ORIGINAL ARTICLE

Hepatoprotective activity studies of herbal formulations

Manisha Bhagwat Kamble[1], Rahul Kondaji Dumbre[1], Vinod D. Rangari[2]

[1]Department of pharmacognosy, Rajgad Dyanpeeth's College of Pharmacy, Bhor, Tal-Bhor, Pune - 412 206, [1]Seth Govind Raghunath Sable College of Pharmacy, SASWAD, Pune - 412 301, [2] Principal, JL Chaturvedi College of Pharmacy, 846, New Nandanvan, Nagpur - 440 009, Maharashtra, India

Traditional system of medicine recommends various hepatoprotective agents and preparations to treat hepatic disorders. Polyherbal formulations F1 and F2 were developed for treatment of liver disorders by exploiting the knowledge of traditional system of medicine and evaluated for hepatoprotective activity using acute liver toxicity models of CCl_4 and Paracetamol induced liver damage in rats. The rats were monitored for morphological changes in liver, biochemical parameter Serum Glutamate Oxaloacetate Transaminase, Serum Glutamate Pyruvate Transaminase, Serum Alkaline Phosphatase, and Serum bilirubin, histopathological studies, and pentobarbitone sleeping time. Both of these formulations F1 and F2 showed significant hepatoprotective activity at dose of 400 mg/kg, which was comparable to silymarin at 6 mg/kg. Formulations F1 and F2 are effective both as prophylactic and therapeutic in experimental liver damage. Biochemical parameters showed better results for formulation F2 but morphological, pentobarbitone sleeping time and hisptopathological observation were similar for both the groups.

Key words: CCl_4 induced toxicity, paracetamol induced toxicity, polyherbal formulations, traditional system of medicine

INTRODUCTION

Any changes in anatomy or functions of liver are characterized as liver disease. Liver has tremendous capacity to detoxify toxic principle and synthesize useful principles. Therefore damage to the liver inflicted by hepato-toxic agents is of grave consequences. Various types of liver disorders are characterized by cirrhosis, jaundice, tumors, metabolic and degenerative lesions, liver cell necrosis and hepatitis etc. Besides viruses, liver disorders can arise due to xenobiotics, excessive drug therapy and environmental pollution and alcohol intoxication. The management of liver diseases is still a challenge to the modern medicine. The modern allopathic drugs have very little to offer for alleviation of hepatic ailments and some of these drugs adversely affect the liver function.[1,2]

The traditional system of medicine like Ayurveda and Siddha system of medicine, Unani system, Chinese system of medicine, Kampoo (Japanese) system of medicine have a major role in the treatment of liver ailments.

A great deal of research has been carried out to evaluate scientific basis for the claimed hepatoprotective activity of herbal agents as single agent or in formulation. The herbal formulations (F1 and F2) under study contain plant ingredients like aqueous extract of *Acacia catechu, Allium sativum* and ethanolic extract of *Andrographis paniculata, Azadirachta indica, Boerhaavia diffusa, Curcuma longa, Eclipta alba, Luffa echinata, Emblica officinalis, Phyllanthus amarus, Picrorrhiza kurroa.* The form of extract whether aqueous or ethanolic and content in dose is based on the traditional knowledge and reports present on these plants. Formulations are developed based on Ayurvedic principles where plants are included for antioxidant activity, hepatoprotective, bioavalibility enhancement and specific activity in modulation of different liver disease conditions as many of these herbal ingredients are known to have liver modifying activity. *Acacia catechu* is reported to prevent fibrosis,[3] *Allium sativum* is known for its antioxidant property and protect liver microsomal enzyme,[4] *Andrographis paniculata* is found to be beneficial in infective hepatitis,[5-7] *Azadirachta indica* is found to be protective in paracetamol induced liver damage.[8] *Boerhaavia diffusa* is known traditionally for its effect in jaundice,[4] *Curcuma longa* is known for its anti-infective, antioxidant, stomachic and modifier of hepatic enzyme,[9] *Eclipta alba* is known for its deobstruent property in enlarged liver and pancreatic conditions,[4] *Emblica officinalis* is added for its general tonic properties known traditionally.[4] *Luffa echinata* is known for its use as appetizer and benefits in conditions of jaundice,[4] *Phyllanthus amarus* is found to be beneficial in jaundice.[10-12] *Picrorrhiza kurroa* is known for its hepatoprotective activity traditionally as well as by various studies.[4,13-15] We have undertaken this study to

