International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.3, pp 1924-1931, July-Sept 2010

Hepatoprotective Effect of *Flemingia Strobilifera* R.Br. on Paracetamol induced Hepatotoxicity in Rats

K.V.Anil Kumar*[1], R.Satish[1], T. Rama[2], Anil kumar[1], D .Babul [1], J .Samhitha[1]

[1]Department of Pharmacology, Visveswarapura Institute of Pharmaceutical Sciences, BSK 2nd Stage, Bangalore-70, India.

[2]Department of Biotechnology, Government Science College, Nrupatunga road, Bangalore-01, India.

*Corres.author: anilkumargcp@rediffmail.com
Mobile. +91-9844419736.

Abstract: The present study was carried out to evaluate the hepatoprotective and antioxidant effect of the Chloroform extract of *Flemingia Strobilifera* R.Br. leaves (CEFS) in albino wistar rats. Antioxidant was studied using Nitric oxide scavenging assay (NO). CEFS did not exhibited potent NO scavenging activity. Protective action of CEFS extract was evaluated using animal model of hepatotoxicity induced by Paracetamol. Liver marker enzymes were assayed in serum and antioxidant status was assessed in liver tissue. Levels of marker enzymes such as alanine transminase (ALT), aspartate transaminase (AST) alkaline phosphatase (ALP), total bilirubin were increased and that of total protein was decreased significantly in Paracetamol treated rats. CEFS leaf at both the doses did not reduced 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 Paracetamol treated rats while the activities of reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were decreased. CEFS leaf treatment did not decreased the elevated levels of lipid peroxide and did not increases the activity of antioxidant enzymes. Histopathological changes of liver sample were compared with respective control. From this study it can be concluded that the CEFS is did not showed effective hepatoprotective and antioxidant action against paracetamol induced hepatic injury in rats.
Key Words: CEFS leaf, Flavonoid, Paracetamol, 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] *Flemingia 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

Plant material

The roots 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, Foundation for Revitalization of Local Health Traditions (FRLHT), and preserved a specimen sample of the same in the herbarium section of the FRLHT, Bangalore, India with the voucher No. 100154 for future reference.

Extraction

The collected leaves were shade dried, powdered and then macerated with chloroform for 7 days 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 testing 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

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

Phytochemical evaluation

Chloroform leaves extract of *Flemingia Strobilifera R.Br.* were subjected to qualitative analysis for various phytoconstituent like alkaloids, glycosides, saponins, phytosterols, phenolic compound, tannins, proteins and amino acids[10].

Acute Toxicity Studies

The acute toxicity was determined on nulliparous, non pregnant 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 of CEFS leaf 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

Nitric oxide scavenging assay is carried out as per the method of Sreejayan and Rao[11]. 4 mg of CEFS leaf was dissolved in 0.2ml of methanol and the volume was made up to 10ml in the phosphate buffer saline. The stock was diluted to the required concentration and used in the assay. 50µl of 10mM sodium nitroprusside and 50µl of demineralized water / CEFS leaf solution / Curcuminoids of various concentrations are illuminated (using fluorescence light) at room temperature (25-30°C) for 15mins. Following incubation, 125µl of Griess reagent was added and incubated for 10mins at 25°C. The color developed was measured at 546 nm. The percentage inhibition was calculated using the formula. IC_{50} values were calculated as the average of triplicate analyses.

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 care facility 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. Group– I served as normal control received 10% tween 80 (1 ml/kg) once daily for 14 days. Group– II served as Paracetamol control, administered with paracetamol (2 gm/kg) once daily for 14 days[12]. Group– III Reference control, Silymarin (100 mg/kg) once daily for once daily for 14 days. Group– IV received CEFS leaf (30mg/kg) once daily for 14 days. Group–V received CEFS leaf (60mg/kg) once daily for 14 days. 24 hours after the treatments blood was obtained from all animals by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500rpm at 30^0C for 15 min and utilized for the estimation of various biochemical parameters namely SGOT[13], SGPT [14], ALP[15], total bilirubin[16] and total protein[17]. 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[18]. A part of homogenate after precipitating proteins with Trichloroacetic acid (TCA) was used for estimation of glutathione[19]. The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 40^oC. The supernatant thus obtained was used for estimation of SOD[20] and CAT activities[21].

