ISSN: 0974-6943

Jitendra Vaghasiya et al. / Journal of Pharmacy Research 2009, 2(4), 610-614

Research Article

Available online through
www.jpronline.info

Protective Effect of Polyherbal Formulation on Isoniazid Induced Hepatotoxicity in Rats

Jitendra Vaghasiya[1*], Yagnik Bhalodia[1], Malaviya Shailesh[1], Nurudin Jivani[1], Shivkumar Rathod[2]

*[*1]Department of Pharmacology,Smt. R.B.Patel Mahila Pharmacy College, S.N.D.T Women's University, Atkot, Gujarat, India.*

[2]Department of Pharmacology,M. S. University of Baroda, Vadodara, Gujarat, India.

***For correspondence:*Jitendra Vaghasiya,Department of Pharmacology, Smt. R. B. Patel Mahila pharmacy College, Atkot, Gujarat, India.**

*E-mail:*j_vaghasiya@yahoo.com

Received on:11-12-2008; Accepted on :04-02-2009

ABSTRACT

Objective: To evaluate effect of Polyherbal Formulation (PHF) on isoniazid induced hepatotoxicity in rats and assessment of any possibility of co administration of PHF along with such hepatotoxic drug.*Materials and Methods:* Hepatotoxicity in rat was induced by Isoniazid (INH) (200mg/kg/p.o. for 24 days) and protective effect of PHF (0.25ml/kg/p.o. and 0.5ml/kg/p.o. either along with hepatotoxic drugs or followed by inducing hepatotoxicity) was measured by estimating marker enzymes for liver function like Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase and g glutamic transpeptidase. Oxidative stress markers like lipid peroxidation, reduced glutathione, super oxide dismutase and catalase. Protein profile likes Total bilirubin, direct bilirubin, Total albumin and Total protein. Histopathological study was carried out to confirm hepatotoxicity. *Results and Discussions:* Isoniazid induced hepatotoxicity characterized by significant increase in marker enzymes for liver function and oxidative stress along with depletion of proteins. Administration of PHF either along with Isoniazid or followed by inducing hepatotoxicity significantly improved the level of marker enzymes for liver function, oxidative stress and depleted proteins profile. *Conclusion:* The study suggest protective role of PHF and it can be utilized to treat the hepatotoxicity with long-term clinically useful drugs, which are at the risk of developing hepatotoxicity.

Key words: Hepatotoxicity; Isoniazid; Polyherbal Formulation; Oxidative stress; Protein profile.

INTRODUCTION

Drug-induced Hepatotoxicity (DIH) account for 9.5% of all suspected adverse drug reaction, and are the most common reason for withdrawal of drug from the market [1]. The liver is central metabolizing organ, so it more susceptible to metabolism-dependant injury. Thus injury may be a direct toxic effect or immunological reaction to either of the drug or an active metabolite formed by bioactivation [2]. It is reported that 62% of withdrawn drugs having toxic metabolite [3]. Although, with the exception of rare cases, DIH subsides after cessation of treatment with the drug, this represents an important diagnostic and therapeutic challenge for physicians.

Drug-induced immune-mediated liver injury occur by hapten-like reaction in which low molecular weight drugs or its metabolites may covalently bind to macromolecules such as liver protein, alter that protein and become immunogenic[4]. Dose-dependent hepatotoxicity is due to prolong administration or single toxic dose. The predominant clinical presentation is acute hepatitis and/or cholestasis, although almost any clinical pathological pattern of acute or chronic liver disease can occur.

Susceptibility to drug-induced hepatotoxicity is also influenced by genetic and environmental risk factors. Unpredictable, low-frequency, idiosyncratic reactions often occur on a background of a higher rate of mild asymptomatic liver injury and, although difficult to predict, they may be detected by monitoring serum alanine aminotransferase levels. Recent and future advances in toxicogenomics and proteomics should improve the identification of risk factors and the understanding of idiosyncratic hepatotoxicity [3].

