International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-2, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

In vivo Study of Hepatoprotective Activity of *Hygrophila Schulli* Leaves on Liver Damage in Male Wistar Rats

S. Vijayakumar*, A. Sumathi, L. Surendran

Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore-641028, Tamilnadu, India.

Manuscript No: IJPRS/V3/I2/00291, Received On: 04/06/2014, Accepted On: 15/06/2014

ABSTRACT

The objective of this study was investigated the hepatoprotective activity of methanol extract of leaves of *Hygrophila schulli* against paracetamol induced hepatotoxicity. The plant leaves was dried in shade, they were powdered and extracted with methanol. The hepatoprotective activity of the methanol extract was assessed in paracetamol induced hepatotoxic rats. Alteration in the levels of biochemical markers of hepatic damage like Total protein, Albumin, Total bilurubin, ACP and LDH, were tested in both paracetamol treated and normal groups. Treatment of Methanolic extract of *Hygrophila schulli* leaves (500 mg/kg) has brought back, the altered levels of biochemical markers to the near normal levels in the dose dependent manner. Our findings suggested that *Hygrophila schulli* methanol leaf extract possessed hepatoprotective activity.

KEYWORDS

Hepatoprotection, Methanol, *Hygrophila schulli*, Paracetamol, Silymarin

INTRODUCTION

Medicinal plants play a vital role for the development of new drugs[1]. The World Health Organization has defined traditional medicine as comprising therapeutic practices that have been in existence for hundreds of years[2]. The traditional preparations comprise medicinal plants, minerals and organic matter. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy[3].

Liver is a vital organ present in vertebrates and other animals[4]. It is the second largest organ in human body and is the most complex organ[5]. It produce bile, an alkaline compound which acids indigestion via the emulsification of lipids.

***Address for Correspondence:**
S. Vijayakumar
Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore-641028, Tamilnadu, India.
E-Mail Id: svijayakumar39@gmail.com

It also performs and regulates a wide variety of high volume biochemical reaction requiring specialized tissue[6].

Ayurvedic medicine is essential preventive medicine in therapeutic approach. Many Ayurvedic medicines are used for treating liver disorders. Thus search for crude drugs of plant origin with antioxidant activity has become a central focus of study of hepatoprotection[7].

Hygrophila schulli (Buch. – Ham.), Almedia and Almedia seeds, *Hygrophila auriculata* (Schum) Heine (Syn), *Asteracantha longifolia* Nees, Acanthaceae is described in ayurvedic literature as Ikshura, Ikshugandha, and Kokilasha, as like having eyes as Indian Cuckoo.

The plant is widely distributed throughout India, Srilanka, Burma, Malaysia and Nepal. The whole plant, roots, seeds, and ashes of the plant

are extensively used traditional system of medicine for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction, pain, urinary infections, oedema and gout. It is classified in ayurvedic system as Seethaveeryam, mathuravipaka and used for the treatment of permecham (Diabetes), athisaram (Dysentry)[8,9] etc., hypoglycaemic[10], antibacterial[11,12] and hepato protective[13] activities.

MATERIALS AND METHODS

Plant Material

The fresh plant leaves of *Hygrophila schulli* were collected from Narasipuram, Coimbatore, Tamil Nadu, India. The plant material was taxonomically identified by the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, and India.

Preparation of Extract

The *Hygrophila schulli* leaves was collected and dried under shade and powdered leaves (200 gm) were extracted with methanol in a Soxhlet extractor for 36hrs[14]. The extract was concentrated and at last trace of solvent was removed by Rotary Vacuum evaporator and used for further investigation.

Animals

Male Wistar albino rats weighing 150 – 200 g were obtained from the small animals breeding station, Mannuthy, Kerala, India. All the animals were housed in clean polypropylene cages and maintained under standard environmental conditions (14 h dark/10 h light cycles; Temp 25 ± 2°C; 35-60% humidity, air ventilation). The animals were fed with standard pellet diet (M/s. Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. The experimental protocol was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the Institutional Ethical Committee.

Statistical Analysis

The results were expressed as mean ± SD of six animals from each group. A column means followed by different superscript are significant at 5% DMRT[15].

Experimental Design

Rats were divided into five groups, each group consisting of six animals.

Group I: Control received the vehicle viz.,

Group II: Paracetamol Treated (750 mg/kg P.O.) 13 at every 72h for 21 days

Group III: Treatment with methanolic extract of *Hygrophila schulli* 250mg/kg P.O. for 21 days and simultaneously administered paracetamol 750 mg/kg every 72h.