Histopathiological 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 value of < 0.05 was considered as statistically significant**.**

Results

Preliminary phytochemical investigation

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

In-vitro antioxidant activity

The CEFS leaf extract did not showed NO free radical scavenging activity in a concentration dependent manner. Where only 11.08 % inhibition was noted with 200 µg /ml of CEFS leaf. Thus IC_{50} value could not be determined for NO radical inhibition assay. (Table 1)

Serum biochemical parameters

The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) showed a significant ($p<0.001$) increase in Paracetamol treated rats as compared to Vehicle control group (Table 1). Administering CEFS leaf (30/60mg /kg) did not reduced the elevated levels of AST, ALT and ALP as compared to Paracetamol treated rats. When compared to Vehicle control group the total protein concentration was significantly ($p<0.001$) lower and total billirubin was significantly ($p<0.001$) increased in Paracetamol treated group (Table 1) CEFS leaf (30/60mg /kg) did not restore the levels of total protein and total billirubin towards normalcy when compared to Paracetamol 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 Paracetamol treated rats when compared to Vehicle control group. Administering CEFS leaf (30 and 60 mg/kg) did not decreased the elevated levels of malondialdehyde (MDA) content and did not elevated the levels of GSH, CAT and SOD (Table 2).

Histopathology

The CEFS leaf treated group showed the periportal inflammation and lymphocytic infiltrate around portal triad compared to group II. Patchy hemorrhagic necrosis of hepatocytes, sinusoidal dilatation and necrosis seen around central vein and also around portal triad were observed in group treated with paracetamol. The Silymarin treated showed the normal hepatic cells with portal vein (V) and portal artery. (Fig 1). The liver sections of the rats treated with CEFS leaf followed by Paracetamol intoxication did not showed a sign of protection as it was evident the presence of inflammation and infiltration.

Table 1. IC_{50} data of the CEFS leaf in Nitric oxide scavenging assay

Sample	Concentration tested (µg/ml)	% inhibition	IC_{50} (µg/ml)
Curcuminoids	10	37.262	16.88 µg/ml
	20	55.714	
	40	68.571	
	60	75.476	
CEFS leaf	3.125	0.00	-*
	6.25	0.00	
	12.5	0.00	
	25	0.00	
	50	0.00	
	100	0.00	
	200	11.084	

*Activity was not observed in the concentration range tested, hence IC_{50} not calculated.

Table 2: Effects of CEFS leaf on serum Biochemical markers in Paracetamol induced Hepatotoxicity.

Groups	SGOT (U/L)	SGPT (U/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total protein (gm/dl)
Vehicle Control	91.21±4.860	41.32±2.066	191.42±9.571	0.319±0.015	7.91±0.395
Paracetamol (2gm/kg)	282.46±13.123[a]	147.53±7.376[a]	270.21±12.960[a]	0.652±0.032[a]	5.06±0.255[a]
Silymarin (100mg/kg)	105.92±5.290***	59.90±2.995***	196.21±9.143***	0.412±0.02***	6.98±0.308***
CEFS leaf (30 mg/kg)	257.81±12.959[ns]	131.24±7.212[ns]	240.21±11.951[ns]	0.589±0.032[ns]	5.86±0.251[ns]
CEFS leaf (60 mg/kg)	245.14±12.757[ns]	126.28±7.014[ns]	224.14±12.607[ns]	0.542±0.029[ns]	6.18±0.163[ns]

