International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 3, 2011

Research Article

EVALUATION OF HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *FLEMINGIA STROBILIFERA R.Br.* AGAINST EXPERIMENTALLY INDUCED LIVER INJURY IN RATS

K.V. ANIL KUMAR*[1], VEERE GOWDA K[2]

Asst. Professor, Department of Pharmacology, Visveswarapura Institute of Pharmaceutical Sciences, BSK II stage, Bangalore-70. India, Lecturer, Visveswarapura Institute of Pharmaceutical Sciences, BSK II stage, Bangalore-70. India Email: anilkumargcp@rediffmail.com

Received: 19 Feb 2011, Revised and Accepted: 21 March 2011

ABSTRACT

The present study was carried out to evaluate the hepatoprotective and antioxidant effect of the Chloroform extract of *Flemingia Strobilifera* R.Br. leaf (CEFS) in wistar albino rats. Antioxidant was studied using 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) assay. Protective action of CEFS leaf extract was evaluated using animal model of hepatotoxicity induced by ethanol-Carbon tetrachloride (CCl_4). Liver marker enzymes were assayed in serum and antioxidant status was assessed in liver tissue. Histopathology was also studied. CEFS leaf did not demonstrated in vitro scavenging of DPPH radicals. Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and total bilirubin were increased and the levels of total protein were decreased in ethanol-CCl_4 treated rats. CEFS leaf at both the doses did not decreased the elevated levels of all these biochemical parameters and did not restored the normalcy of total protein significantly. Lipid peroxidation (LPO) was increased significant in liver tissue in the ethanol-CCl_4 treated rats while the activities of reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were decreased. CEFS leaf at both the doses did not decreased the elevated levels of lipid peroxide and did not restored the normalcy of GSH, CAT and SOD. Histopathology's also showing similar results. From this study it can be concluded that the CEFS leaf did not showed significant hepatoprotective and antioxidant action.

Keywords: CEFS leaf Flavonoid ethanol CCl_4 Hepatoprotective Antioxidant.

INTRODUCTION

Liver is the key organ for detoxication and disposition of endogenous substances. It is continuously and widely exposed to xenobiotics, hepatotoxins, and chemotherapeutic agents that lead to impairment of its functions[1]. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages[2]. Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality. Hepatotoxicity in most cases is due to free radical. Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism[3]. Reactive oxygen species mediated oxidative damage to macromolecules such as lipids, proteins and DNA has been implicated in the pathogenecity of major diseases like cancer, rheumatoid arthritis, degeneration process of aging and cardiovascular disease etc. Antioxidants have been reported to prevent oxidative damage caused by free radicals by interfering with the oxidation process through radical scavenging and chelating metal ions[4].

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effect. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity[5].

Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic. etc[6]. *F. strobilifera* R.Br, an important medicinal plant, is commonly known as Kusrunt found in Sind, Rajputana, Bengal, South India and Andaman's[7]. Previous chemical studies showed that flavonoids, flavonoid glycosides, chalkones, epoxychromenes and pterocarpans were the main constituents found in this genus of Flemingia Strobilifera R.Br[8, 9].

MATERIAL AND METHODS

Collection of plant material

The leafs of the plant *Flemingia Strobilifera R.Br.* belonging to family Fabaceae were collected from the Western Ghats of Maharashtra in the month of July 2009. The plant was authenticated by Dr. Jawahar Raveendran, Conservative Research & Action group, FRLHT, and preserved a specimen sample of the same in the herbarium section of the FRLHT, Bangalore, with the voucher No. 100154 for future reference.

Preparation of plant extracts

The collected leaves was shade dried and powdered then macerated with chloroform for 7 days the extract so obtained was filtered and the extract so obtained was filtered. The procedure was again repeated five times using adequate amount of chloroform at an interval of 3 days. The filtrate was evaporated to dryness to get residue. Then the residue was transferred to a china dish and evaporated on thermostat controlled water bath at 40ºC and stored in a refrigerator until further use. The amount of extract collected was 40 gm w/w from the dried powdered leaves of *Flemingia Strobilifera R.Br.*

Animals

Female Albino wistar rats (150-200g) used for the study were obtained from Drug control laboratory Bangalore, Karnataka. After one week of acclimatization the animals were used for experiments. The protocol for the study was approved by institutional animal Ethics committee (Reg. no.152/1999/CPCSEA) as per the Indian CPCSEA guideline.