Polyherbal formulation contains plant extract of Himsra, Kasani, Kakamachi, Arjuna, Kashmarda, Birranjshipa, Jharuka and processsed in 50mg herbs each in powder form of Bhringraja, Punarnava, Guduch, Daruharidra, Mulaka, Amalaki, Chitraka, Vidanga, Haritaki, and Parpata. The main objective of present study was to assess hepatoprotective effect of Polyherbal formulation and assessment of any possibility of co administration along with such hepatotoxic drug.

MATERIALS AND METHODS

Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of M. S. University, Baroda and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy adult male Wistar rats weighing 150-200g were used. Rats were housed in polypropylene cages, maintained under standardized condition (12-h light/dark cycle, 24°C, 35 to 60% humidity) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt. Ltd., Pune) and purified drinking water ad libitium.

Experimental protocol

Animals were divided into seven groups, each having 6 rats and treated accordingly. Group: 1 - rats received normal standard diet for 24 days, Group: 2 - rats received Polyherbal Formulation 0.25ml/kg/p.o. alone for 24 days, Group: 3- rats received isoniazid 200mg/kg/p.o. for 24 days[5], Group: 4 - rats received isoniazid along with PHF 0.25ml/kg/p.o. for 24 days, Group: 5 - rats received isoniazid along with PHF 0.5ml/kg/p.o. for 24 days, Group: 6 - rats received isoniazid followed by PHF 0.25ml/kg/p.o. for 1 week. Group: 7 - rats received isoniazid along with Silymarin (SLM) 20 mg/kg/p.o.

Collection of serum

Blood samples were withdrawn from retro-orbital plexus under light

ether anesthesia without any anticoagulant and allowed for 10 minutes to clot at room temperature. It was centrifuged at 2500 rpm for 20 minutes. The serum obtained was kept at 4ºC until used. All the animals were euthanasiously sacrificed after blood collection with spinal dislocation method and liver removed for study of histopathology.

Estimation of liver function

Estimation of marker enzymes for liver function like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were done by using kit, Span Diagnostic Ltd, India and Gama glutamic transpeptidase (GGTP) was done by using kit, Dade Behring Ltd., UK. Estimation of Lactate dehydrogenase (LDH) was done by using kit, Enzopak-Reckon diagnostics. Protein profile likes Total bilirubin, direct bilirubin, Total albumin and Total protein were done by using kit, Span Diagnostic Ltd, India.

Estimation of oxidative stress markers

All the animals were euthanasiously sacrificed after blood collection with spinal dislocation method under light ether anesthesia and liver removed for study of oxidative stress markers and histopathological evaluation. Liver kept in cold conditions (precooled in inverted petridish on ice). It was cross chopped with surgical scalpel into fine slices in chilled 0.25 M sucrose, quickly blotted on a filter paper. The tissue was minced and homogenized in 10 mM Tris-HCl buffer, pH 7.4 (10%w/v) with 25 strokes of tight teflon pestle of glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for oxidative stress markers assays like lipid peroxidation[6], Reduced Glutathion[7] , Super oxide dismutase [8] and Catlase[9] .

Histopathological study

Liver was collected after the rats were sacrificed. After blotting free of blood and tissue fluids, it was kept in 5% formalin. 5-15µm thick section was serially cut on a leitz microtome in horizontal planeand mounted on glass slide with the help of egg albumin in glycerine solution (50% v/v). They were then stained with 10% hematoxylin for 3-5 minutes and placing in running water intensified the staining. The hematoxylin stained sections were stained with 10% eosin for 2 minutes. The sections were observed and desired areas were photographed in an Olympus photomicroscope. The sections were viewed under 40X magnifications.

Statistical analysis

The results were expressed as mean ± SEM and analysed by unpaired student's *t*-test using the NCSS statistical computer package. Level of significance was set at $P < 0.05$ [10].

