Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:

Protective and curative effect of poly herbal formulation containing indigenous medicinal plants against various hepatotoxic agents in rats

Sunil Mistry[*,1], Kunduri Rajeswar Dutt[2], Sashi Bhusan Biswal[3], Jyotirmoyee Jena[3]

[1]*Gayatri College of Pharmacy, Gayatri Vihar, Sambalpur- 768200, India*
[2]*CMR College of Pharmacy, Kandlakoya, Medchan Road, Hyderabad - 501401, India*
[3]*Department of Pharmacology, VSS Medical College, Sambalpur University, Sambalpur- 768017, India*

ARTICLE INFO

Article history:
Received 18 August 2012
Received in revised from 8 September 2012
Accepted 5 December 2012
Available online 28 December 2012

Keywords:
CCl_4
paracetamol
hepatoprotective activity
HPTLC fingerprinting

ABSTRACT

Objective: To evaluate the protective and curative potential of PHF, a polyherbal formulation, against two experimentally induced hepatotoxicity models in rats. **Methods:** Hepatoprotective activity of the PHF containing three indigenous medicinal plants extracts *Coccinia indica*, *Sida cordata* and *Scoparia dulcis*, was screened against CCl4 and paracetamol induced hepatotoxicity in rats. **Result:** Administration of hepatotoxins (CCl4 and paracetamol) shows significant morphological, biochemical and histopathological deteriorations in the liver of experimental animals. Pretreatment with PHF had significant protection against hepatic damage by maintaining the morphological parameters within normal range and normalizing the elevated levels of biochemical parameters (SGPT, SGOT, ALP and total bilirubin), which were evidently showed in histopathological study. **Conclusions:** The PHF has highly significant hepatoprotective effect at 100 and 200 mg/kg, p.o. on the liver of all the two experimental animal models. The liver protection due to combined action of all plant extracts along with their phytoconstituents.

1. Introduction

Liver is a key organ that regulates metabolism, secretion, storage, and detoxifying functions in the body, and hepatic damage is often associated with distortion of these functions[1]. The damage to the liver caused by hepatotoxic agents is of grave consequences[2]. In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are in rise. Jaundice and hepatitis are two major hepatic disorders that account for the high death rate[3]. There are potent indigenous herbal medicines available for the treatment of liver disorders in various parts of the world and most of them have not yet scientifically been validated. If they are conducted, it could lead to the development of cost effective drugs[4]. In the absence of reliable modern hepatoprotective drugs, there are a number of traditional medicines recommended for treatment of liver diseases. Many herbs such as *Silybum marianum*[5], *Tridax procumbens*[6], and *Andrographis paniculata*[7] have been reported to possess hepatoprotective activity. Plants contain wide variety of bioactive molecules including terpenoids, steroids, phenols, and flavonoids. In addition to their nutritional value these phytoconstituents exhibit a wide array of pharmacological properties such as anti-inflammatory, antiviral, anti-proliferative, and anti-carcinogenic[8]. Plant derived phenolic, flavonoid, and polyphenolic compounds are considered to contribute to the prevention of diseases associated with oxidative stress. 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 polyherbal formulation (PHF) under study contains plant ingredients *Coccinia indica*, *Sida cordata* and *Scoparia dulcis*. These drugs are used in Indigenous and Ayurvedic system of medicine for the treatment of diseases such as liver, diabetes, gonorrhea, abdominal pain, diarrhea, fever and inflammation[9-10]. The ingredients and content in dose is based on the traditional knowledge on these plants. Formulation is 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 *Scoparia dulcis* and *Coccinia indica* of these herbal formulation are known to have liver modifying activity[10-12]. The present study was undertaken to explore the hepatoprotective effects of PHF against CCl4 and paracetamol induced hepatotoxicity in rats as well as to identify the active constituents responsible for hepatoprotection.

