Original Research Paper Pharmaceutical sciences

Evaluation of Antioxidant and Hepatoprotective Activity of Abrus Precatorius Leaf Extracts in Mice

Ravat Nirav M. Student, Department of Zoology, School of Sciences, Gujarat University, Ahmedabad, India.

*** Dr. H. N. Highland** Professor, Department of Zoology, School of Sciences, Gujarat University, Ahmedabad, India. * correspondence Author

ABSTRACT

Liver failure is one of the most severe diseases now days. Millions of people are affected worldwide because of infected water, food, alcohol and unnecessary drug treatment which are the most common reasons. Due to the higher cost of advanced techniques for treatment and side effects of drugs, people have moved on to ancient therapies like house remedies or herbal medicines. But lack of scientific data is one of the major drawbacks of these drugs. Medicinal plant products, some of which may become potential drug candidates which play major roles to maintain the health of human body include some of these major functions like carbohydrate, fat and protein metabolism, bile secretion and detoxification. In the present study, a selected plant extract was investigated for possible liver protection as they have potential phyto-constituents which have not been screened till date.

KEYWORDS Abrus precatorius, Carbon tetrachloride, Hepatotoxicity

Introduction

The liver is a site of intense metabolism and excretion, as the largest organ of the human body. It maintains and regulates the homeostasis of body. It is continuously affianced with nutrient supply, growth of biochemical pathways, reproduction and energy provision (Ahsan ***et at***., 2009). Detoxification, fat, protein and carbohydrate metabolism, storage of vitamin and bile secretion are its fundamental functions (Ward, 1999). Liver failure is one of the most severe diseases now days. It causes more than 2% of all deaths, and ratio may be increasing in next coming years. Millions of people are affected worldwide because of infected water and food which are the most common reasons (Dahanukar et al, 2000). If we consider gender as one of the parameter, men are more affected in comparison to women (Koul and Kapil, 1993). It also affects the younger age too, death ratio is more than 5 % in the age group of 40s caused by different liver disease. Hepatotoxicity contributes a major role in liver disease (Subramonium and Pushpangadan, 1999). It is because of increased amount of AST and ALT in serum and ill functioning of hepatocyte at its base ground level. Unfortunately poor habits of eating, alcohol and unnecessary drug treatment give damages at micro level to the liver (Bezenjani, 2012). That leads the liver damage micro to macro level and also affects morphological as well as histological changes of liver.

Advanced technology and techniques are established for treatment and can give satisfactory results. But on the other hand cost of these techniques and side effects of drugs cannot be afforded and preferred by all and that is why people moved on to ancient therapies like house remedies or herbal medicines. Ancient literature is flooded with therapies of plants having liver tonic (hepatoprotective) properties.

Synthesized and herbal origin compounds have been tested for their potentiality for protection against hepatotoxicity in various experimental systems, both ***in vivo*** and ***in vitro*** (Bhatt and Bhatt, 1996). But herbal drugs or drugs of herbal origin have gained tremendous popularity and market as they have been sold as safe drugs. Conventional medicine or herbal drugs as they are popularly known are being used by a large population (Treadway, 1998). Lack of scientific data is one of the major drawbacks of these drugs. Researchers from all over the world are trying to study these medicinal plant products, some of which may become potential drug candidates. It plays major roles to maintain the health of human body which include some of these major functions like carbohydrate, fat and protein metabolism, bile secretion and detoxification. Thousands of plants with their extracts and fractions have been tested for hepatoprotective properties (Jalapure et al, 2003). Screening of these plants gave so many compounds which gives tremendous result, silymarin is one of the best example. Some plants which can exhibit good activity and have potential phyto-constituents but have not been screened till date (Bone, 1997).

The plant ***Abrus precatorius*** commonly known as, Ratti or Gunchi, Indian liquorice, Crab's eye, Jequirity, rosary peas, it is a leguminous plant of the fabacea family (Mann et al, 2003). It grows widely in dry climates of subtropical and tropical regions worldwide. Leaves and seeds of this plant are used for medicinal purposes, a practice most probably dating back to antiquity (Ivan, 2003).