Drugs and chemicals

Silymarin was gifted from Micro labs, Bangalore, India. SGOT, SGPT, ALP, total Bilirubin and Total Protein kits were procured from Prism Diagnostics Pvt Ltd., Mumbai, India. Thiobarbituric acid (TBA) were purchased from Spectrum (P) Ltd, Mumbai, Nitro blue tetrazolium chloride (NBT) and Phenazine methosulphate were purchased from SD fine chem. Ltd. Mumbai, India and 5,5'- dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Himedia laboratories (P) Ltd, Mumbai, India and the rest of the chemicals utilized were of analytical grade.

Phytochemical evaluation

CEFS were subjected to qualitative analysis for various phytoconstituent like alkaloids, glycosides, saponins, phytosterols, phenolic compound, tannins, proteins and amino acids.

Acute toxicity studies

The acute toxicity was determined on virgin female albino wistar rats by fixed dose method of OECD Guide line no 420 given by CPCSEA. Groups of 6 rats were administered test drug by oral route in the range of 2000-300 mg/kg and mortality was observed after 24 hr. The safe dose was found to be 300 mg/kg body weight. For the study two doses were selected, 30 mg/kg body weight and 60 mg/kg body weight ($1/10^{th}$, $1/5^{th}$ of the maximum safe dose).

Assessment of in-vitro antioxidant activity

DPPH assay was evaluated using the method[10]. In brief, 200µl of methanol / vehicle buffer / positive control/various concentration of CEFS leaf solution and 50µl of DPPH (0.659mM) solution were incubated at 25°C for 20minutes. Following incubation the absorbance was read at 510 nm. A control reaction was carried out without the test sample. The % inhibitions were calculated as follows.

Percentage inhibition = (1– absorbance of test/absorbance of control) × 100

Assessment of hepatoprotective activity

Female Albino wistar rats weighing 150– 200 g were maintained in animal house and they were divided in to 5 groups of 6 animals in each. The weight range of the animals was equally distributed throughout the groups. They were acclimatized to housing conditions for at least one week prior to use. The animals of group I were treated with vehicle for 21 days and animals in group II to group V were treated 40%v/v ethanol (2ml/100g body wt. p.o) for 21 days. On 20th day they were injected with CCl_4 (1:1 in groundnut oil, 0.1 ml/kg body wt. s.c.)[11]. In addition to this, the following treatments were given to the animals of group III, IV and V from day 15^{th} to 21^{st} day. Group III were treated with Silymarin 100 mg/kg Group IV and V were treated with CEFS leaf 30 mg/kg and 60 mg/kg respectively**.** The blood was obtained from all animals by puncturing retro-orbital plexus.

The blood sample was allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30^0C for 15 min and utilized for the estimation of various biochemical parameters namely SGOT[12], SGPT [12], ALP[13], total bilirubin[14] and total protein[15]. After collection of blood samples the rats in different groups were sacrificed and their livers were excised immediately and washed in ice cold normal saline, followed by 0.15 M Tris-Hcl (pH 7.4) blotted dry and weighed. A 10%w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation[16]. A part of homogenate after precipitating proteins with Trichloroacetic acid (TCA) was used for estimation of glutathione[17]. The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 40^0C. The supernatant thus obtained was used for estimation of SOD[18] and CAT activities[19].

Histopathological examination

Liver pieces were preserved in 10% formaldehyde solution. The pieces of liver processed and embedded in paraffin wax. Sections of about 4-6 microns were made and stained with hematoxylin and eosin and photographed.

Analysis of results

The results were expressed as mean ± SEM and were analyzed for statistically significant difference using one-way ANOVA followed by Tukey's Kramer post hock test. P values < 0.05 were considered significant.

RESULTS

Preliminary phytochemical investigation

The Preliminary phytochemical investigation of the CEFS leaf showed moderate presence of flavanoids.

In-vitro antioxidant activity

The CEFS leaves did not showed antioxidant activity in DPPH free radical scavenging model. Only 19.33 inhibitions were noted with 200 µg/ml of CEFS leaf. Thus IC_{50} value could not be determined for DPPH radical inhibition assay. (Table 1)

Serum biochemical parameters

The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), total bilirubin were significantly ($P<0.001$) increased and the levels of total protein were significantly ($P<0.001$) decreased in ethanol-CCl_4 treated rats when compared to control group (Table 2). Administering CEFS leaf (30 and 60mg /kg) did not reduced the elevated levels of AST, ALT ALP and total bilirubin levels as well as not restore the levels of total protein towards normalcy when compared to ethanol-CCl_4 treated rats.