Group IV: Treatment with methanolic extract of *Hygrophila schulli* 500 mg/kg P.O. for 21 days and simultaneously administered paracetamol 750 mg/kg every 72 h.

Group V: Silymarin Treated 50 mg/kg (P.O.) for 21 days and simultaneously administered Paracetamol 750 mg/kg every 72 hrs.

Estimation of Total Protein

The blue colour developed in this reaction is due to the reaction of peptide bond with copper sulphate in alkaline medium and due to the reaction tyrosine and tryptophan with phenol reagent[16].

Estimation of Albumin

Albumin binds with bromocresol green (BCG) to produce a blue-green color with an absorbance maximum at 628 nm. The intensity of the color produced is directly proportional to the albumin concentration in the sample.

Estimation of Total Bilirubin

Serum is diluted with water and methanol added in an amount insufficient to precipitate the proteins, yet sufficient to ensure that all the bilirubin reacts with the diazo reagent to form azobilirubin. The resulting red purple colour is measured calorimetrically at 540 nm[17].

Estimation of LDH

The lactate is acted upon by lactate dehydrogenase to form pyruvate in the presence of NAD. The pyruvate forms pyruvate phenyl hydrozone with 2, 4-dinitrophenyl hydrazine.

The colour developed is reed in a spectrophotometer at 440 nm[18].

Estimation of ACP

The method used was that of King and Armstrong in which disodium phenyl phosphate is hydrolyzed with the Liberation of phenol and inorganic phosphate. The Liberated phenol is measured at 700 nm with Folin ciocalteu reagent[19].

RESULTS

The level of total protein and Albumin in serum decrease slightly in paracetamol induced rats (Fig: 1), but total bilirubin, Acid phosphatase (ACP) and lactate dehydrogenase (LDH) levels were increased in the paracetamol induced rats, when compared to the normal control (Fig: 2,3,4). The paracetamol with methanolic extract of *H.schulli* (250 mg/kg) the levels of total protein, Albumin slightly were increased, but the level of Bilirubin, ACP and LDH levels were decreased when compare to the paracetamol treated. The High dose of paracetamol with methanolic extract of *H.schulli* (500mg/kg) the level of protein, albumin, bilirubin, ACP and LDH levels were more or less similar to the Standard (Silymarin 50mg/kg)(Fig: 1,2,3,4).

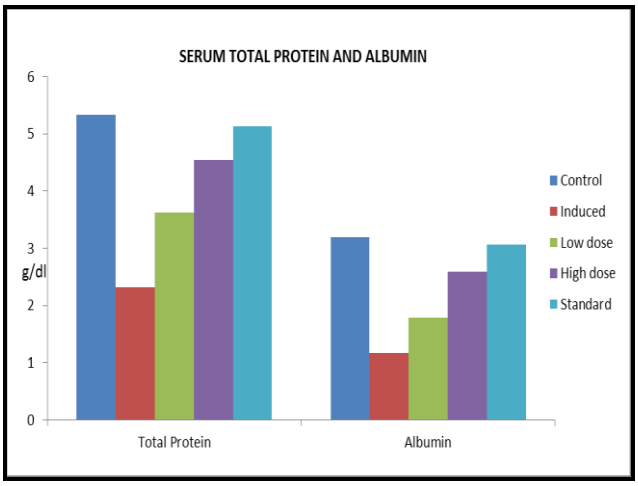

Figure 1: Serum Total Protein and Albumin

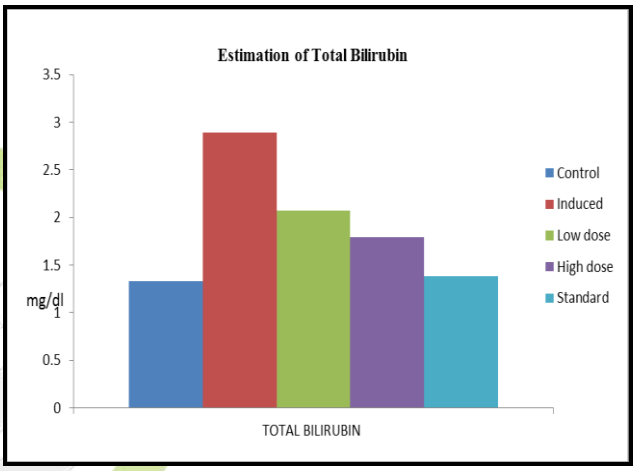

Figure 2: Estimation of Total Bilirubin

Table 1: Effect of Methanolic extract of *Hygrophila schulli* on serum biochemical parameters