*Corresponding author: Sunil Mistry, Asst. Professor, Gayatri College of Pharmacy, Gayatri Vihar, Sambalpur, Orissa, India.
Tel.: +91 9040582818
E-mail: mistrysunil80@gmail.com

2. Materials and methods

2.1. Collection of the plant materials

The plant materials used for the PHF preparation were *Coccinia indica*, *Sida cordata* and *Scoparia dulcis*. The plants were collected from Raipur (Lat. 22°33′N; Long. 82°38′E) Chhattisgarh, India. They were identified and authenticated by office of the Additional Director, Central National Herbarium, Govt. of India, Howarh and specimen samples were preserved in the herbarium section of the Department, with voucher no. CNH/I-304/2010/Tech.II /360,61,62 for future reference.

2.2. Extraction and preparation of PHF

The plant parts were washed, shade dried and powdered. In order to prepare the PHF, about 75 g of *Coccinia indica* leaves, 45 g of *Sida cordata* leaves and 30 g of *Scoparia dulcis* whole plant powders were soaked overnight in 750 ml of 95% ethanol. This suspension was filtered and the residue was resuspended in an equal volume of 95% ethanol for 48 h and filtered again. The two filtrates were pooled and the solvent were evaporated in a rotary evaporator, to get a yield of 26 g. This extract was dissolved in distilled water and this was administered orally to the rats.

2.3. Phytochemical analysis and HPTLC fingerprinting

All the plant extracts were identified according to the chemical test[13] and fingerprinting data (Co-TLC with marker). HPTLC is one of the standardization parameter useful for qualitative and quantitative determination of phytoconstituents present in the herbal extract/formulation. HPTLC was performed on TLC plate precoated with silica gel 60 GF 254 as stationary phase. Various pure solvents of varying polarity were tried in different proportions as mobile phase for the development of chromatogram[14]. It was also identified by compounds like Cucurbitacin B[15] from *Coccinia indica*, and Scoparic acid A[9] from *Scoparia dulcis* Linn.

2.4. Drugs and chemicals

Silymarin procured from Micro labs, Banglore and all biochemical kits were purchased from Span Diagnostics Ltd Surat, India. All other chemicals and reagents were of analytical grade and purchased from local firms. The HPTLC plate silica gel 60GF-254 (20X20 cm2) was purchased from *E. Merck* (Darmstadt, Germany), supplied by Anchrom Technologies, Mumbai.

2.5. Experimental animals

Male wistar albino rats of 200-230 g were acclimatized for 7 days under standard husbandry conditions, i.e., room temperature of (26±10)℃, relative humidity of 45-55% and light: dark cycle of 12:12 h. All the experimental protocols were approved by the institutional Animal Ethics Committee (IAEC) of Gayatri College of Pharmacy, Sambalpur (Registration No. 1339/ac/10/CPCSEA). They were allowed to take standard pellet food (Anjali Animal Food, Indore) and water ad libitum. All animal experiments were performed according to the Committee for the Purpose of the Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

2.6. Acute toxicity study

Swiss albino mice were divided into test group comprising of six animals in each group. The test was performed using increasing oral dose of herbal extracts from 500 to 5000 mg/kg b.w. The mice were observed continuously for 1 h and then half hourly for 4 h for any gross behavioral change and general motor activities like writhing, convulsion, response to tail pinching, gnawing, pupil size, fecal output, feeding behavior, etc., and further up to 72 h for any mortality. All the extracts did not cause any significant behavioral changes and no mortality was observed[16].

2.7. Experimental Procedure

Hepatoprotective activity of the PHF was demonstrated by using two experimentally induced hepatotoxicity models.

