Nirali Prakashan, 2004, 149-60.
27. Anonymous. OECD guidelines for the testing of Chemicals, Acute oral toxicity up and down procedure, 2006, 1-8.
28. Qureshi MS, Reddy AV, Kumar GS. Protective effect of *Hydrolea zeylanica* Vahl. Leaf extract in ethanol and cold restraint stress induced ulcers in rats. Res J Pharm Techno., 2017, 10: 49-54.
29. Janbaz KH, Gilani AH. Studies on preventive and curative effects of berberine on chemical-induced hepatotoxicity in rodents. Fitoterapia, 2000, 71: 25-33.
30. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetate transaminase. American Journal of Clinical Pathology, 1957, 28: 53–56.
31. King J. The hydrolases-acid and alkaline phosphatases, Practical Clinical Enzymology, Nostrand Company Limited, London, 1965, 191–208.
32. Malloy HT, Evelyn KA. The determination of bilirubin with the photometric colourimeter. Journal of Biological Chemistry, 1937, 119: 481–490.
33. Prasanna H, Hukkeri VI, Hunasagi BS, Mirihal SC, Kulkarni RV, Patel CC. Screening of Hedychiumspicatum for hepatoprotective activity in albino rats. Indian Drugs, 2002, 39 (2): 117-119.
34. Hirayama C, Morotami I, Hiroshige K. Quantitative and metabolic changes of hepatic collagens in rats after carbon tetrachloride poisoning. Biochem J., 1979, 118: 229-232.
35. Ala-Kokko L, Stenback F, Ryhanen L. Preventive effect of malotilate on carbon tetrachloride induced liver damage and collagen accumulation in the rat. Biochem J., 1987, 246: 503-509.
36. Recknagel R.O. Carbon tetrachloride hepatotoxicity. Pharmacological Reviews, 1967, 19(2): 145-208.
37. Ashok S.K., Somayaji S.N. and Bairy K.L. Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride induced hepatic injury in rats. Indian J Pharmacol., 2001, 33(2): 260-6.

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