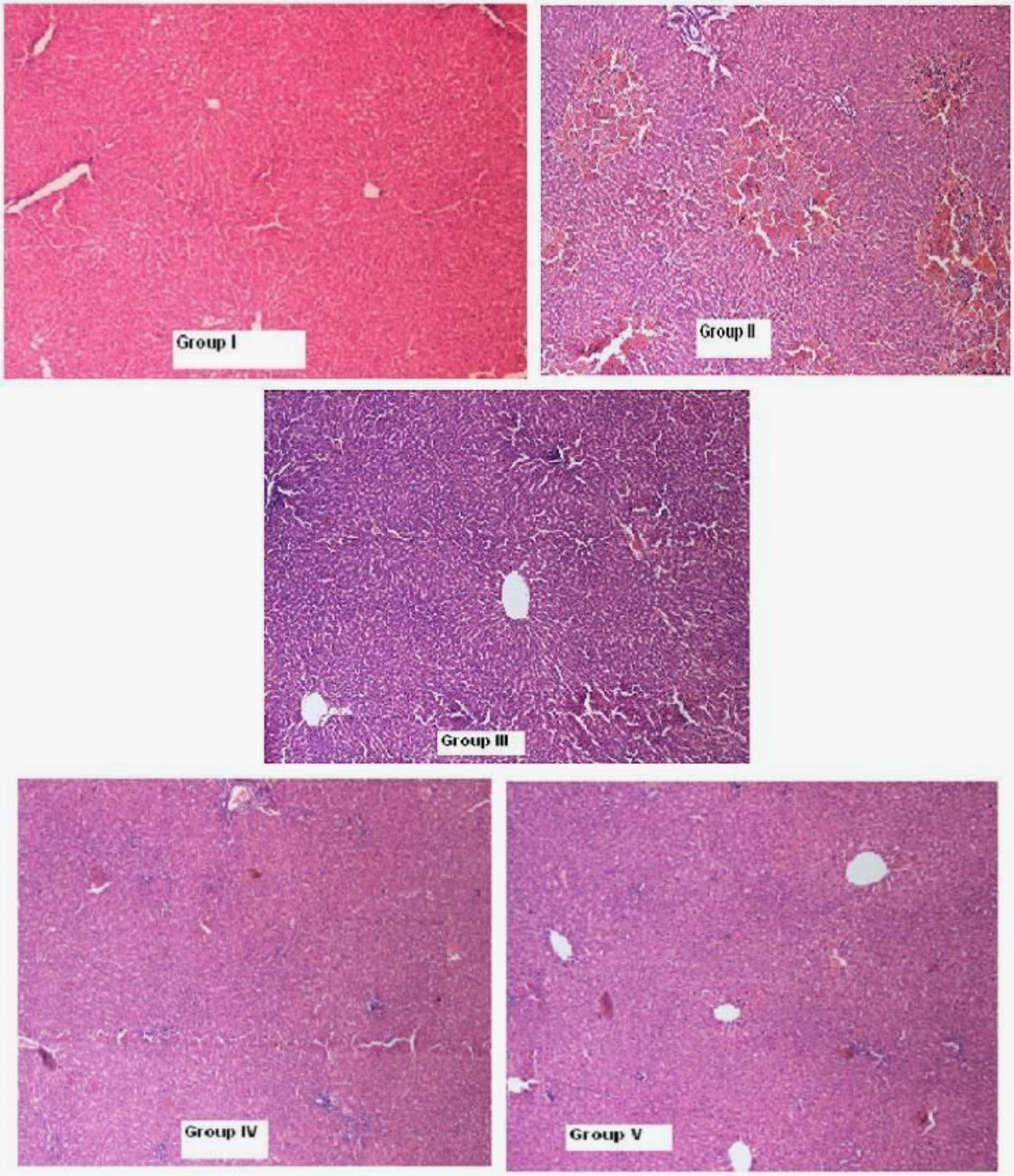
All values are expressed as MEAN ± SEM, one way analysis of variance (ANOVA) followed by Tukey's Kramer post hock analysis.[a] $p<0.001$ Vs. vehicle control. [ns] $p>0.05$, [***] $p<0.001$ Vs. Paracetamol control.