2.7.1. Carbon tetrachloride (CCl4) induced acute hepatotoxicity in rats

Wistar rats of 200-220 g were divided in to five groups with six animals each (n=6). Group-1 and group-2 were served as normal control and disease control respectively. Group-3, 4 and 5 corresponded to reference standard (Silymarin-100mg/kg/day, p.o., PHF-100mg/kg/day, p.o. and PHF-200mg/kg/day, p.o. respectively). The treatment lasted for 7 days and on the seventh day's night all the animals were fasted for 12 h. Then all the animals except those in groups-1 were treated with 1 mL of CCl4 in liquid paraffin (1:1). 24 h after CCl4 administration, body weight recorded and blood samples were collected for the estimation of biochemical parameters, namely SGPT, SGOT, ALP and total bilirubin. All the animals were sacrificed and the liver weight was recorded. Liver tissues collected were subjected to histopathology[17].

2.7.2. Paracetamol-induced hepatotoxicity in rats

Wistar rats of 200-220 g were divided into five groups with six in each (n=6). Group-1 and group-2 were served as normal control and disease control respectively. Group-3, 4 and 5 corresponded to reference standard (Silymarin-100mg/kg/day, p.o., PHF-100mg/kg/day, p.o. and PHF-200mg/kg/day, p.o. respectively). The treatment was carried out for 7 days and on seventh day's night all the animals were fasted for 12 h and all the animals except those in group-1 were treated with paracetamol (2g/kg, p.o.) in sucrose solution (40%v/v) in three divided doges. 48 h after paracetamol administration, body weight was documented and blood samples were collected for the estimation of biochemical parameters, namely, SGPT, SGOT, ALP and total bilirubin. All the animals were sacrificed and the liver weight was recorded. Liver tissues collected were subjected to histopathology[18].

2.8. Histopathological Studies

For histological studies, the liver tissues were fixed with 10% phosphate buffered neutral formalin, dehydrated in graded (50-100%) alcohol and embedded in paraffin. Thin sections (5M) were cut and stained with routine hematoxylin and eosin (H & E) stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

2.9. Statistical analysis

Values were expressed as mean±SEM. Statistical difference in mean was analyzed using one way ANOVA and followed by turkey's multiple comparison tests. P<0.05 were considered statically significant.

3. Results

3.1. Phytochemical analysis and HPTLC figure printing

All the extracts showed the presence of glycosides, flavonoids, alkaloids, proteins, carbohydrates and phenolic compounds. According to TLC results, n-hexane: ethyl acetate: methanol (6:3:0.5) solvent system for Cucurbitacin B scanned over the wavelength 280nm and pet-ether: diethyl ether: methanol (5:4:1) for Scoparic acid A at 254 nm. Results of HPTLC fingerprinting showed the presence of Cucurbitacin B at Rf 0.78, and Scoparic acid A at Rf 0.37 in PHF of *Coccinia indica*, and *Scoparia dulcis*, respectively (Figure 1).

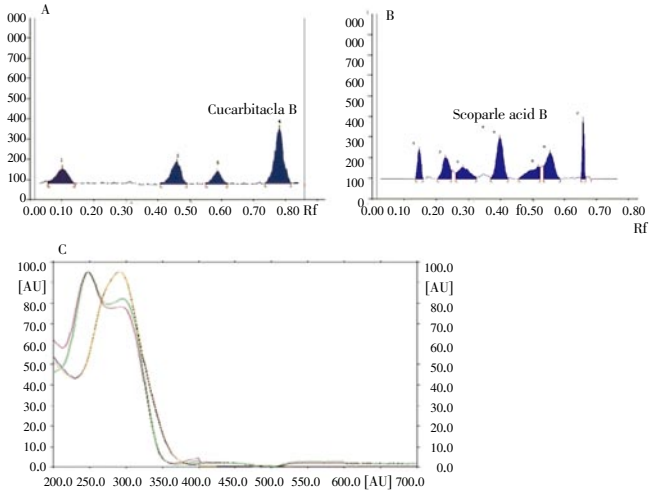

Fig. 1.HPTLC finger printing of Compounds.

A) HPTLC densitometric scan of Cucurbitacla B in *Coccinia indica* sample.