Hepatic Oxidative Stress parameters

Malondialdehyde (MDA) level was significantly ($P<0.001$) increased and the levels of GSH, CAT and SOD were significantly ($P<0.001$) decreased in ethanol-CCl_4 treated rats when compared to control group. Administering CEFS leaf (30 and 60 mg/kg) did not decreased the elevated levels of malondialdehyde (MDA) content as well as not rises the antioxidant levels. (Table 3).

Histopathology

Histopathological studies also provided supportive evidence for the biochemical analysis. The CEFS treated group showed the fatty change and lymphocytic infiltrate around portal triad compared to group II (Fig. 1). Fatty change, structure of focal necrosis with portal traiditis and congestion were observed in group treated with ethanol-CCL_4. Treatment with silymarin exhibited almost normal architecture (Fig. 1).

Table 1: DPPH free radical scavenging activity

Treatment	Concentration tested (µg/ml)	% inhibition	IC_{50} (µg/ml)
Gallic acid	0.5	12.97	
	1.5	39.77	
	2.5	55.35	1.73 µg/ml
	5.0	95.21	
CEFS leaf	12.5	4.45	*
	25	7.17	
	50	7.80	
	100	9.26	
	200	19.33	

IC 50 values were calculated by log probit analysis. *Activity was not observed in the concentration range tested, hence IC_{50} not calculated.

Table 2: Effects of CEFS leaf on serum Biochemical markers

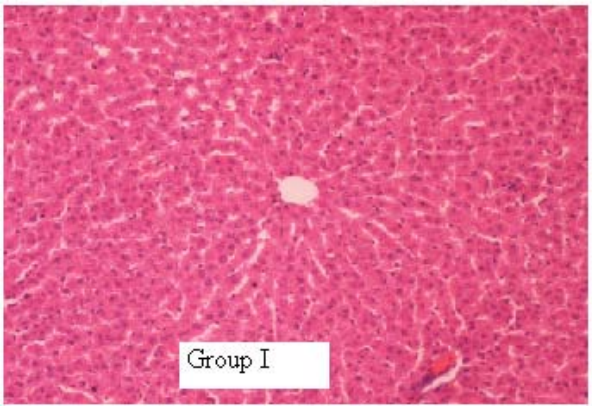
Treatments	SGOT U/L	SGPT U/L	ALP IU/L	Total Bilirubin mg/dl	Total protein g/dl
Vehicle Control	86.28 ± 3.261	37.92±1.876	202.71±8.572	0.292±0.014	6.94±0.323
Ethanol+CCl_4 (2ml/100gm +0.1 ml/kg s.c.)	295.70 ±10.785[a]	182.45±6.124[a]	308.21±14.410[a]	0.714±0.035[a]	4.91±0.245[a]
Silymarin (100 mg/kg)	116.41±5.820***	67.99±2.993***	214.19±8.723***	0.405±0.020***	6.82±0.328***
CEFS (30 mg/kg)	272.71±14.571 [ns]	170.28±7.914 [ns]	272.62±14.281 [ns]	0.657±0.035 [ns]	5.54±0.248 [ns]
CEFS (60 mg/kg)	254.24±13.262 [ns]	160.52±8.676 [ns]	260.23±13.961 [ns]	0.615±0.031 [ns]	5.90±0.25 [ns]

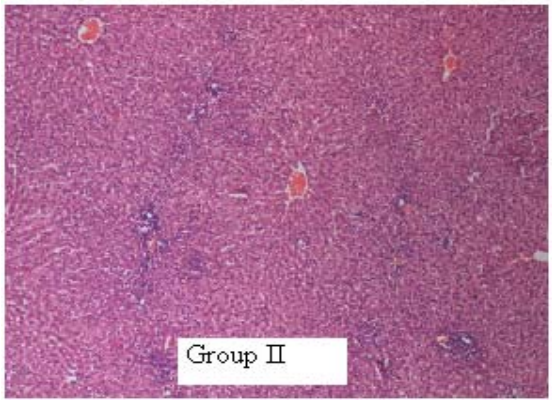
All values are expressed as MEAN ± SEM, one way analysis of variance (ANOVA) followed by Tukey's Kramer post hock analysis.[a] P<0.001 vs. vehicle control. [ns] P>0.05, ***P<0.001 vs. Ethanol+CCl_4