For correspondence: Dr. Manisha Bhagwat Kamble, Department of pharmacognosy, Rajgad Dyanpeeth's College of Pharmacy, Bhor Tal-Bhor, Dist-Pune - 412 206, Maharashtra, India. E-mail: manik76@indiatimes.com
Received: 11-03-08; **Accepted:** 12-06-2008

evaluate the efficacy of these formulations in rats in which acute hepatotoxicity was induced by carbon tetrachloride and Paracetamol treatment.

MATERIALS AND METHODS

Preparation of Formulations

Formulation F1 and F2 are a uniform mixture of dried and pulverized herbal extracts [Table 1]. It contains a mixture derived from aqueous extract of *Acacia catechu*, *Allium sativum*, and alcoholic extracts of *Andrographis paniculata, Azadirachta indica, Boerhaavia diffusa, Curcuma longa, Eclipta alba*, *Emblica officinalis*, *Luffa echinata*, *Picrorrhiza kurroa, Phyllanthus amarus*. All the crude material were procured from market and authenticated.

Chemicals

Carboxy methyl cellulose, Paracetamol, Pentobarbitone was purchased from Sigma Chemicals USA. Olive oil, Carbon tetrachloride, Formalin was purchased from SD Fine chemicals, Chennai.

Choice of Animal

Male albino rats (strain Wistar) with weight range 125-175 gm were obtained from Indian Institute of Toxicology. All animals were kept under condition of temperature of 20-25°C and light of 12h dark and 12h light. All the animals received standard diet (Amruta Labs, Pune) and water ad libitum. The rats were randomly selected and were divided into different groups with six animals in each group.

***In-vivo* animal models for hepatoprotective activity**

Method 1: Carbon Tetrachloride Induced Toxicity[12]

The animals were divided into various groups containing six animals in each group. Except normal control all other groups (CCl_4 treated, Formulations F1 and F2 treated, standard) received carbon tetrachloride (CCl_4) 30% v/v in olive oil (1.25 ml/kg of body wt) orally daily for 5 days to induce hepatotoxicity. Normal control group received plain olive oil orally. From 6th day till 12th day (total 7 days) animals received treatment of herbal formulations F1 and F2 suspended in vehicle at dose of 400 mg/kg orally. The standard group received Silymarin 6mg/kg orally while the control group received vehicle (1% carboxy methyl cellulose). On the 13th day blood was collected from each animal for serum analysis. The rats were sacrificed, liver removed and observed for weight, volume and appearance and then fixed in 10% formalin for histopathological studies of the liver to determine the degree of hepatic damage.

Method 2: Paracetamol Induced Toxicity[8]

The animals were divided into various groups with six animals in each group. They received prophylactic treatment of either F1 or F2 suspended in vehicle at a dose of 400 mg/kg for five days. The standard group received silymarin 6 mg/kg and the control groups (normal and negative) received vehicle (1% carboxy methyl cellulose). After administration of the fifth dose of formulations, hepatotoxicity was induced in treatment, standard and negative control groups by feeding paracetamol (2 gm/kg) suspended in water orally. After 48h of paracetamol feeding, blood was collected from each rat for serum analysis. The rats were sacrificed, liver removed and observed for weight, volume and appearance and then fixed in 10% formalin for histopathological studies of the liver to determine the degree of hepatic damage.

Pentobarbitone Sleeping Time Test[16]

The rats were kept on standard diet. Experiments were set as mentioned above for CCl_4 and Paracetamol. Twenty four hours after the last treatment by formulations F1 and F2 in CCl_4 model and 48h after Paracetamol treatment, pentobarbitone sodium in water for injection (35 mg/kg b.w) was administered intraperitoneally. Food was withdrawn and water given ad libitum 12h before pentobarbitone injection. All the experiments were conducted between 09.00am to 5.00pm in temperature controlled room. The