B) HPTLC densitometric scan of Scoparle acid B in *Scoparia dulcis* sample.

C) A typical overlain spectrum of standard drugs and drug extracted from PHF.

3.2. Effect of PHF against CCl4-induced hepatotoxicity in rats

Table 1

Effect of PHF on CCl4- induced hepatotoxicity in rats.

Group	Liver weight(g)	Serum biochemical parameters			
		SGPT	SGOT	ALP	Bilirubin
G1	6.67±0.23	51.52±07.3	158.8±15.12	427.6±51.4	0.27±0.05
G2	8.27±0.14 α	628±15.3*	355.3±18.42*	665.3±17.5*	0.61±0.07*
G3	6.92±0.17 γ	190.5±10.5 α , γ	585.4±22.52 α , γ	497.3±28.7 β	0.57±0.03*
G4	7.12±0.24 γ	327.7±07.4 α , γ , δ	560±12.81 α , γ , δ	388.7±19.3 γ	0.69±0.06*
G5	7.02±0.12 γ	73.5±05.7 γ , δ	146.7±11.63 γ , δ	435.4±31.6 β	0.64±0.03*

All values are Mean±SEM, n=6.* *P*<0.05 when compared with normal group, α *P*<0.01 when compared with normal group, β *P*<0.05 when compared with disease control group and γ *P*<0.01 when compared with disease control group, δ *P*<0.01 when compared with Silymarin treated group.

Table 2

Effect of PHF on paracetamol-induced hepatotoxicity in rats.

Group	Liver weight(g)	Serum biochemical parameters			
		SGPT	SGOT	ALP	Bilirubin
G1	6.67±0.22	53.65±1.22	158.4±16.13	429.6±42.46	0.28±0.04
G2	8.02±0.13 α	387.15±17.04 α	643.7±32.11 α	167.2±33.75 α	1.6±0.23 α
G3	6.22±0.16 γ	78.42±6.87 γ	186.4±9.05 γ	434.7±7.11 γ	0.24±0.12 γ
G4	7.33±0.19 γ	115.5±12.07 β	235.2±26.20 β	574.9±23.51 β	0.63±0.07 γ
G5	6.75±0.11 γ	69.11±3.54 γ , δ	167.6±17.9 γ , δ	412.3±15.32 γ	0.50±0.18 γ

All values are Mean±SEM, n=6.* P<0.05 when compared with normal group, α P<0.01 when compared with normal group, β P<0.05 when compared with disease control group and γ P<0.01 when compared with disease control group, δ P<0.01 when compared with Silymarin treated group.

CCl4 per se treated animals showed significant elevation of serum biochemical parameters, such as SGPT, SGOT, ALP and total bilirubin. The liver weight was increased compared to normal control group, and the pathological lesion of the liver was evident. Pretreatment with silymarin (100mg/kg, p.o.) and PHF at 100 and 200 mg/kg, p.o. for 7days, had produced significant protective effect on CCl4-induced hepatic damage by maintaining the morphological changes and normalizing the elevation of serum biochemical parameters (SGPT, SGOT, ALP and total bilirubin), and therefore inhibited the histopathological abnormalities caused by CCl4. PHF showed dose dependent protection against CCl4 induced hepatic damage (Table 1 & Figure 2).

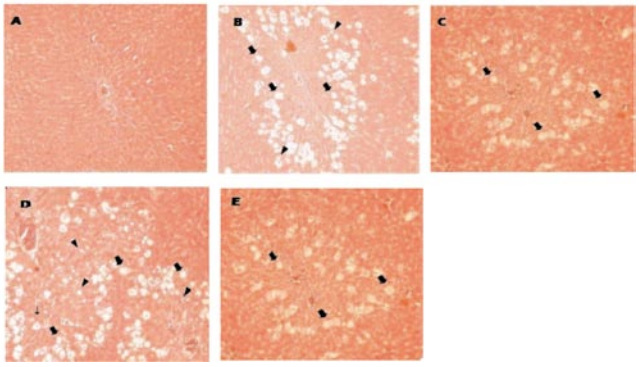

Fig. 2. Effect of PHF on CCl4-induced hepatotoxicity in rats (histopathology)