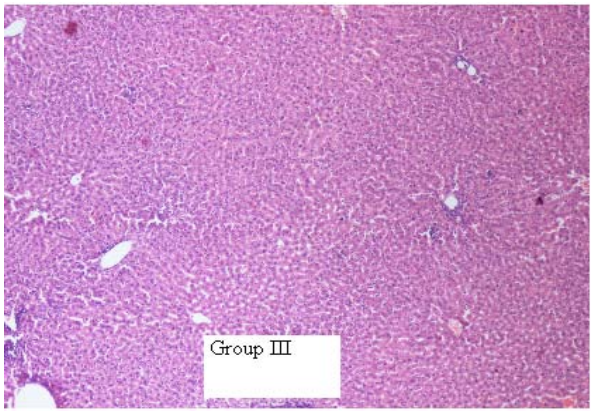
Table 3: Effects of CEFS leaf on Hepatic oxidative stress.

Treatments	MDA (nM/g)	GSH (µM/g)	CAT (µM /min/g)	SOD (unit /g)
Vehicle Control	189.47±8.472	15.22±0.661	197.42±8.872	93.71±3.685
Ethanol+CCl_4 (2.0ml/100gm +0.1 ml/kg s.c.)	435.10±19.785 [a]	7.31±0.365 [a]	119.12±4.956 [a]	36.70±1.735 [a]
Silymarin (100 mg/kg)	233.60±10.68***	13.51±0.675***	182.07±8.843***	84.52±3.226***
CEFS (30 mg/kg)	390.21±18.56 [ns]	8.52±0.371 [ns]	135.21±5.010 [ns]	41.29±1.814 [ns]
CEFS (60 mg/kg)	372.21±17.261 [ns]	9.25±0.379 [ns]	145.72±6.386 [ns]	45.18±2.109 [ns]

All values are expressed as MEAN ± SEM, one way analysis of variance (ANOVA) followed by Tukey's Kramer post hock analysis.[a] P<0.001 Vs. vehicle control. [ns] P>0.05 , ***P<0.001 Vs. Ethanol+CCl_4







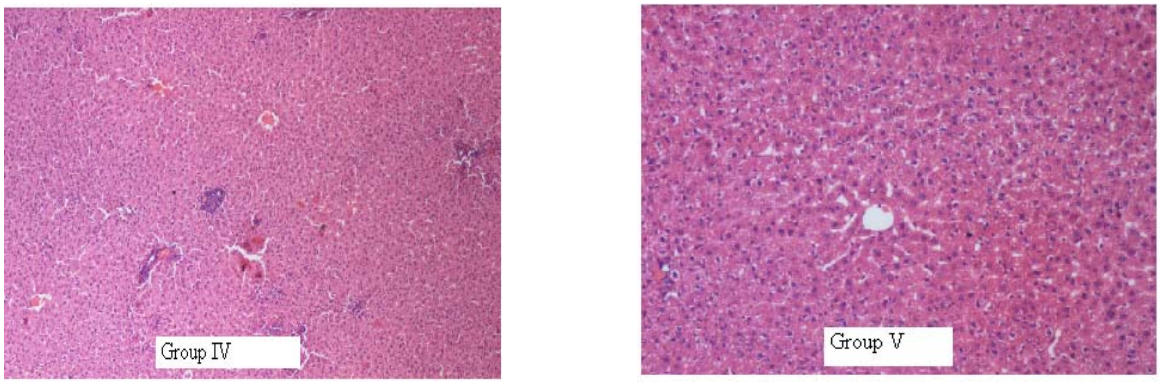


Fig.1: Effect of CEFS on Histopathological changes in rat liver after 21days treatment of Ethanol-Carbon tetrachloride

Group I -Vehicle Control, Group II -Ethanol-CCL_4 (2.0ml/100gm + 0.1 ml/kg s.c.), Group- III Silymarin -100 mg/kg, Group IV -CEFS 30 mg/kg, Group V -CEFS 60 mg/kg.

DISCUSSION

Alcohol administration in moderate doses *per se* is not known to be markedly hepatotoxic beyond causing the production of fatty liver[20]. However, it is well known to potentiate the hepatotoxicity of various xenobiotics in alcoholics and the information about interaction between alcohol and the hepatotoxins is well documented[21]. The potentiation of CCl_4 & its hepatotoxicity is supposed to be produced by enhanced production of active metabolites by the ethanol activated mixed function oxidase system[22]. This eventually leads to hepatocellular necrosis and is reflected in our experiment by marked changes in various enzymatic and non-enzymatic parameters of alcohol-$CC1_4$, treated rats. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. Hepatocellular necrosis or membrane damage leads to very high levels of serum SGOT and SGPT released from liver to circulation. Among the two, GPT is a better index of liver injury, since SGPT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner, thus liver GPT represents 90% of total enzyme present in the body[23].