Table 1: Composition of formulations F1 and F2

Plant	Formulation F1			Formulation F2		
	Composition wt in gms	Treatment wt in mg	%	Composition wt in gms	Treatment Wt in mg	%
Acacia catechu	0.57	7.61	1.9	0.36	3.91	0.98
Allium sativum	10.00	133.33	33.33	15.00	164.47	41.12
Andrographis paniculata	1.04	13.87	3.47	4.16	45.61	11.40
Azadirachta indica	3.67	48.93	12.23	7.34	80.48	20.12
Boerhaavia diffusa	2.50	33.33	8.33	1.87	20.50	5.13
Curcuma longa	0.40	5.28	1.32	0.42	4.56	1.14
Eclipta alba	3.90	52.00	13.00	2.34	25.66	6.41
Emblica officinalis	3.80	50.67	12.67	2.00	21.93	5.48
Luffa echinata	1.00	13.33	3.33	0.83	9.13	2.28
Picrorrhiza kurroa	0.63	8.35	2.09	0.50	5.48	1.37
Phyllanthus amarus	2.50	33.33	8.33	1.66	18.20	4.55
Total	30	400	100	36.48	400	100

animals were placed on table after loss of righting reflex. The time interval between loss and regain of righting reflex was measured as Pentobarbitone Sleeping Time (PST). This functional parameter was used to determine the metabolic activity of the liver.

Statistical Analysis

Data was presented as mean ± Standard Error and analyzed using one way ANNOVA with Dunnett test.

Assessment of Hepatoprotective Activity

The term "Liver function tests" refers to a group of biochemical investigations useful in confirming that the liver is diseased and in indicating whether the hepatic cells (parenchymal liver disease) or the biliary tract (obstructive or cholestatic liver disease) is primarily involved in giving an indication of the extent of liver damage and in assessing the progress. Activity was assessed by using 4 parameters viz. morphological, biochemical, histopathological and functional parameter.

1) Morphological Parameter: changes in color, weight, and volume of liver are determined.
2) Biochemical parameter (Liver function tests): blood samples collected were examined for changes in Serum Glutamate Oxaloacetate Transaminase (SGOT) (IU/L) and Serum Glutamate Pyruvate Transaminase (SGPT) (IU/L),[17] Serum Alkaline Phosphatase (SALP) (IU/L)[18] and Serum bilirubin (mg/ml).[19]
3) Histopathological Parameters**:** This included the histological changes in the liver architecture like architecture of hepatic lobules, swelling of liver cells, fatty change, focal necrosis, inflammatory cell infiltration around portal areas, Kupffer cell hyperplasia etc.
4) Functional Parameters: Pentobarbitone sleeping time was used as a functional parameter for ensuring the liver damage or efficacy of test formulation.

RESULTS

Carbon Tetrachloride Induced Hepatotoxicity

CCl_4 in dose of 1.25 ml/kg b.w. po produced acute hepatic damage in negative control (carbon tetrachloride treated) when compared with normal control. There is significant rise in levels of enzymes (biochemical parameters) SGOT, SGPT, SALP and serum bilirubin, as compared to normal control. There is increase in liver weight and volume (morphological parameter) and appears pale reddish brown. Further the physiological parameter, pentobarbitone sleeping time was prolonged when compared to normal control [Table 2].

Treatment with formulations F1 and F2 at dose of 400 mg/kg for 7 days after CCl_4 intoxication showed decrease in levels of enzymes SGOT, SGPT, SALP and bilirubin. The values for SGOT, SAP and bilirubin are near normal for F2. Liver weight and volume were observed to be reduced and appeared normal. The standard silymarin showed significant reduction in all parameters when compared to CCl_4 treated group [Table 2].

Paracetamol Induced Hepatotoxicity

Paracetamol in dose of 2 gm/kg b.w., produced acute hepatic damage. Paracetamol significantly increased levels of SGOT, SGPT, SALP and serum bilirubin. Paracetamol prolonged duration of sleeping time after Pentobarbitone injection. There is significant increase in liver weight and liver volume [Table 3].