A: Liver section of normal rat (group-1);

B: Liver section of CCl4-treated rat (group-2);

C: Liver section of rats treated with CCl4 and 100mg/kg of Silymarin (group-3);

D: Liver section of rats treated with CCl4 and 100mg/kg of PHF (group-4);

E: Liver section of rats treated with CCl4 and 200mg/kg of PHF (group-5).

3.3. Effect of PHF against paracetamol-induced hepatotoxicity in rats

Administration of the paracetamol per se at a dose of 2 g/kg, p.o. showed cetrilobular necrosis in histopathological studies in animal and its association with elevation of serum biomarkers for liver function, such as SGPT, SGOT, ALP and total bilirubin. The liver weight was also increased. Pretreatment with PHF at 100 mg/kg and 200mg/kg, p.o. for 7days offered significant protection against paracetamol-induced hepatic damage by inhibiting the morphological changes and maintaining the serum biochemical parameters (SGPT, SGOT, ALP and total bilirubin). Histopathological analysis demonstrated that the pathological lesion caused by paracetamol were very minimal in PHF pretreated groups. PHF showed dose dependent protection against paracetamol -induced hepatic damage and the protective effect of PHF -200 mg/kg, p.o. was comparable with silymarin-100 mg/kg, p.o. (Table 2 & Figure 3).

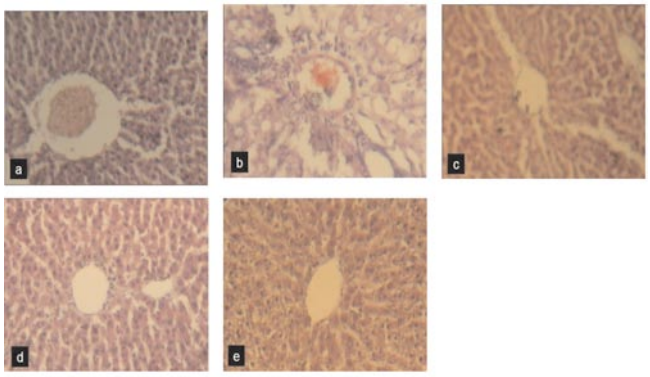

Fig. 3. Effect of PHF on paracetamol-induced hepatotoxicity in rats (histopathology).

a: Liver section of normal rat (group-1);

b: Liver section of paracetamol-treated rat (group-2);

c: Liver section of rats treated with paracetamol and 100mg/kg of Silymarin (group-3);

d: Liver section of rats treated with paracetamol and 100mg/kg of PHF (group-4);

e: Liver section of rats treated with paracetamol and 200mg/kg of PHF (group-5).

4. Discussion

Liver plays an important role in the metabolism of drug and nutrients. Because of its central role in drug metabolism, it is the most vulnerable tissue for drug toxicity. According to the reports published by USFDA, more than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for 20-40% of all instances of hepatic failure[19].

Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. About 75%-80% of blood coming to the liver arrives directly from gastrointestinal organs and then spleen via portal veins which brings drugs and xenobiotic in concentrated form[20]. Many chemicals damage mitochondria, an intracellular organelle that produces energy, its dysfunction release excessive amount of oxidants which in turn damage hepatic cells. Activation of some enzymes in the cytochrome P-450 system, such as CYP2E1, also leads to oxidative stress[21]. Injury to hepatocyte and bile duct cells leads to accumulation of the bile acid inside the liver, which promotes further liver damage. Non-parenchymal cells, fat storing stellate cells and leukocytes (i.e. neutrophil and monocyte) also have roles in the mechanism. In present study the hepatoprotective effect of PHF, was evaluated against CCl4 and paracetamol induced hepatotoxicity models in rats.

