International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
February - March, 2013, Vol. 2, No.2, pp 321-329
ISSN: 2278-0238

Research Article

ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF AN ETHANOL EXTRACT OF PIPER CUBEBA FRUITS

Amol P. Pachpute[1]*, Tushar A. Deshmukh[2]

1. Department of Pharmacology, Shri Jagdishprasad Jhabarmal Tibrewal University, Jhunjhunu, Rajasthan - 333001, India.
2. College of Pharmacy, Faizpur, Jalgaon, Maharashtra - 425503, India

***Corresponding Author: Email**: amolpachpute@gmail.com

(Received: November 05, 2012; **Accepted:** January 03, 2013**)**

ABSTRACT

The fruits of the *Piper cubeba* plant were chosen and studied for antioxidant and hepatoprotective activity. The antioxidant potential of the ethanol extract was examined using a 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, reducing power, hydroxyl radical scavenging activity, nitric oxide radical scavenging activity and hydrogen peroxide radical scavenging activity. The extract had significant dose-dependent antioxidant activity in all in vitro experiments. Hepatoprotective activity of the extract was evaluated in rat model of carbon tetrachloride (CCl_4) induced liver damage. CCl_4 significantly altered serum marker enzymes and total protein. The ethanol extract of *Piper cubeba* attenuated CCl_4 induced serum marker enzymes and total protein. Histology of liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration which further evidence the hepatoprotective activity.

Keywords: *Piper cubeba*, CCL4, antioxidant, hepatoprotective activity.

INTRODUCTION

Liver is the major site of xenobiotic metabolism in any vertebrate living system. Therefore, injury to liver caused by toxic chemicals, drugs and virus infiltration from ingestion or infection may be harmful and can lead to various complications (Ghosh, 1996). Many people die every year due to liver anomalies. Among them, most common are cirrhosis, cholestasis, hepatitis, portal hypertention, hepatic encephalopathy, hepatic failure and certain tumors like hepatoma. As one of the aspects of the body's natural ecosystem, it is being realized that the majority of the diseases/disorders are mainly due to the hyper physiological burden of free radicals, causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. Alternative medicines like Ayurveda, Siddha and Unani provide a holistic approach to treat hepatic disorders with success (Nadkarni et al., 2007).

Piper nigrum and *Piper cubeba* are the two flowering vine in the family Piperaceae. Cubeb (*Piper cubeba*), or tailed pepper, is a plant in genus Piper, cultivated for its fruit and essential oil. The fruits were traditionally used as stimulant, carminative, expectorant, stomachic, and also used in the treatment of

gonorrhoea, especially in gleet, and in the discharge present after acute prostatitis, especially if purulent in character (Mukherjee, 2002). In addition, the antioxidant activity of 16 isolated compounds from *Piper cubeba* was identified (Aboul-Enein et al., 2011). Also, it has been reported that fruits possesses anti-inflammatory activities (Eun-Mi Choi et al., 2003). Further, higher free radical scavenging activity in ethanolic extracts of *Piper cubeba* fruits in comparison to *Piper nigrum* was demonstrated (Gayatri et al., 2011).

In view of above understanding, it can be hypothesized that fruits of *Piper cubeba* has strong free radical scavenging activity, and therefore may also possess the hepatoprotective potential. Hence, the present study was aimed to investigate the antioxidant and hepatoprotective activity of fruits of *Piper cubeba* in CCl_4 induced hepatic model in rats (Kokate et al., 2007).

Material and Methods

The fruits of *Peper cubeba* were collected from Mahavir Ayurvedic Bhandar, Mumbai, India. The fruits were identified and authenticated by Department of life sciences, Ramnarainruiya College, Mumbai, India. Due care has been taken to avoid substitutions. The fruits were cleaned and washed thoroughly in water and then shade dried for a week at 35-40°C. After drying, the fruit material was pulverized in an electrical grinder and stored in a well closed container for future use (Kokate et al., 2007).

Extraction of fruits material

Dried and pulverized fruits powder was defatted with petroleum ether. The defatted material was extracted with ethanol using a Soxhlet extractor. The solvent from extract was evaporated using a vacuum rotary evaporator under reduced pressure and a semi solid mass 16.22 % w/w in respect of dry materials was obtained. Finally, the extract was stored in vacuum desiccators (Kokate et al., 2007).

Procurement of experimental animals

Female swiss albino mice (20-25grams) and Wistar rats (150-200 grams) were obtained from National toxicology centre, Pune. All experimental protocols were approved by Institutional Animal Ethics Committee of CPCSEA, Govt. of India (SSDJ/IAEC/2011-03 dated 25/02/2011) after scrutinization. The animals received the drug treatment by oral gavage tube.

Evaluation of In Vitro Antioxidant Activity

DPPH (1, 1-Diphenyl-2-Picryl Hydrazyl) free radical scavenging activity

The free radical scavenging activity of extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (Badami et al., 2005; Joaquin et al., 2002).

The scavenging percentage on the DPPH radical was 61.80 % for *Peper cubeba* extract at the concentration level of 120 µg and 65.72 % for ascorbic acid at the concentration level 80 µg. The half-effective concentration (EC_{50}) for *Peper cubeba* extract was found to be 85.30 µg and for ascorbic acid 52.9 µg.

Reducing Power Assay

The reducing power of extract was determined using potassium ferricyanide. The reducing powers of the extracts and natural-based antioxidants like ascorbic acid on Fe^{3+} were concentration dependent (Hiramoto et al., 1995).