RESULTS

Effect of polyherbal formulation on Marker Enzymes of Liver Function

Serum levels of ALT, AST, GGTP, ALP and LDH were significantly (P<0.001) increased after treatment with Isoniazid compared to control. PHF (0.25ml/kg) perse has no effect on ALT, AST, GGTP, ALP and LDH but PHF (0.25ml/kg and 0.5 ml/kg) when administrated along with or after treatment with Isoniazid produced significant (P<0.001) decreased in the levels of ALT, AST, GGTP, ALP and LDH. Silymarin treatment also produced significant (P<0.001) decreased in the levels these enzymes but was less than that of PHF. Correspondingly, there was significantly (P<0.001) increased in the ratio of ALT/AST after Isoniazid, which was significantly (P<0.001) decreased after treatment with PHF and Silymarin (Table: 1).

Effect of Polyherbal formulation on Protein Profile

Isoniazid administration caused significant (P<0.001) increase in serum Total Bilirubin and significant (P<0.001) decrease in Direct Bilirubin, Total Protein and Albumin compared to control. There was no effect of PHF (0.25ml/kg) alone on serum Total Bilirubin, Direct Bilirubin, Total Protein and Albumin. PHF (0.25ml/kg and 0.5ml/kg) along with Isoniazid, PHF (0.25ml/kg) followed by Isoniazid and Silymarin showed significant (P<0.001) decrease in Total Bilirubin increase in Direct Bilirubin. PHF (0.25ml/kg and 0.5ml/kg) along with Isoniazid, Silymarin and PHF (0.25ml/kg) followed by Isoniazid treatment were found to caused significant (P<0.001, P<0.05 respectively) increase in Total Protein and Albumin compared to Isoniazid control. Correspondingly the changes were observed in the ratio of Albumin/Globulin (Table: 2).

Effect of Polyherbal formulation on Oxidative stress markers

Isoniazid significantly (P<0.001) increased lipid peroxidation and caused significant (P<0.001) decrease in GSH, SOD and CAT compared to control. There was no significant effect of PHF (0.25ml/kg) alone on lipid peroxidation, Reduced glutathione (GSH), Super oxide dismutase (SOD), Catalase (CAT). PHF (0.25ml/kg and 0.5ml/kg) along with Isoniazid and PHF (0.25ml/kg) followed by Isoniazid was found to caused significant (P<0.001) decrease in lipid peroxidation and significant (P<0.001) increase in GSH, SOD and CAT as compared to Isoniazid control. Silymarin along with Isoniazid produced effect similar to PHF (Table: 3).

Effect of polyherbal formulation on Histopathological changes

Liver section of control rats revealed the normal hepatic hexagonal lobules and normal morphology. Liver tissue of Isoniazid treated rats showed vacuolation, degeneration of hepatocyte, mild inflammation and piecemeal necrosis. PHF and Silymarin treatment improved structural integrity of liver cells (figure 1).

DISCUSSION

ALT and AST are enzymes produced within the cells of the liver, as the cells are damaged, leaks into the bloodstream leading to a rise in the serum levels. ALP is an enzyme, which is associated with the biliary tract, and is elevated; biliary tract damage and inflammation should be considered. It is used often times to confirm that the alkaline phosphatase is of the hepatic etiology by GGTP. Mild to moderate elevation of ALT, AST, ALP (1-3 times) are usually seen in drug toxicity [11,12,13].

Elevated serum ALT, AST, ALT/AST, GGTP, ALP, LDH and CPK levels in Isoniazid treated animals compared to control animals is attributed as damage to the structural integrity of liver[14], and presumptive markers of drug induced necrotic lesions in the hepatocyte. This decrease in elevated serum ALT, AST, ALT/AST, GGTP, ALP and LDH levels in PHF along with or followed by Isoniazid treated animals in part may be due to the protective effect of PHF on liver cells following restoration of liver cell membrane permeability [15] .

This protective effect indicates reduction in enzymes present in the extra cellular milieu as liver cell. Protective effect of component of PHF has also been observed in several experimental studies [16,17,18] . It can be stated that PHF contains *Tamarix gallica*, and crude herbal extracts of *Capparis spinosa*, *Cichorium intybus*, *Solanum nigrum*, *T. arjuna*, and *A. millefolium*, these medicinal herbs alone or in combination can influence in restoration of the cellular functions and structural integrity of liver.