In CCl4-induced hepatotoxicity model, upon administration of CCl4 to animals, it undergoes enzymatic activation, majorly by CYP2E1, into the trichloromethyl free radical ($CCl_3^{\cdot}$) within the membrane of the endoplasmic reticulum. This is followed by chloromethylation, saturation, peroxidation and progressive destruction of the unsaturated fatty acid of the endoplasmic reticulum membrane phospholipids. These processes are known as lipid peroxidation, leading to functional and structural disruption

of hepatocytes[22].

During hepatic damage, cellular enzyme like SGPT, SGOT, ALP, bilirubin (Direct & Total) will leak into the serum resulting in the elevation of their serum concentration, the increase of the liver weight and volume. Histopathology of the damaged liver showed histological changes, such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis was observed[23].

Pretreatment with silymarin (100mg/kg, p.o.), PHF (100 and 200 mg/kg, p.o.) for 7 days offered significant protection against the CCl4-induced hepatic damage. Both the doses of PHF prevented the histological changes caused by CCl4, which further confirmed its hepatoprotective activity against CCl4-induced hepatic damage. The possible mechanism of action may be associated with inhibition of CYP2E1 activity or scavenging of free radicals responsible for CCl4 toxicity. Paracetamol induced hepatotoxicity is one of the well-known and commonly used animal model for studying the hepatoprotective property of drugs.

Administration of paracetamol at a dose of 1-3 gm/kg/day, p.o. results in hepatic damage. The toxic metabolite N-acetyl-p-benzoquineimine is an oxidative product of paracetamol formed by the action of cytochrome P-450 and it reacts with reduced glutathione (GSH) to yield non-toxic-3-GS-yl-paracetamol. Depletion of GSH causes the remaining quinone to undergo covalent bonding with cellular macromolecules (sulphydryl groups of protein) and leads to cell death. Histopathology of the liver shows necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis, eosinophilic cytoplasm and large excessive hepatic lesions[24].

Pretreatment with sylimarin (100 mg/kg, p.o.) and PHF (100 & 200 mg/kg, p.o.) for 7 days had significant protection against the paracetamol induced hepatic damage. The possible mechanism of action behind the hepatoprotective activity of PHF in this model may be associated with the antioxidant property.

In conclusion, our findings clearly state that PHF extract, at 100mg/kg and 200mg/kg dose level offers significant dose dependent protection against experimentally induced hepatotoxic models. The resulting hepatoprotective activity of PHF could be attributed to phytochemicals like Cucurbitacin B and Scoparic acid A.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The work was carried out with research facilities provided by Gayatri College of Pharmacy, Sambalpur and project was funded with some amount by Neelkant herbal Formulation, is gratefully acknowledged (File number: NHP/RG-0141/2011).

References

[1] Wolf PL. Biochemical diagnosis of liver diseases. *Indian J Clin Biochem* 1999; **14**: 59-90.

[2] Shanmugasundaram P, Venkataraman S. Hepatoprotective and antioxidant effects of Hygrophila auriculata (K. Schum) Heine Acanthaceae root extract. *J. Ethnopharmacol* 2006; **104**: 124-128.

[3] Nazeema TH, Brindha V. Antihepatotoxic and antioxidant defense potential of Mimisa pudica. *Inter J Drug Discovery* 2009; **1**: 1-4.

[4] Jamal AK, Yaacob WA, Laily BD. A chemical study on Phyllanthus Columnaris. *Eur J Scientific Research* 2009; **28**: 76-81.

[5] Flora K, Hahn M, Rosen H, Benner K. Milk thistle (Silybum marianum) for the therapy of liver disease. *Am J Gastroenterol* 1998; **93**: 139-43.

[6] Ravikumar V, Shivashangari KS, Devaki T. Hepatoprotective activity of Tridax procumbens against D-galactosamine/lipopolysaccharide-induced hepatitis in rats. *J Ethnopharmacol* 2006; **101**: 55-60.