After five days of treatment with F1 and F2 at 400 mg/kg

Table 2: Carbon tetrachloride induced liver toxicity in rats

Groups	Liver wt in g	Liver vol in ml	PBT sleeping time in min	Liver enzymes			Bilirubin (mg%)	
				SGPT(IU/l)	SGOT(IU/l)	SAP(U/l)	Total	Direct
Control	6.42±0.07	7.46±0.09	138.33±6.67	84.26±8.39	163.07±7.3	114.43±7.99	0.52±0.05	0.39±0.02
CCl4	8.47±0.36	10.26±0.45	235.83±13.10$^{\#}$	243.41±14.89$^{\#}$	284.91±14.77$^{\#\#}$	289.53±13.19$^{\#}$	1.24±0.07$^{\#}$	0.59±0.03$^{\#}$
CCl4+400mg F1	6.7±0.16	7.41±0.12	155.83±7.73**	143.02±8.85**	193.82±10.80***	162.46±11.4**	0.62±0.03**	0.45±0.01**
CCl4+400mg F2	6.67±0.08	7.39±0.12	185.16±7.93**	195.19±10.22*	165.19±8.04***	125.48±9.64**	0.57±0.02**	0.39±0.01**
Silymarin	6.65±0.09	7.3±0.13	162.33±6.42**	116.73±8.74**	164.32±7.05***	131.30±8.56**	0.53±0.02**	0.42±0.01**

N = 6; values are mean ± standard error; *P < 0.05; **P < 0.01; ***P<0.001 when compared with CCl_4 Negative control. $^{\#}P$ < 0.01; $^{\#\#}P$ < 0.001 when compared with normal control. PBT = pentobarbiton

Table 3: Paracetamol induced liver toxicity in rats

Groups	Liver wt in g	Liver vol in ml	PBT sleeping time in min	Liver enzymes			Bilirubin (mg%)	
				SGPT(IU/l)	SGOT(IU/l)	SAP(U/l)	Total	Direct
Control	6.35±0.13	7.45±0.17	91.00±4.46	97.2±4.94	154.5±6.30	122.67±9.95	0.55±0.01	0.43±0.05
Paracetamol	8.0±0.17	10.99±0.28	201.5±9.47$^{\#}$	205.13±10.09$^{\#}$	283.96±6.35$^{\#}$	291.93±11.58$^{\#}$	1.065±0.06$^{\#}$	0.63±0.02$^{\#}$
Para+400mg F1	6.80±0.12	6.93±0.10	132.5±6.16**	125.14±6.94**	176.65±5.82**	151.33±5.84**	0.88±0.07	0.58±0.02
Para+400mg F2	6.48±0.17	6.76±0.16	134.5 ±6.42**	136.33±7.84**	182.35±9.53**	135.15±6.69**	0.62±0.03**	0.50±0.01**
Silymarin	6.64±0.12	6.98±0.11	153.00±3.88**	115.02±8.61**	168.26±6.08**	122.02±3.86**	0.615±0.02**	0.48±0.007**

N = 6; values are mean ± standard error; ** = P<0.01 when compared with Paracetamol negative control. $^{\#}$ = P<0.01 when compared with normal control. PBT = pentobarbitone

Paracetamol was given as a toxicant. It was observed that both formulations significantly lowered paracetamol induced levels of SGOT, SGPT and SAP. Pentobarbitone sleeping time was lowered compared to paracetamol treatment. Morphology of liver appeared normal and weight and volume of liver were near normal value when compared to paracetamol treated group. However the bilirubin levels were markedly reduced in F2 and standard but did not reduced significantly in F1 treated rats [Table 3].

Sub acute CCl_4 treatment caused marked congestion of central vein and portal triads, presence of prominent Kupffers cells and cloudy degeneration indicating fibrosis. However necrosis was not observed in any group which indicates that sufficient hepatoxicity does not seem to have developed in the animals so as to cause the necrosis of liver. While paracetamol treated groups showed less of congestion of portal triad but severe congestion of central vein, but presence of prominent Kupffer cells, cloudy degeneration was observed. Both formulations prevented Paracetamol-induced changes in liver where central vein and portal triads appear normal with slight unclearity for F2, but absence of prominent of Kupffer cells and cloudy degeneration. The CCl_4 and formulations F1 and F2 treated groups showed excellent protection to liver architecture.

DISCUSSION

Herbal principles are coming up as an effective source of disease treatment. Ayurvedic System of medicine has always used this hidden potential. But it is very essential to mould this system with modern standards. Multi-component drug formulations F1 and F2 contain the extracts of several medicinal plants that contain specific therapeutically active principles and are traditionally used in liver disorders and screened for various hepatoprotective mechanisms. The crude drugs used are *Acacia catechu*, *Allium sativum*, *Andrographis paniculata, Azadirachta indica, Boerhaavia diffusa, Curcuma longa, Eclipta alba, Emblica officinalis, Luffa echinata, Picrorrhiza kurroa, Phyllanthus amarus*. Combined action of all the ingredient helps to normalize the liver function and thus cure complex liver disorders.