Total bilirubin may rise in irritation of liver. Direct bilirubin fraction is that portion of bilirubin that has undergone metabolism by the liver, if the direct bilirubin is low, while the total bilirubin is high; this reflects liver cell damage or bile duct damage within the liver itself. Albumin is

ISSN: 0974-6943

Table: 1 Effect of INH, PHF alone and INH along with PHF on Marker enzymes of liver function

Groups	ALT (IU/L)	AST (IU/L)	ALT/AST	GGTP (IU/L)	ALP (IU/L)	LDH (IU/L)
CONTROL	35.17 ± 2.613	40.00 ± 1.414	0.8800 ± 0.028	40.83 ± 1.014	152.5 ± 1.478	345.3± 4.702
PHF(0.25ml)	32.50 ± 2.262	37.33 ± 1.476	0.7767 ± 0.097	39.00 ± 0.966	151.0 ± 2.221	340.0 ± 3.347
INH	200.7± 3.499^{+++}	79.67 ± 1.358^{+++}	2.522 ± 0.046^{+++}	146.7 ± 4.3^{+++}	350.2± 3.01^{++}	518.2± 8.8^{+++}
INH+ PHF(0.25ml)	59.00± 2.793***	69.00 ± 3.276***	0.8650 ± 0.066***	56.33± 2.9***	171.5± 2.12***	370.2± 4.65***
INH+PHF(0.5ml)	53.33 ± 2.404***	55.17 ± 2.676***	0.9700 ± 0.038***	52.17± 2.5***	161.0± 2.86***	358.3± 5.27***
INH followed by PHF	80.50± 2.849***	74.83 ± 3.410***	0.9967 ± 0.051***	71.67± 3.6***	222.5± 4.47***	415.0± 6.58***
INH +SLM	55.50 ± 2.849***	56.17 ± 2.786***	0.9983 ± 0.06***	63.67± 1.6***	190.3± 3.41***	379.8± 3.62***

[Values are mean ± SEM of 6 animals in each group]. Data analysed by unpaired student's t-test, ***, +++ p<0.001, + compared with control, * compared with INH control

Table: 2 Effect of INH, PHF alone, along with and followed by INH on Protein Profile

Groups	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Total Protein (mg/dl)	Albumin (mg/dl)	A/G
CONTROL	0.7033± 0.008	0.3017± 0.006	7.497± 0.116	4.995± 0.058	2.037± 0.128
PHF(0.25ml)	0.6900± 0.023	0.3100± 0.009	7.330± 0.075	4.900± 0.096	2.043± 0.126
INH	1.918± 0.216^{+++}	0.1100± 0.023^{+++}	5.200± 0.159^{+++}	2.100± 0.23^{+++}	0.7517± 0.159
INH + PHF(0.25ml)	0.9017± 0.024***	0.2517± 0.020**	6.850± 0.275***	4.70 ± 0.20***	2.598± 0.525
INH + PHF(0.5ml)	0.8500± 0.028***	0.2500± 0.029**	7.133± 0.233***	4.917± 0.20***	2.555± 0.543
INH followed by PHF	1.115± 0.050***	0.2283± 0.026*	6.117± 0.208*	4.533± 0.11***	3.558± 0.78***
INH +SLM	0.9800± 0.037***	0.2600± 0.0279***	6.517± 0.202***	4.717± 0.22***	2.645± 0.182

[Values are mean ± SEM of 6 animals in each group]. Data analysed by unpaired student's t-test, ***, +++ p<0.001, ++, ** p<0.001 +, * p<0.001 + compared with control, * compared with INH control

Table:3 Effect of INH, PHF alone, along with and followed by INH on oxidative stress markers