Table 3: Effects of CEFS leaf on Hepatic oxidative stress markers in Paracetamol induced Hepatotoxicity

Groups	MDA (n M/g)	GSH (µ M/g)	CAT (µM /min/g)	SOD (Unit /g)
Vehicle Control	198.62±9.831	14.12±0.704	212.24±9.612	99.45±3.972
Paracetamol (2gm/kg)	322.54±15.127[a]	7.91±0.385[a]	146.28±7.214[a]	55.64±2.782[a]
Silymarin (100mg/kg)	218.26±10.913***	12.41±0.615***	205.18±9.853***	90.21±4.510***
CEFS leaf (30 mg/kg)	290.25±11.921[ns]	8.97±0.321[ns]	155.28±6.514[ns]	61.31±2.866[ns]
CEFS leaf (60 mg/kg)	275.90±13.645[ns]	9.05±0.401[ns]	169.52±6.726[ns]	68.24±2.212[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. Paracetamol control.

Fig 1. Effect of CEFS leaf on histopathological changes in rat liver after 14 days of Paracetamol treatment.



Group I- Vehicle Control, Group II- Paracetamol, Group III- Silymarin 100 mg/kg, Group IV- CEFS 30mg/kg Group- V-CEFS 60 mg/kg.

Discussion

Paracetamol is a widely used antipyretic and analgesic, produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450, to a highly reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI). NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or –SH group of protein and alters the homeostasis of calcium after depleting GSH[22]. The compound, Sodium nitroprusside is known to decompose in aqueous solution pH (7.2) producing $NO^{\cdot}$. Under aerobic conditions, $NO^{\cdot}$ reacts with oxygen to produce stable products (nitrate and nitrite) ions. This leads to reduction of nitrite concentration in the assay media[23]. In the present study the CEFS leaf did not competes with oxygen thus not inhibits the generation of the anions. 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 GOT and GPT 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[24].

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 (30/60mg/kg, p.o,) did not reduced the 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] .In the present study indicates that CEFS leaf at both the doses not reduces the serum ALP and total bilirubin. The CEFS leaf (30/60mg/kg, p.o,) does not improve the secretory mechanism of hepatic cells. 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. The lowered level of total proteins recorded in the serum as well as liver of CCl4 treated rats reveals the severity of hepatopathy. CEFS leaf treated rats did not maintained near the normalcy of total protein level. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the production of liver cells[27]. Lipid peroxidation has been postulated to be the destructive process in liver injury due to Paracetamol administration[28]. In the present study, an elevation in the levels of MDA in liver of animals treated with Paracetamol was observed. The increase in MDA levels of liver suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals. Treatment with CEFS leaf did not significantly 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 Paracetamol intoxicated groups of rats. Treatment with CEFS leaf not increased the level of glutathione in a dose dependent manner. 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 pre-treatment with CEFS leaf did not caused a significant rise in hepatic SOD and CAT activities. This suggests that CEFS leaf cannot reduce ROS that may lessen the oxidative damage to the hepatocytes and which may improve the activities of the liver antioxidant enzymes, thus protecting the liver from Paracetamol.

Preliminary phytochemical screening of the chloroform extract of the leaves of CEFS leaf revealed moderate presence of flavonoids. In the present state of

knowledge of the chemical constituents, it is not attribute for its hepatoprotective effect since the flavonoids is found moderately in leaves as shown by phytochemical investigations. It can be concluded that the data obtained in the present study suggest that the CEFS leaf did not showed significant hepatoprotective and antioxidant activities on Paracetamol induced hepatic damage in rats. *Flemingia Strobilifera* R.Br is unable to block the bioactivation of Paracetamol by cytochrome P_{450} enzyme and did not prevented the formation free radical or scavenges the free radicals and also not inhibited the lipid peroxidation.

It can be concluded that the data obtained in the present study suggest that the CEFS leaf does not have hepatoprotective and antioxidant activities Paracetamol induced hepatic damage in rats.