Groups	Treatment	Total protein	Albumin	Total Bilirubin	ACP	LDH
I	Control	5.33±0.14[a]	3.20±0.06[a]	1.33±0.50[a]	15.75±0.23[a]	375.10±3.21[a]
II	Paracetamol (750 mg/kg)	2.32±0.96[b]	1.17±0.03[b]	2.89±0.52[b]	23.30±0.06[b]	817.31±3.53[b]
III	Paracetamol+ *Hygrophila schulli* (250mg/kg)	3.62±0.07[c]	1.79±0.16[c]	2.07±0.47[c]	15.85±0.75[c]	646.54±4.89[c]
IV	Paracetamol+ *Hygrophila schulli* (500mg/kg)	4.54±0.26[d]	2.59±0.02[d]	1.79±0.44[d]	9.40±0.23[d]	416.56±2.89[d]
V	Paracetamol+ Silymarin (50mg/kg)	5.13±0.09[e]	3.09±0.02[e]	1.38±0.56[e]	7.26±2.25[e]	391.13±3.21[e]

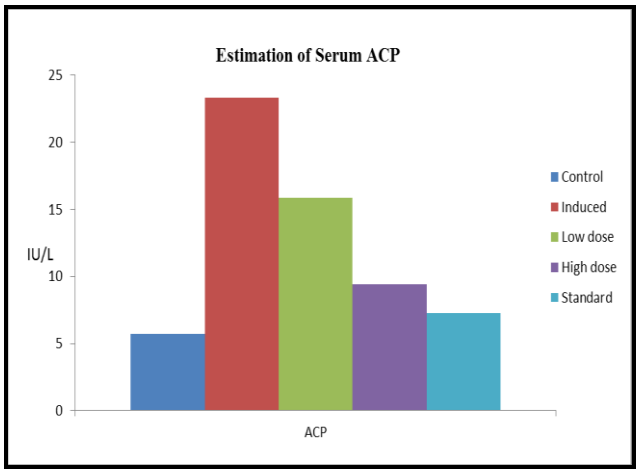

Figure 3: Estimation of Serum ACP

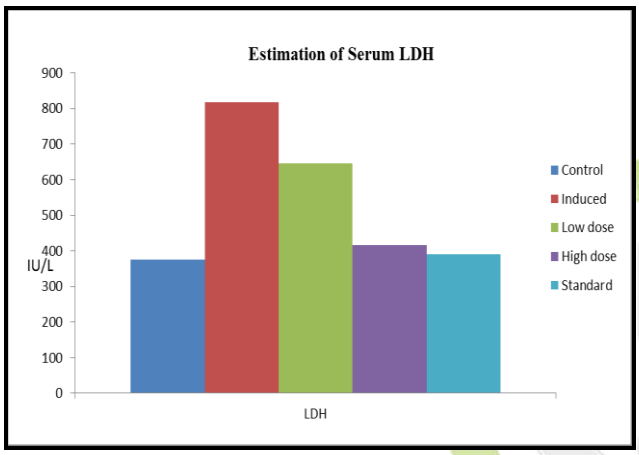

Figure 4: Estimation of Serum LDH

Control: Normal

Induced: Paracetamol

Low dose: Paracetamol+ *Hygrophila schulli* (250mg/kg)

High dose: Paracetamol+ *Hygrophila schulli* (500mg/kg)

Standard: Paracetamol+ Silymarin (50mg/kg)

DISCUSSION

Paracetamol is remarkably safe drug at therapeutic doses but is also the drug most commonly consumed by patients in gross therapeutic for over dosage which may be responsible for the development of acute liver failure[20]. Considerable interest has been shown in the study of toxicity of paracetamol because of its clinical use as antipyretic as well as analysis in large doses, paracetamol is known to produce hepatotoxicity both in experimental animals and also in human beings.

The level of total protein and albumin were reduced due to the paracetamol induced hepatotoxicity. The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulum which result in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to the fatty leaver. A high concentration of bilirubin investigation serum is indication for increased erythrocyte degradation rate[21]. Free bilirubin in lipophilic and therefore insoluble in aqueous solution. It is conjugated with glucornic acid in the liver and thus converted into unconjugated bilirubin which is soluble in water. The levels of bilirubin were determined in the experimental animal.

ACP is a lysosomal enzyme. It activities are related to the fractioning of hepatocytes. LDH is a marker enzyme for cell lysis. LDH activity is elevated in most all causes of liver disease but not as great as the increase seen in aminotransferase activity[22].