The elevated levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver[24]. ALP activities on the other hand are related to functioning of hepatocytes, its increase in serum is due to increased synthesis in the presence of increased biliary pressure[25].

In the present study, Treatment with CEFS leaf (30 and 60mg/kg p.o,) did not suppressed the elevated serum levels of SGOT, SGPT towards the respective normal value this clearly indicates that the plant extract has not stabilizes the plasma membrane as well as not helped in healing of the hepatic tissue damage.

Serum ALP and total bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis, in presence of increasing biliary pressure[26]. The CEFS leaf (30 and 60mg/kg p.o,) did not able to improve the secretory mechanism of hepatic cells and did not reduces the elevated levels of ALP and total bilirubin The site specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis[25]. Hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases. Hence decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the production of liver cells[27]. The lowered level of total proteins recorded in the serum as well as liver of ethanol-CCl_4 treated rats reveals the severity of hepatopathy. CEFS leaves has failed to restore the normalcy of total protein level.

Lipid peroxidation has been postulated to be the destructive process in liver injury due to ethanol-CCl_4 administration[28]. The increase in MDA levels content suggest enhancement of lipid peroxidation which is leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with CEFS leaves did not reduced the levels of lipid peroxidation.

Glutathione (GSH) is one of the most abundant naturally occurring tripeptide, non-enzymatic biological antioxidant present in liver[29]. Its functions are concerned with the removal of free radicals such as H_2O_2 and superoxide radicals, maintenance of membrane protein, detoxification of foreign chemicals and biotransformation of drugs[30]. In the present study, the decreased level of GSH has been associated with an enhanced level of lipid peroxidation in ethanol-CCl_4 intoxicated groups of rats. Treatment with CEFS leaf has not increased the level of glutathione. Serum activities of superoxide dismutase (SOD) and catalase (CAT) are the most sensitive enzymatic index in liver injury caused by ROS and oxidative stress. SOD is one of the most abundant intracellular antioxidant enzymes present in all aerobic cells and it has an antitoxic effect against ROS[31]. CAT is a haemoprotein; it protects the cells from the accumulation of H_2O_2 by dismutating it to form H_2O and O_2 [32]. Therefore reduction in the activities of these enzymes may indicate the toxic effects of ROS produced by toxicants. In the present study, it was observed that CEFS leaves did not cause a significant rise in hepatic SOD and CAT activities. This suggests that CEFS leaf has not diminished the ROS generated that may lessen the oxidative damage to the hepatocytes and which may improve the activities of the liver antioxidant enzymes.

Preliminary phytochemical screening of the CEFS leaf revealed the moderate presence of flavonoids. In the present state of knowledge of the chemical constituents of CEFS leaf, it is not attributing for its hepatoprotective and antioxidant effect since the flavonoids is found moderately in leaves as shown by phytochemical investigations. CEFS leaf was unable to block the bioactivation of ethanol-$CC1_4$ by cytochrome P_{450} enzyme and did not inhibit the lipid peroxidation and also not prevented the formation of free radical or scavenges the free radicals.

It can be concluded that the data obtained in the present study suggest that the CEFS leaf do not have significant hepatoprotective and antioxidant activities on ethanol-CCl_4 induced hepatic damage in rats.

ACKNOWLEDGEMENT

The authors are thankful to the Dr. Vijay Balaji and Mrs. Padmaja, Natural Remedies Pvt. Ltd., Bangalore, for their valuable support and help in carrying out in vitro antioxidant activity.