References

1. Preussmann R., Hepatocarcinogens as potential risk for humanliver cancer. In: Remmer H, Bolt HM, Bannasch P, editors. Primary liver tumors. Lancaster: MTP Press; 1978. 11-29.
2. Chattopadhyay R.R., Possible mechanism of hepatoprotective activity of *Azadirachta* indica leaf extract. J of Ethanopharmacol. 2003, 89, 217-219.
3. Tiwari A., 2001 Curr sci. 81, 1179-1187.
4. Buyukokuroglu M.E., Gulcin I., Okaty M., Kufrevioglu OI., Pharmacol res 2001,44,491-495.
5. Chatterjee T.K., Medicinal plants with hepatoprotective properties. In: Herbal Options. 3rd Edn. Calcutta Books & Allied (P) Ltd. 2000, 135.
6. Miller A.L., Antioxidant flavonoids: structure, function and clinical usage. Alt. Med. Rev. 1996, 1,103-111.
7. Duthic J.S., Flora of upper gangetic plain. Bishen sing Mohendra pal singh new connerght place. Dheradoon.1994.
8. Madan S., Singh G.N., Kumar Y., Kohli K., Singh R.M., Mir S.R., Ahmad S., 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 N.S., Kanchan K., Mohammed A., Yatendra K., Raman M.S., Prakash O., Isoflavonoids from *Flemingia Strobilifera* (L) R.Br. roots. Acta Polon Pharma-Drug research. 2009, 66(3), 297-303.
10. Kokate C.K., Purohit A.P., Gokhale S.B., Text book of pharmacognosy.4th ed. Pune: Nirali Prakashan; 1996, 510-511.
11. Shreejayan., Rao M.N.A., Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol. 1997, 49, 105-107.
12. Ayodele O., Kolawole., Mary T., Olaleye., joshua O., Ajele., Antioxidant Properties and Glutathione S Transferases Inhibitory Activity of *Alchornea cordifolia* Leaf Extract in Acetaminophen-Induced Liver Injury. Iranian J Pharmacol & Therapeut. 2007, 6, 63-66.
13. IFCC. Clin Chim Acta. 1976, 70/2, F19.
14. Bowers G.N., McCommb R.B., Clin. CHEM. 1972, 18, 97.
15. Jendrassik L., Grof P., Biochem. 1938, 2, 297, 81.
16. Strickland R.D., Freeman M., Gurule E.T., Anal Chem. 1961, 33,545.
17. Ohkawa H., Onishi N., Yagi K., Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem. 1979, 95, 351-358.
18. Ellman G.L., Tissue sulphydryl groups. Arch Biochem Biophys 1959, 82, 70-77.
19. Kakkar P., Das B., Viswanathan P.N., A modified spectropho-tometric assay of superoxide dismutase. Ind J Biochem Biophys, 1984, 21,131-132.
20. Aebi H., Catalase. In: Bergmeyer HV, editor. Methods in enzy-matic analysis. Vol 2, New york: Acadamic press; 1974, 674-684.
21. Murugesh K.S., Yeligar V.C., Maiti B.C., Maity T.K., Hepatoprotective and antioxidant role of *Berberis tinctoria* Lesch leaves on paracetamol induced hepatic damage in rats. Iranian J Pharmacol Therapeut. 2005, 4, 64-69.
22. Jayprakash G.K., Singh R.P., Sakariah K.K,, Antioxidant activity of grape seed extracts on peroxidation models *in vitro*. J Agric Food Chem. 2001,55,1018.
23. Achliya G.S., Kotgale S.G., Wadodkar A.K., Dorle A.K., Hepatoprotective activity of *Panchgavya Gritha* in carbon tetrachloride induced hepatotoxicity in rats. Indian J Pharmacol. 2003, 35, 311.
24. Drotman R.B., Lawhorn G.T., Serum enzymes are indicators of chemical induced liver damage.Drug Chem Toxicol.1978, 1, 163.
25. Moss D.W., Butterworth P.J., Enzymology and medicine (Pitman Medical, London) 1974, 39.

26. Willianson E.M., Okpako D.T., Evans F.J., 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 *A*ntioxidants, oxidants and free radicals, S I Baskin and H Salem edtitors.1997, 237.
29. Gul M., Kutay F.Z., 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 A.F., Glutathione depletion, its effect on other antioxidant systems and hepatocellular damage, Xenobiotica. 1991, 21, 1067.
31. Rajmohan T., Anthony L.L., 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 K.M., Kakali M., Banerjee S., Subhash C.M., Tapan KM., Pal M., Saha B.P., Evaluation of hepatoprotective activity of *Cassia fistula* leaf extract. J Ethnopharmacol. 1999, 66, 227.

*****