Groups	MDA (Nmoles/gm of tissue)	GSH (µmole/gm of tissue)	SOD (Unit/gm of tissue)	CAT (µmole H_2O_2 consumed/ min/gm of tissue)
CONTROL	5.102± 0.09453	106.0 ± 1.633	43.67± 1.282	295.0 ±1.592
PHF(0.25ml)	5.100 ± 0.09309	104.0 ± 2.266	44.00 ± 1.673	308.7 ±16.87
INH	28.22± 1.887^{+++}	31.83± 2.212^{+++}	19.67± 2.679^{+++}	60.17± 4.086^{+++}
INH + PHF(0.25ml)	7.383± 0.3572***	91.17± 4.715***	37.17± 2.638***	280.2± 5.326***
INH + PHF(0.5ml)	6.900± 0.2556***	99.17± 2.810***	49.00± 3.317***	279.2± 5.753^{G**}
INH followed by PHF(0.25ml)	10.60± 0.2266***	80.00± 2.933***	31.00± 1.653***	250.0± 4.830***
INH +SLM	8.400± 0.2828***	85.00± 2.933***	32.17± 2.638***	270.5± 4.682***

[Values are mean ± SEM of 6 animals in each group]. Data analysed by unpaired student's t-test, ***, +++ p<0.001, + compared with control, * compared with INH control

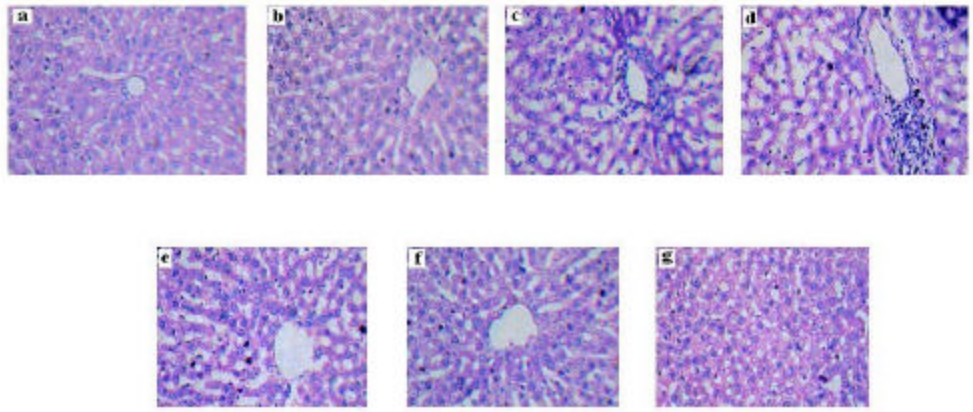

Figure 1 Hematoxyline and eosin stained sections of rat liver tissue (magnification X 40) (a) normal rat liver, (b) isoniazide treated rat liver, (c) polyherbal formulation (0.25 ml/kg) treated rat liver, (d) Isoniazide along with polyherbal formulation (0.25 ml/kg) treated rat liver, (e) Isoniazide along with polyherbal formulation (0.5 ml/kg) treated rat liver, (f) Isoniazide followed by polyherbal formulation (0.25 ml/kg) treated rat liver, (g) Isoniazide along with silymarin treated rat liver.

synthesized by the liver; it represents a major synthetic protein and is a marker for the ability of the liver to synthesize proteins. Low level indicates that the synthetic function of the liver has been markedly diminished [19].

Isoniazid treated animals significantly increased Total Bilirubin and decreased in Direct Bilirubin, Total Protein and Albumin reflects liver cell damage or bile duct damage and synthetic function of the liver has been markedly diminished, indicated drug induced hepatotoxicity [19].

PHF along with and followed by Isoniazid significantly normalized Total Bilirubin, Total Protein, Direct Bilirubin and Albumin indicating liver curative effect. The curative and hepatoprotective effect *Cassia occidentalis, Cichorium intybus* and *Solanum nigrum* of PHF were observed against chemically induced liver damage in experimental animals [20]. The diuretic effect of *T. arjuna* and anti-inflammatory and anti-immunotoxicity effect of *Cichorium intybus* have been shown in clinical and experimental studies [21,22].

In present study increased in lipid peroxidation and depletion of antioxidant enzymes such as GSH, SOD and CAT in Isoniazid treated animals compared to control animals indicate generation of oxidative stress.