CONCLUSION

The current research explained the hepatotoxicity induced by paracetamol and its prevention through hepatoprotective drugs such as plant extract of *H.schulli* and Silymarin (A Standard hepatoprotective drug) were analyzed in low and high dosage. The result showed that high dosage of *H.schulli* (500 mg/kg) prevents liver damage against paracetamol.

REFERENCES

1. Sharma, V., and Agrawal, R. C. (2014). *In vivo* antioxidant and hepatoprotective potential of *Glycyrrhiza glabra* extract on carbon tetra chloride (CCl_4) induced oxidative-stress mediated hepatotoxicity. *Int J Res Med Sci. 2(1),* 314-320.
2. Kamboj, V.P. (2002). Herbal medicine. *Current Science, 78,* 35-7.
3. Hota, N. P. and Pathi, M. M. (2003). Typical uses of certain common and uncommon plants. *Ancient Science of Life*. 1-6.

4. Maton, A., H. Jean, C. W. McLaughlin, M. Q., Warner, D. Lattart and Wright, J. D. (1993). *Human Biology and Health.* Eaglewood Cliffs, New Jersey, Prentice Hall, USA.
5. Singh, A., & Handa, S. S. (1995). Hepatoprotective activity of Apium graveolens and Hygrophila auriculata against paracetamol and thioacetamide intoxication in rats. *Journal of Ethno-pharmacology*, *49*(3), 119-126.
6. Jones, E. A. and Summerfield, J. A. (1988). Kupffer cells, *The Liver Biology & Pathobiology*. 638-74.
7. Venkumar, M. R. and Latha, M. S. (2002). Antioxidant activity of *Curculigo orchiodies* in carbon tetra chloride induced hepatopathy in rats. *Ind. J. Clin. Bio-chem., 17-2*, 807.
8. Nadkarni, K.M. (1978). *Indian Materia Medica. 1*, 667-669
9. Chopra, R. N., Nayar, S. L., and Chopra, I. C. (1986). *Glossary of Indian Medicinal Plants*, 29.
10. Fernando, M. R., Wickramasinghe, S. M. D., Thabrew, M. I., Ariyananda, P. L., & Karunanayake, E. H. (1991). Effect of Artocarpus heterophyllus and Asteracanthus longifolia on glucose tolerance in normal human subjects and in maturity-onset diabetic patients. *Journal of Ethno-pharmacology*, *31*(3), 277-282.
11. Boily, Y., and Vanpuyvelde, L. (1986). Screening of medicinal plants of Rwanda (Central Africa) for antimicrobial activity. *Journal of Ethnopharmacology*. *16*, 1-13.
12. Vlietinck, A. J., Van Hoof, L., Totte, J., Lasure, A., Berghe, D. V., Rwangabo, P. C., & Mvukiyumwami, J. (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *Journal of Ethnopharmacology*, *46*(1), 31-47.
13. Singh, A., & Handa, S. S. (1995). Hepatoprotective activity of Apium graveolens and Hygrophila auriculata against paracetamol and thioacetamide in toxiation in rats. *Journal of Ethno-pharmacology*, *49*(3), 119-126.
14. Vasantharaj, S., Sathiyavimal, S., & Hemashenpagam, N. (2013). Phytochemical and Antibacterial activity of Eucalyptus. *International Journal of Pharmaceutical Sciences Review & Research*, *21*(2), 237-239.
15. Woolson, R. F., and William, R. C. (1987). *Statistical methods for the Analysis of Biochemical Data*. John wiley & Sons Inc, New York. pp. 146-54.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, *193*(1), 265-275.
17. Malloy, H. T., & Evelyn, K. A. (1937). The determination of bilirubin with the photoelectric colorimeter. *Journal of Biological Chemistry*, *119*(2), 481-490.
18. King, J. (1965b). *The dehydrogenase or oxidoreductase- lactate dehydrogenase. In; Practical clinical Enzymology- Van, D.* (EDS), Norstand company Ltd., London. pp. 83-93.
19. King, E. J., & Armstrong, A. R. (1934). A convenient method for determining serum and bile phosphatase activity. *Canadian Medical Association Journal*, *31*(4), 376-381.
20. Grudy, J. G. (1997). Paracetamol hepatotoxicity; how to prevent. *J .R. Soc. Med. 90*, 368.
21. Singh, B., Saxena, A., Chandan, B. K., (1998). Hepatoprotective activity of verbenalin on experimental liver damage in rodents. *Fitoterapia*, *69(2)*, 135–140.
22. John, A., Moss, D. W., and Ralph Handesson, R. (1986). Enzyme in text book of Clinical Chemistry Tietz-N.W.W.B. Saunder Company. Chapter 5.