Seeds are used traditionally to treat scratches, asthma (Saganuwan et al., 2009), diabetes, wounds caused by cats, dogs and mice and sores. The leaves are used to treat colic, convulsion (Iwu, 1993), coughs, sore throat and as they have antisuppurative properties (Rajaram and Janardhana, 1992) and also insomnia (Mann, et al., 2003). Antimicrobial activity of the aqueous extract of the plant was reported (Saganuwan, and Gulumbe, 2005 [a]; Saganuwan and Gulumbe, 2005 [b]) against ***Salmonella typhimurium***, ***Escherichia*** coli, ***Streptococcus pyogenes, Klebsiella pneumoniae***, and ***Streptococcus pneumoniae***. Other uses of the plant are in abortion, malaria, anaemia (Saganuwan, 2011), erectile dysfunction, cancer (Duke et al., 2002), ophthalmia (Windholz, 1983) and night blindness.

Phytochemical constituents of the plant are abrin, abricin, abraline, abrusgenic acid-methylester, abrasine, abruslectone, anthocyanins, abrussic acid, ash, campestrol, hypaphorine, N, choline, n-dimethyl tryptophan-methanocationmethyl-ester, cycloartenol, pectin, delphinidine, glycyrrhizin, gathic acid, P-coumaroylgalloyl-glucodelpinidin, calcium, pentosans, precatorine, phosphorus, picatorine, precasine, polygalacturonic acid, large quantities of oil protein trigonelline (Budavari, 1989; Windholz, 1983; Gosselin et al., 1984) and proteins (Saganuwan, 2011).

Materials and Methods

Extract preparation

Plant material was collected authentication carried out at the department of Botany, Gujarat University, Gujarat. The leaves were washed with double distilled water, shaded dried at room temperature, then it was powdered with a mechanical grinder and stored in air tight containers at room temperature. This powder material of leaves was extracted with Soxhlet extraction method using hydro-alcohol (in ratio of 50%) and methanol as solvent. These extracts were filtered and concentrated by rotary evaporator (Buchi, USA) to obtain the crude extract. The final concentrated extracts were lyophilized and stored at -20 °C.

Phytochemical Analysis

Preliminary Phytochemical screening was carried out of methanolic and hydro-alcoholic extracts. This analysis was carried out for the detection of the presence of different constituents such as saponins, alkaloids, flavonoids, tannins and terpenoids (Harborne, 1984).

Animals

Swiss albino mice weighing between 20-30 gm (2-3 months old) were used. Animals were housed 3 per cage in polypropylene cages and the general environmental condition was strictly controlled in the colony, such as 10% air exhaust in the air conditioning unit, relative humidity 60 ± 5 %, temperature 25 ± 3 ˚C and a 14 hr light and 10 hr dark cycle. Amrut certified rodent diet and RO (Reverse Osmosis) water was provided to animals ***ad libitum***. Animal housing and handling were performed in accordance with Good Laboratory Practice (GLP) mentioned in CPCSEA guidelines. Animal house is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, vide registration no. 167/1999/CPCSEA. Experimental protocols used in this study were reviewed and approved by the institutional animal ethics committee (IAEC).

CCl_4 induced hepatotoxicity

Animals (n=6) were divided in seven groups. Group 1 served as Control, received vehicle (0.2% agar, orally). Group 2 (Positive control) received Silymarin (50 mg/kg, orally). Group 3 (Disease control) received CCL_4 (1 ml/kg in 1:1 proportion with olive oil, ip.). Group 4 (AbHy – 150) received hydro-alcoholic extract (150 mg/kg, orally). Group 5 (AbHy – 200) received hydro-alcoholic extract (200 mg/kg, orally). Group 6 (AbMe – 150) received methanolic extract (150 mg/kg, orally) and Group 7 (AbMe – 200) received methanolic extract (200 mg/kg, orally). All the treatments were given daily except carbon tetrachloride, was given on every 24 hrs for 7 days. Blood samples were collected post 24 hrs of last treatment. After that animals were sacrificed for liver samples (Rao et al., 2006).

Assessment of Hepatoprotective activity

At the end of treatment animals were fasted overnight and blood samples were collected. Serum was separated analyzed for biochemical parameters such as ALT, AST, ALP, total bilirubin, direct bilirubin, and GGT in automated biochemistry analyzer.

Assessment of Oxidative stress markers:

Estimation of lipid peroxidation (LPO): Liver tissue was homogenized in 5mL of Hank's balanced salt solution (pH 7.4), centrifuged at 3500 rpm for 10 minutes. Lipid peroxidation was measured in terms of malondialdehyde (MDA): thiobarbituric acid (TBA) reaction (Ohkawa et al., 1979).