[7] Pramyothin P, Udomuksorn W, Poungshompoo S, Chaichantipyuth C. Hepatoprotective effect of Andrographis paniculata and its constituent andrographolide on ethanol hepatotoxicity in rats. *Asia Pac J Pharmacol* 1994; **9**: 73-8.

[8] Eastwood MA. Interaction of dietary antioxidant in vivo: how fruits and vegetables prevent diseases. *QJM* 1999; **92**: 527-30.

[9] Praveen TK, Dharamaraj S, Bajaj Jitendra, Dhanabal SP, Manimaran S, Nanjan MJ, Razdan Rema. Hepatoprotective activity of petroleum ether, diethyl ether, and methanol extract of *Scoparia dulcis* L. against CCl4- induced acute liver injury in mice. *Indian Journal of Pharmacology*, 2009; **41**: 110-114.

[10]Rao GMM, Vijayakumar M, Rao ChV, Rawat AKS, Mehrotra S. Hepatoprotective effect of *Coccinia indica* against CCl4 induced hepatotoxicity. *Natural Product Sci*, 2003; **9**(1): 13-17.

[11]Mohamed V, Mohammed ABS. Hepatoprotective studies of *Coccinia indica* linn in CCl4 induced hepatic injury. *Eth pharm j.* 2005; **23**: 39-44.

[12]Gnanasekaran D et al., In vitro Hepatoprotective activity against CCl4 induced toxicity of some selected siddha medicinal plants. *American Journal of Pharm Tech Research* 2012: **2**(1): 466-473.

[13]Khandelwal KR. Practical Pharmacognosy: techniques and experiments. 9th ed. New Delhi: Nirali prakashan; 2008, p.149-156.

[14]Wagner H, Baldt S, Zgainski EM. Plant drug analysis. Berlin: Springer; 1996. p. 54, 35,118.

[15]Tiwari V, Tiwari A, Madhavan V. Preliminary phytochemical analysis, HPTLC studies and antipyretic activity of alcohol and aqueous extract of helicteres isora L. root. *Int J Pharm Pharm Sci* 2010; **2**(2): 74-79.

[16]Ghosh MN. Fundamentals of experimental Pharmacology. 3 rd ed. Kolkata: Hilton and Company; 2005. p. 190-7.

[17]Devaraj VC, Gopala KB, Viswanatha GL, Jagadish KV, Kumar S. Hepatoprotective activity of Hepax-A polyherbal formulation. *Asian Pac J Trop Biomed* 2011;142-146.

[18]Chanchal KR, Jagadish VK, Mohammed A. Hepatoprotective activity of Psidium guajava Linn. Leaf extract. *Ind J Exp Biol* 2006; **44**: 305-311.

[19]Soni M, Mohanty PK, Jaliwala YA. Hepatoprotective activity of fruits of Prunus domestica. *Int J Pharm Biosci* 2011; **2**(2): 439-453.

[20]Vikramjit M, Mrtcalf J. Functional anatomy and blood supply of the liver. *Anaesthesia & Intensive Care Medicine* 2009; **10**: 332-333.

[21]Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. *Toxicol Sci* 2002; **65**: 166-176.

[22]Tatiya AU, Surana SJ, Sutar MP, Gamit NH. Hepatoprotective effect of poly herbal formulation against various hepatotoxic agents in rats. *Phacog Res* 2012; **4**:50-56.

[23]Rajib Am, Monirul IK, Jahan BI, Ashik MM, Ekramul H. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *Eur J Scientific Res* 2009; **37**:302-310.

[24]Maiti K, Mukherjee K, Murugan V, Saha BP, Mukherjee PK. Enhancing bioavailability and hepatoprotective activity of andrographolide from Andrographis paniculata, a well-known medicinal food, through its herbosome. *J Sci Food Agric* 2010; **90**: 43-51.