Isoniazid may cause oxidative stress mediated hepatotoxicity because the activation of CYP2E1 which generates the ROS [23]. PHF along with and followed by Isoniazid significantly reduced lipid peroxidation and increased antioxidant enzymes such as GSH, SOD and CAT as compared to Isoniazid treated animals indicated modification of oxidative stress by PHF. The protection of liver cells against toxic materials including drugs, lipid peroxidation and free radical injury may decrease inflammation [24]. Immune dysfunction is component of liver disease and thus immunomodulation by herbal therapy prevent oxidative stress, inflammation and strengthens the detoxifying power of liver cell [25].

All these effects strengthen liver and regulate body metabolism and ultimately inhibit further liver cell damage in the favor of their regeneration.[26] The anti-oxidative and anti-hepatotoxic property of esculetin and p-methoxybenzoic acid the main constituent of *Cichorium intybus* and *Capparis spinosa*, respectively, have been reported in chemically induced hepatotoxicity in experimental animals [27,28]. *Achillea millefolium*, another component of PHF contains several bioactive constituents including flavonoids and terpenoids with anti-oxidative and anti-inflammatory properties [29,30,]. Furthermore anti-oxidative property of flavonoid content of *Tamarix gallica* and inhibitory effect *Solanum nigrum* crude extracts on free radical-mediated DNA damage increase the hepatoprotective effect of PHF[31]. In addition, the antioxidative, anti-lipoproxidative and increase in glutathione content of the liver cells was observed with arjunolic acid and flavonoids present in *T. arjuna*.[32].

Although there is insufficient information to establish the mechanism of action of PHF protection, this could be due to its anti-inflammatory, anti-oxidative, immunomodulating as well as restorative effects.

CONCLUSION

The study suggest protective role of Polyherbal Formulation along with or followed by Isoniazid induced hepatotoxicity and this effect may be due to its anti-inflammatory, anti-oxidative, immunomodulating as well as restorative effects. So Polyherbal Formulation can be utilized to treat the hepatotoxicity with long term clinically useful drugs, which are at the risk of developing hepatotoxicity.

ACKNOWLEDGEMENT

We are deeply indebted to Unichem Lab. and Olcare Lab for providing gift samples of Isoniazid and Silymarin.