Estimation of Superoxide Dismutase Activity (SOD): Liver tissue was homogenized at 13,000 rpm in 4mL of chilled Tris buffer (50mM, pH 8.2). Homogenate was treated with 1mL of 0.1% Triton X 100 (v/v) for 20min at 4 ºC. Sample was centrifuged at 10,000 rpm at 4 ºC for 30min. The supernatant was used for the assay of superoxide dismutase (SOD) activity (Marklund and Marklund, 1974).

Estimation of reduced glutathione activity (GSH): Tissue was homogenate with 5 ml of 0.2 M phosphate buffer (pH 7.6). Then added 0.1 ml of 25% trichloroacetic acid and centrifuged at 3900 g for 10 min at 25ºC. 1 ml of sample supernatant was added to 1 ml of 0.2 M of phosphate buffer (pH 7.6) solution followed by the addition of 1 ml of DTNB solution. Prepared solution was used for the assay of reduced glutathione activity (GSH) activity (Ellman and Boyne, 1972).

Statistical analysis

Data were expressed as mean ± SEM (n=6). Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnet's test. All statistical analyses were performed using the statistical software GraphPad Prism 6. Mean values were considered as statistically significant at ***P*** value <0.05.

Result

Preliminary Phytochemical analysis revealed that the different groups like carbohydrates, phenolic compounds, tannins, alkaloids and Flavonoids are present. The present study had been designed to demonstrate the hepatoprotective activity of crude extracts of ***A. precatorius*** in carbon tetrachloride induced hepatotoxic experimental animals at different dose levels. Result of this study of both crude extracts, hydro-alcoholic and methanolic of this plant at a dose of 150 mg/kg and 200 mg/kg body weight on mice intoxicated with CCl_4 was illustrated in the Table 1. Table 1 shows the comparative effect among control, CCl_4 treated - disease control and extract treatment group in two doses. Disease hepatotoxic control group showed significantly increased activity of ALT, AST, ALP, GGT and biliribun levels when compared with control group ($p<0.05$). In hydro-alcoholic and methanolic leaf extract groups 4 & 6 show curtailed level reduction in the different biochemical parameters, while dose group 5 & 7 show statistically significant reduction when compared with disease hepatotoxic control for 7 day treatment period ($p<0.05$).

Effect of oral administration of both extracts for oxidative stress markers such as LPO, SOD and GSH were shown in Table 2. In the disease control - CCl_4 treated group GSH and SOD level was reduced while LPO level increased when compared with untreated control group. Administration of the hydro-alcoholic extract, at both dose levels decreased the LPO and increased the activity of SOD and GSH level respectively in comparison to disease hepatotoxic control. Moreover, treatment with the methanolic extract (group 7) showed significant increase in SOD, GSH and decreased in LPO level respectively when compared with disease control group ($p<0.05$).

Discussion

Literature review revealed that diverse biological and chemical investigations were carried out for ***A. precatorius***. CCl_4 toxicity has been used as a test for potential hepatoprotective activity by investigations for the past several years. As proved in this study also CCl_4 has been shown to cause oxidative stress such as glutathione depletion, lipid peroxidation and also effect on superoxide Dismutase activity (Recknagel, 1967). It increases the ALT, AST, GGT levels as well as damaged structural integrity of liver a finding that corroborates with our data also. It is metabolically activated from cytochrome P-450 and causes the cellular damage after released (DeGroot and Noll, 1986) and because of it structural changes occurred in endoplasmic reticulum and other membrane. The data obtained in this study indicated that both leaf extracts have good antioxidant property and alleviates the oxidative stress which plays an important role in pathogenesis of hepatotoxicity. Treatment with ***A. precatorius*** methanolic and hydro-alcoholic leaf extracts recovered the imbalanced biochemical and vital functional parameters in a dose dependent manner that validates the hepatoprotective effect of both leaf extracts.

Our study suggests that ***A. precatorius*** leaf extracts have good antioxidant and hepatoprotective effect which is comparable to Silymarin. As Silymarin is not native to this area, it is difficult and costly to obtain. Hence a potent extract of ***A. precatorius*** proves to be a viable alternative.