CCl_4 is routinely used hepatotoxin for screening studies. Administration of CCl_4 orally causes acute liver damage that mimics natural causes. It mediates changes in liver function that ultimately leads to destruction of hepatocellular membrane. Cytochrome P-450 activates CCl_4 to form various free radicals (trichloromethyl, Cl_3C-CCl_3 (hexachloroethane), $COCl_2$ (phosgene), etc.) which are involved in pathogenesis of liver damage in chain reactions results in peroxidation of lipids, covalent binding to macromolecules, disruption of metabolic mechanisms in mitochondria, decrease levels of phospholipids, increase triglyceride levels, inhibition of Calcium pump of microsomes thus leading to liver necrosis.[20] Extent of hepatic damage is assessed by elevated levels of marker enzymes mainly SGPT, SGOT, SALP and total bilirubin. Decrease levels of transaminases indicate stabilization of plasma membrane and protection of hepatocytes against damage caused by hepatotoxin. SAP is related to functioning of hepatocytes and increase in its activity is due to increased synthesis in presence of biliary pressure.

Toxic metabolite of Paracetamol, N-acetyl, p-benzoquinone imine, covalently interact with thiol groups in proteins and causes liver cell damage by causing depletion of glutathione levels and thiol proteins of liver and consequent stimulation of lipid peroxidation. Due to exhaustion of detoxifying agent glutathione, hepatocytes are vulnerable to the excess of toxic metabolite resulting into liver cell necrosis.[21]

As both the hepatotoxic agents are known to cause toxicity, CCl_4 was given for 5 days (1.25 ml/kg b.w. po) prior to the drug formulation to assess the therapeutic effect to already started damage process. Paracetamol was given in a single dose (2 gm/kg) after 5 days administration of drug formulation to assess prophylactic resistance to toxic changes induced by paracetamol. Formulations F1 and F2 were able to reduce levels of enzymes especially SGOT in both experiments, indicating that they were protective to hepatocytes and maintained normal liver physiology and further cause stabilization of plasma membrane and regeneration of damaged liver cells.

Drug metabolizing capacity of liver is severely affected due damaging effects of CCl_4 and Paracetamol on liver microsomal enzyme system, thus resulting in prolongation of Pentobarbitone induced sleeping time [Tables 2 and 3]. It indicates physiological parameter which highlights the normal or delayed metabolic activity of liver. Herbal drug formulation F_1 (155.83 min for CCl_4 and 132.5 min for Paracetamol) and F_2 (185.16 min for CCl_4 and 134.5 min for Paracetamol) reduced elevated levels of pentobarbitone induced sleep time, indicating protective effect on metabolic functions of liver [Tables 2 and 3].

CONCLUSION

The herbal drug formulations F1 and F2 designed and developed for the treatment of liver disease are prepared from the dry extracts of the abundantly available Indian medicinal plants.

These formulations have shown very significant hepatoprotective activity in therapeutic and prophylactic mode in acute toxicity models and are found to be comparable with the results of Sylimarin. However formulation F2

has demonstrated slightly better results as compared to formulation F1 when biochemical parameters are taken into consideration. The formulation found to modulate bilirubin hence; it can be proposed to be beneficial in obstructive jaundice and hepatitis condition especially formulation F2 and the reason may be due to antioxidant activity or presence of higher quantity of *Andrographis paniculata* and *Azadirachta indica* in formulation. But no significant difference was observed in morphological, histological and physiological parameters. Other mechanisms may be anti-inflammatory; immunomodulatory or direct modulation of certain microsomal system. This can only be confirmed in further studies for chronic hepatotoxicity and mechanism of hepatoprotection.

On conclusion, the prepared multicomponent herbal drug formulation has demonstrated a very good hepatoprotection against the CCl_4 and paracetamol induced liver damage. Further evaluation of these formulations is going on in our laboratory.

ACKNOWLEDGEMENTS

We are thankful to Dr. Gokhale S. K. (MD pathologist) for performing the histopathological studies of liver samples.