REFERENCES

1. Preussmann R. Hepatocarcinogens as potential risk for human liver cancer. In: Remmer H, Bolt HM, Bannasch P, editors. Primary liver tumors. Lancaster: MTP Press; 1978. P. 11-29.
2. Chattopadhyay RR. Possible mechanism of hepatoprotective activity of *Azadirachta* indica leaf extract. J Ethnopharmacol 2003; 89: 217- 219.
3. Tiwari A Curr Sci 2001; 81: 1179-1187.
4. Buyukokuroglu ME, Gulcin I, Okaty M, Kufrevioglu OI. Pharmacol Res 2001; 44: 491-495.
5. Chatterjee TK. Medicinal plants with hepatoprotective properties. In: Herbal Options. 3rd ed. Calcutta Books & Allied (P) Ltd; 2000. P. 135.
6. Miller AL. Antioxidant flavonoids: structure, function and clinical usage. Alt. Med. Rev. 1996; 1: 103-111.
7. Duthic JS. Flora of upper gangetic plain. Bishen singh Mohendra pal singh new connerght place. Dheradoon; 1994. P. 91-92.
8. Madan S, Singh GN, Kumar Y, Kohli K, Singh RM, Mir SR *et al*. A New Flavanone from *Flemingia strobilifera* (Linn) R.Br. and its antimicrobial activity. Trop J Pharm Res 2008 Mar; 7 (1): 921-927.
9. Madan S, Gyanendra NS, Kanchan K, Mohammed A, Yatendra K, Raman MS *et al*. Isoflavonoids from *Flemingia Strobilifera* (L) R.Br. roots. Acta Pol Pharm -Drug research 2009; 66(3): 297-303.
10. Vani T, Rajini M, Sarkar S, Shishoo CJ. Antioxidant properties of the Ayurvedic formulation-Triphala and its constituents. Int J Pharmacol 1997; 35(5): 313-317.
11. Tripathi SC, Patnaik GK, Dhawan BN. Hepatoprotective activity of Picroliv against Alcohol-carbon tetachloride induced damage in rat. Indian J Pharmacol 1991; 23: 143-148.
12. IFCC. Clin Chim Acta 1976; 70/2: F19.
13. Bowers GN, McCommb RB. Clin Chem 1972; 18: 97.
14. Jendrassik L, Grof P. Biochem 1938; 2: 297: 81.
15. Strickland RD, Freeman M, Gurule ET. Anal Chem 1961; 33: 545.
16. Ohkawa H, Onishi N, Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
17. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959; 82: 70-77.
18. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984; 21:131-132.
19. Aebi H. Catalase. In: Bergmeyer HV, editor. Methods in enzymatic analysis. Vol 2, New york: Acadamic press; 1974. P.674-684.
20. Lieber CS, Decarli LM, Rubin E. Sequential production of fatty liver hepatitis and cirrhosis in subhuman primates fed with ethanol and adequate diet. Proc Nat Acad Sci 1973; 72: 437-441.
21. Zimmerman HJ. Effects of alcohol on other hepatotoxins. Alcohol Clin Exp Res 1986; 10: 311-315.
22. Johansson I. lagelman-Sundberg M. Carbon tetrachloride induced lipid peroxidation dependent on an ethanol inducible form of rabbit liver microsomal cytochrome P 450. FEBS Lett 1982; 183: 265-269.
23. Achliya GS, Kotgale SG, Wadodkar AK, Dorle AK, Hepatoprotective activity of *Panchgavya Gritha* in carbon tetrachloride induced hepatotoxicity in rats. Indian J Pharmacol 2003; 35: 311.
24. Drotman RB, Lawhorn GT, Serum enzymes are indicators of chemical induced liver damage. *Drug Chem Toxicol* 1978; 1: 163.
25. Moss DW, Butterworth PJ. Enzymology and medicine Pitman Medical, London; 1974; 39.
26. Willianson EM, Okpako DT, Evans FJ. Selection, preparation and pharmacological evaluation of plant material, John Wiley: England 1996; 13.
27. Awang D. Milk Thistle. Can Pharm J. 1993; 23 : 749-754.
28. Muriel P, Peroxidation of lipids and liver damage. In: Antioxidants, oxidants and free radicals. S I Baskin and H Salem editors. Washington, DC: 1997. P. 237.
29. Gul M, Kutay FZ, Temocin S, Hanninen O. Cellular and clinical implications of glutathione. Indian J Exp Biol 2000; 38: 625.
30. Comporti M, Maellaro E, Del Bello B, Casini AF. Glutathione depletion, its effect on other antioxidant systems and hepatocellular damage. Xenobiotica 1991; 21: 1067.
31. Rajmohan T, Anthony LL. Hepatoprotective and antioxidant effect of tender coconut water on carbon tetrachloride induced liver injury in rats. Indian J Biochem Biophys 2003; 40: 354.
32. Bhakta T, Pulok KM, Kakali M, Banerjee S, Subhash CM, Tapan KM, Pal M Saha B P, Evaluation of hepatoprotective activity of Cassia Fistula leaf extract. J Ethnopharmacol 1999; 66: 227.