Conclusion

From the analysis of presented data it can be concluded that the hydro-alcoholic and methanolic leaf extracts of ***A. precatorius*** have shown significant hepatoprotective activity which was demonstrated by amelioration of CCL_4 hepatotoxic animals. In addition the extracts prove effective in a dose dependant manner as compared with disease control group.

Table 1: Showed the comparison of biochemical parameters for both extracts at different dose with disease control group.

Group	Dose	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)	BID (mg/dL)	BIT (mg/dL)
1	Control(Vehicle)	42.70±3.11	85.55±4.30	44.70±3.43	3.02±0.30	0.08±0.01	0.16±0.02
2	Positive Control (50mg/kg)	59.59±10.00	98.20±2.81	56.46±3.77	4.55±0.81	0.09±0.01	0.14±0.01
3	Disease Control (1 ml/kg)	97.15±3.90	160.13±7.10	94.73±5.11	11.47±2.20	0.40±0.14	0.97±0.34
4	AbHy (150mg/kg)	76.05±3.30	140.52±8.99	90.80±4.61	10.48±2.04	0.34±0.03	0.81±0.24
5	AbHy (200 mg/kg)	67.52±1.88*	131.10±8.51*	78.50±2.33*	7.27±0.62*	0.27±0.05*	0.61±0.12*
6	AbMe (150 mg/kg)	87.47±4.15	155.97±5.40	88.90±4.99	6.70±0.94	0.24±0.04	0.52±0.10
7	AbMe (200 mg/kg)	61.60±2.59*	114.12±6.62*	66.50±4.77*	4.83±0.65	0.13±0.01*	0.25±0.04*

Results are expressed as Mean ± SEM. AbHy = ***A. precatorius*** hydro-alcoholic extract, AbMe = ***A. precatorius*** methanolic extract. *= P<0.05 when compared with Disease Control group.

Table 2: Showed the comparison of biochemical assay for both extracts at different dose with disease control group.

Group	Dose	Reduced Glutathione (GSH) (mM/100 mg tissue wt.)	Lipid Peroxidation (LPO) ($X10^{-5}$ nm/ gm)	Superoxide dismutase (SOD) (Units/ 100 mg tissue wt.)
1	Control (Vehicle)	1.00±0.13	3.21±0.25	71.12±6.92
2	Positive Control (50mg/kg)	0.94±0.09	2.83±0.23	62.50±1.78
3	Disease Control (1 ml/kg)	0.53±0.03	6.43±0.21	34.61±2.9
4	AbHy (150mg/kg)	0.62±0.08	5.16±0.61	48.10±2.82
5	AbHy (200 mg/kg)	0.77±0.04*	4.21±0.59	58.22±4.07*
6	AbMe (150 mg/kg)	0.61±0.05	5.75±0.39	53.69±4.08
7	AbMe (200 mg/kg)	0.86±0.05*	3.53±0.29*	64.23±1.71*

Results are expressed as Mean ± SEM. AbHy = ***A. precatorius*** hydro-alcoholic extract, AbMe = ***A. precatorius*** methanolic extract. *= P<0.05 when compared with Disease Control group.

References:

1. Bhatt AD, Bhatt NS. (1996). Indigenous drugs and liver disease. Ind J Gastroenterology 15: 63-67.
2. Bone K. Ginger. (1997). Brit J Phytother 4:110-120.
3. Budavari S. (1989). The Merck Index: An Encyclopaedia of Chemicals, Drugs and Biologicals, 10th ed. Rahway New Jersey Merck and Co. Inc. USA.
4. Dahanukar SA, Kulkarni RA, Rege NN. (2000). Pharmacology of medicinal and natural plants and natural products. Ind J Pharmacol 32: 81-118.
5. DeGroot, H. and T. Noll. (1986). The crucial role of low steady state oxygen partial pressures in haloalkan free-radical mediated lipid peroxidation. Biochemical Pharmacology, 35: 15-19.
6. Duke JA, Bogenschutz-Godwin MJ, duCellier J, Duke PAK. (2002). Handbook of Medicinal Herbs, 2nd ed. CRC Press, Boca Raton London New York Washington DC.
7. Ellman, G. L. and Boyne, A. F. (1972). A Methodology for analysis of tissues sulfhydryl components. Anal. Biochem. 46(2): 639-53.
8. Gosselin RE, Smith RP, Hodge HC. (1984). Clinical Toxicity of Commercial Products, Williams and Wilkins, Baltimore London.
9. H. Ohkawa, N.Ohishi, and K. Yagi. (1979). "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," Analytical Biochemistry, vol. 95, no. 2, pp. 351–358.
10. Harborne, J. B. (1984). Phytochemical methods (Vol. 278): Springer.
11. Ivan AR. (2003). Medicinal plants of the world. Chemical constituents, traditional and modern medicinal uses. pg 18.
12. Iwu MM. Malaria. (1993). Handbook of African Medicinal Plants. CRC Press Boca Raton Ann Arbour London Turkey; 53:347.
13. Jalapure SS, Patil MB, Prakash NS, Hemlata K, Manvi FV. (2003). Hepatoprotective activity of the fruits of Piper longum Linn. Ind J Pharm Sci 65:363-366.
14. Koul IB, Kapil A. (1993). Evaluation of the liver protective potential of piperine an active principle of black and long peppers. Planta Med 59:413-417.
15. Mann A, Gbate M, Nda Umar A. (2003). Abrusprecatorius: Papilionaceae. Medicinal and Economic Plants of Nupeland. Jube-Evans Books and Publications, Bida, Nigeria; 191.
16. Rajaram N, Janardhana K. (1992). The chemical composition and nutritional potential of the tribal pulse, Abrus precatorius L. Plant Food Human Nutr.; 42(4):285-290.
17. Rajib Ahsan, Km Monirul Islam, A. usaddik and E. Haque. (2009). Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride induced hepatotoxicity in albino rats. Global journal of pharmacology 3(3); 116-122.
18. Rao, G.M.M., Rao, Ch.V., Pushpangadan, P., Shirwaikar, A. (2006). Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn. Journal of Ethnopharmacology 103, 484–490.
19. Recknagel RO. (1967). Carbon tetrachloride Hepatotoxicity. Pharmacol Rev 19:145-208.
20. S.Marklund and G.Marklund, (1974). "Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase," European Journal of Biochemistry, vol. 47, no. 3, pp. 469–474.
21. Saganuwan SA. (2011). Toxicological and antimalarial effects of aqueous stem bark extract of Abrus precatorius (Jequirity bean) leaf in Swiss albino mice. PhD Thesis Usmanu Danfodiyo University, Sokoto, Nigeria; 250.
22. Saganuwan SA, Gulumbe ML. (2005). In vitro antimicrobial activities testing

of Abrus precatorius cold water leaf extract on Salmonella typhimurium, Escherichia coli and Klebsiella pneumoniae. J Technol Res; 4(3):70-3.
23. Saganuwan SA, Gulumbe ML. (2005). In vitro antimicrobial activities testing of Abrus precatorius cold-water leaf extract on Streptococcus pyogenes and Streptococcus pneumoniae. Proceedings of the 2nd Annual Conference of the Nigeria Soc. Indigenous Knowledge Dev, Cross River State Univ. Technol. Obubra, 9th – 12th Nov.; 93-97.
24. Saganuwan SA, Onyeyili PA, EtukUE. (2009). Acute toxicity and haematological studies of aqueous extract of Abrus precatorius leaf in Musmusculus. Abstract of African Education Initiative Conference; 65.
25. Sedighe Nasiri Bezenjani, Iran Pouraboli, Reza Malekpour Afshar and Gholamabbas Mohammadi. (2012). Hepatoprotective effect of otostegia persica boiss. Shoot extract on carbon tetrachloride-induced acute liver damage in rats. Iran J Pharm 11(4): 1235–1241.
26. Subramonium, A. and P. Pushpangadna. (1999). Development of phytomedicine for liver disease. Indian J. Pharmaco., 31:24-30.
27. Treadway S. (1998). An Ayurvedic herbal approach to a healthy liver. Clin Nutr Insights 6:1-3.
28. Ward, F.M. and M.J. Daly. (1999). Hepatic Disease. In: clinical pharmacy and therapeutics (Walker R. and C. Edwards Eds.). Churchill livingstone, New York, pp: 195-212.
29. Windholz M. (1983). The Merck Index: An Encyclopaedia of Chemicals, Drugs and Biologicals, 10th ed. Rahway New Jersey, Merck and Co. Inc.