REFERENCES

1. Zimmerman H. Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. 2nd ed. Philadelphia: Lippincott, Williams & Wilkins, 1999.
2. Kaplowitz N. Biochemical and cellular mechanisms of toxic liver injury. Semin Liver Dis 2002; 22:137–44.
3. Temple RJ, Himmel MH. Safety of newly approved drugs: implications for prescribing. JAMA 2002; 287: 2273 - 2275.
4. Knowles S, Uetrecht J, Shear N. Idiosyncratic drug reactions: the reactive metabolite syndrome. Lancet 2000; 356:1587–91.
5. S. Santhosth., Effect of chitosan supplementation on antitubercular drugs-induced hepatotoxicity in rats. Toxicol 2006; 53-59.
6. Slater TF, Sawyer, BC. (1971): The stimulatory effects of carbon tetrachloride and other halogenoalkanes or peroxidative reactions in liver fractions in vitro. Biochem J 1971; 123: 805–814.
7. Moran MS, Depierre JW, Mannervik B. (1979): Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica ACTA 1979; 582: 67
8. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay of SOD. J Biol.Chem 1972; 247: 3170.
9. Colowick SP, Kaplan NO, Packer L. Methods in Enzymology, 105. Academic Press, London 1984; 121.
10. Hintze JL: Copyright C. 865, East 400. North Kaysville, Utah 1986; 84: 546–0445
11. Rosen HR, Keefe EB. Evaluation of abnormal liver enzymes, use of liver tests and the serology of viral hepatitis: Liver disease, diagnosis and management. 1st ed. New York; Churchill livingstone publishers 2000; 24-35.
12. Friedman SF, Martin P, Munoz JS. Laboratory evaluation of the patient with liver disease. Hepatology, a textbook of liver disease. Philedelphia; Saunders publication, 2003; 1: 661-709.
13. Simko V. Alkaline phosphatases in biology and medicine. Dig Dis 1991; 9: 189-193.
14. Chenoweth, M.B., Hake, C.L the smaller halogenated aliphatic hydrocarbons. Ann. Rev. Pharmacol 1962; 2: 363–398.
15. Kalab, M., Krechler, T. The effect of the heptoprotective agent Liv-52 on liver damage. Cas. Lek. Cesk. 1997;136: 758–760.
16. Kataria, M., Singh, L.N. Hepatoprotective effect of Liv- 52 and kumaryasava on carbon tetrachloride induced hepatic damage in rats. Indian J. Exp. Biol. 1997; 35: 655–657.
17. Sandhir, R., Gill, K.D, Hepatoprotective effects of Liv-52 on ethanol induced liver damage in rats. Indian J. Exp. Biol 1999; 37: 762–766.
18. Mathur, S. Role of Liv-52 in protection against beryllium intoxication. Biol. Trace Elem. Res. 1994; 41: 201–215.
19. Green RM, Flamm S. AGA techinal review of evaluation of liver chemistry tests. Gastroenterology 2002; 123: 1367-1384.
20. Kanase, A., Patil, S., Thorat, B. Curative effects of mandur bhasma on liver and kidney of albino rats after induction of acute hepatitis by CCl(4). Indian J. Exp. Biol 1997; 35: 754–764.
21. Bharani, A., Ganguly, A., Bhargava, K.D. Salutary effect of Terminalia arjuna in patients with severe refractory heart failure. Int. J. Cardiol 1995;49: 91–99.
22. Kim, J.H., Mun, Y.J., Woo, W.H., Jeon, K.S., N.H., Park, J.S. Effects of the ethanol extract of Cichorium intybus on the immunotoxicity by ethanol in mice. Int. Immunopharmacol 2002; 2 (6): 733–744.
23. Koop, D. R. Oxidative and reductive metabolism by cytochrome P4502E1. FASEB J 1992; 6: 724–730.
24. Yang, H., Chen, Y., Xu, R., Shen, W., Chen, G. Clinical observation on the long-term therapeutic effects of traditional Chinese medicine for treatment of liver fibrosis. J. Tradit. China Med 2000; 20: 247–250.
25. Jiang, W., Li, S., Wang, C., Wang, Y. Comparative study of effects of three kinds of herbal mixture decoctions on improving immune senescence and free radical metabolism. Chin. Med. J 1997; 110: 750–754.

26. Bean, P. The use of alternative medicine in the treatment of hepatitis C. Am. Clin. Lab 2002; 21: 19–21.
27. Gilani, A.H., Janbaz, K.H., Shah, B.H., Martin-Aragon, S., Benedi, J.M., Villar, A.M. Effects of the antioxidant (6,7-dihydroxycoumarin) esculetin on the glutathione system and lipid peroxidation in mice. Gerontology 1998; 44: 21–25.
28. Germano, M.P., De Pasquale, R., D'Angelo, V., Catania, S., Silvari, V., Costa, C. Evaluation of extracts and isolated fraction from Capparis spinosa L. buds as an antioxidant source. J. Agric. Food Chem. 2002; 27: 1168–1171.
29. Glasl, S., Mucaji, P., Werner, I., Presser, A., Jurenitsch, J. Sesquiterpenes and flavonoid aglycones from a Hungarian taxon of the Achillea millefolium group. Z. Naturforsch 2002; 57 (11–12): 976–982.
30. Goldberg, A.S., Mueller, E.C., Eigen, E., Desalva, S.J. Isolation of the anti-inflammatory principles from Achillea millefolium (Compositae). J. Pharm. Sci. 1969; 58 (8): 938–941.
31. McPhail, D.B., Hartley, R.C., Gardner, P.T., Duthie, G.G. Kinetic and stoichiometric assessment of the anti-oxidant activity of flavonoids by electron spin resonance spectroscopy. J. Agric. Food Chem 2003:12: 1684–1690.
32. Sumitra, M., Manikandan, P., Kumar, D.A., Arutselvan, N., Balakrishna, K., Manohar, B.M., Puvanakrishnan, R. Experimental myocardial necrosis in rats: role of arjunolic acid on platelet aggregation, coagulation and antioxidant status. Mol. Cell. Biochem 2001; 224 (1–2): 135–142.

Source of support: Nil, Conflict of interest: None Declared