REFERENCES

1. Meyer SA, Kulkarni AP, Hepatotoxicity. *In*: Hodgson E, Smart RC, editors. Introduction to biochemical toxicology. 3rd ed. New York: A. John Wiley and Sons, Inc; 2001. p. 487-90.
2. Harsh Mohan. Text book of pathology, 4th ed. New Delhi: Jaypee Publishers; 2002. p. 569-630.
3. Rege N, Dahanukar S, Karandikar SM. Hepatoprotective effect of Cynidanol (+) against Carbon tetrachloride induced liver damage. Indian Drugs 1984;22:556-68.
4. Wealth of India. vol 1, A-Z, PID. New Delhi: CSIR; 1985.
5. Trivedi N, Rawal VM. Hepatoprotective and Toxicological evaluation of *Andrographis parniculate* on severe liver damage. Indian J Pharmacol 2000;32:288-93.
6. Mehrotra R, Rawat S, Kulshreshtha DK, Patnaik GK, Dhawan BK. *In vitro* studies on the effect of certain natural products against hepatitis B virus. Indian J Med Res 1992;92:133-8.
7. Handa SS, Sharma A. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbontetrachloride. Indian J Med Res 1990;92:276-83.
8. Chattopadhyay R, Sarkar SK, Ganguly S, Banerjee RN, Basu TK, Mukherjee A. Hepatoprotective activity of *Azadirachta indica* leaves on paracetamol induced hepatic damage in rats. Indian J Exp Biol 1993;30:738-40.
9. Srimal RC. Turmeric a brief review of medicinal properties. Fitoterapia 1997;68:483-93.
10. Sane RT, Chawla JL, Kuber VV. Studies on *Phyllanthus amarus*-Part I. Indian Drugs 1997;34:580-4.
11. Chaudhary K, Singh A, Bagchi GD, Kumar S. Determination of optimum planting and harvesting schedule for high yields of *Phyllanthus amarus* herb containing the hepatoprotective compound phyllanthin. Indian Drugs 1998;35:481.
12. Reddy BP, Murthy VM, Venkateshwarlu V, Kokate CK, Rambhau D. Antihepatotoxic activity of *Phyllanthus amarus, Tinospora cordifolia* and *Ricinum communis.* Indian Drugs 1992;30:338-41.
13. Ansari RA, Aswal BS, Chander R, Dhawan BN, Garg NK, Kapoor NK, *et al.* Hepatoprotective activity of Kutkin-the iridoid glycoside mixture of *Picrorrhiza kurroa*. Indian J Med Res 1988;87:401-4.
14. Saraswat B, Visen PK, Patnaik GK, Dhawan BN. Anticholestatic effect of picroliv, active hepatoprotective principles of *Picrorhiza kurroa* against carbontetrachloride induced cholestasis. Indian J Exp Biol 1993;31:316-8.
15. Visen PK, Saraswat B, Patnaik GK, Agarwal DP, Dhawan BN. Protective activity of Picroliv isolated from *Picrorrhiza kurroa* against ethanol toxicity isolated rat hepatocytes. Indian J Pharmacol 1996;28:98-101.
16. Anand KK, Gupta VN, Rangari VD, Chandan BK. Structure and hepatoprotective activity of a biflavonoid from *Canarium manii*. Planta Med 1992;58:493-5.
17. Reitzman S, Frankel S. A colorimetric methiod for determination of serumglutamic oxaloacetic acid glutamic pyruvate transaminases. Am J Clin Pathol 1957;28:56-63.
18. Bessey OA, Lowery DM, Brock MJ, A method for rapid determination of alkaline phosphatase with five cubic millimeters of serum. J Biol Chem 1964;164:321-9.
19. Malloy HT, Evelyn KA. The determination of bilirubin with photoelectric colorimeter. J Biol Chem 1936;119:481-90.
20. Seakins A, Robinson DS, The effect of the administration of carbon tetrachloride on the formation of plasma lipoproteins in the rats. Biochem J 1963;86:401-7.
21. Portmann B, Talbot IC, Day DW, Davidson AR. Histopathological changes in the liver following a paracetamol overdose: Correlation with clinical and Biochemical parameters. J Pathol 1975;117: 169-80.

Source of Support: Nil, **Conflict of Interest:** None declared.