The reducing power increased with increasing concentration. For instance, the absorbance at 700 nm was found to be 0.0722 and 0.0740 for *Peper cubeba* extract and ascorbic acid at the concentration of 800 µg and 400 µg. This means to reach a similar reducing power, the concentration required for *Peper cubeba* extract was ~2- fold than ascorbic acid.

Nitric Oxide Radical Scavenging Activity

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction as reported previously (Herencia et al., 2002, Marcocci et al., 1994).

The scavenging percentage on the nitric oxide radical was 43.50 % for *Peper cubeba* extract at the concentration level of 60 µg and 44.10 % for curcumin at the concentration level of 40 µg. The half-effective concentration (EC_{50}) for *Peper cubeba* extract was found to be 134 µg and for curcumin was 96.5 µg.

Hydroxyl Radical Scavenging Activity

The scavenging capacity for hydroxyl radical was measured on the basis of Fenton reaction (Lloyd et al., 1997).

The scavenging percentage on the hydroxyl radical was 58.52 % for *Peper cubeba* at the concentration level of 120 µg and 56.84 % for ascorbic acid at the dose 80 µg. The half-effective concentration (EC_{50}) for *Peper cubeba* extract was found to be 78.0 µg and for ascorbic acid was 67.9 µg.

Hydrogen Peroxide Radical Scavenging Activity

The ability of extracts to scavenge H_2O_2 was determined using earlier reported method (Sroks et al., 2003). The scavenging percentage on the hydrogen peroxide radical was 69.12 % for *Peper cubeba* extract at the concentration level of 120 µg and 66.78 % for ascorbic acid at the concentration 80 µg. The half-effective concentration (EC_{50}) for *Peper cubeba* extract was found to be 66.9 µg and for ascorbic acid was 48.1 µg.

LD_{50} Determinations

Acute oral toxicity study was performed using female mice as per OECD (Organization for Economic Co-operation and Development) Guideline 425. (Turner RA. (1965). Female Swiss albino mice, 5 groups of 6 animals, weighing 20-25grams were used. Graded doses of the extract 175-2000 mg/kg were administered orally doses in mg/kg chosen were 175, 550, 1250 and 2000 mg/kg. After dosing, the animals were observed for 2 hours and then intermittently for further 4 hours and finally recording mortality up to 24 hours and keep for observation upto 14 days.

Signs and Symptoms

The animals did not shown any significant changes in body weight, skin and fur, eyes, mucous membranes, and also respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavioral pattern.

Mortality

There was no mortality observed at dosage range from 175 mg/kg to 2000 mg/kg. *Piper cubeba* was found to be safe up to 2000 mg/kg body weight when administered orally.

Evaluation of *In Vivo* Hepatoprotective Activity

Toxin: CCl_4 was injected subcutaneously (s.c.) at a dose of 1 ml/kg in olive oil (1:1).

Standard drug and dosage schedule: Silymarin was administered orally at a dose 25 mg/kg/day (Satyanarayana et al., 2009).

Dose selection: According to the OECD guidelines, the extract was found safe up to 2000 mg/kg dose. Thus, two doses of drug 250 mg/kg (Dose 1) and 500 mg/kg (Dose 2) were selected for the study as shown in **Table 1**.

Experimental design: Wistar rats were divided into five groups of six animals each.

Biochemical Investigations

Blood samples of the rats were withdrawn from ratino bulber venous plexus with the help of a glass capillary under light anesthesia and were kept at room temperature for 2 hours. Serum was separated at 2500 rpm for 15 min and biochemical investigations were carried out as described previously (Satyanarayana et al., 2009).

The biochemical parameters like Alanine aminotransferase (ALT) (IFCC 1986), Aspartate aminotransferase (AST) (IFCC 1986), Alkaline Phosphatase (ALP) (Bowers et al., 1972), Total Bilirubin (TBil), Direct Bilirubin (DBil) (Jendrassik et al., 1938), Serum Triglycerides (STG) (Bucolo et al., 1973; Trinder et al. 1969) and Total proteins (TP) (Doumas et al., 1975) were assayed using assay kits (Varad Diagnostic, Ahmednagar).

The rats were sacrificed by cervical dislocation. Liver samples were dissected out and washed immediately with ice cold saline. Liver homogenates (5% w/v) were prepared in ice cold 50mM potassium phosphate buffer (pH 7.4) using a homogenizer (Remi, India). The supernatant was used for the assay of marker enzymes namely Lipid Peroxidation (LPO) as per earlier described method (Buege et al., 1978), Reduced Glutathione (GSH) determination was carried out according to previous suggested method (Anderson, 1985) and Catalase Level (CAT) was estimated by the standard method (Aebi et al., 1984).

Histology

The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation, including cell necrosis, fatty change, hyaline regeneration, ballooning degeneration (Shanmugasundaram et al., 2006).

Statistical Analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test. The values are expressed as mean ± SEM and $p < 0.05$ was considered significant in all comparisions.

Results

The hepatoprotective effect of ethanolic extract of *piper cubeba* on CCl4-intoxicated rats is shown in **Table 2 and 3.**

Table1. Dosage regimen

Groups	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
CCl_4 control	Water	Water	Water	Water	Water	Water	Olive oil + CCl_4	Water
Vehicle control	Water	Water	Water	Water	Water	Water	Water + Olive oil	Water
Dose 1 250 mg/kg)	Dose 1	Dose 1	Dose 1	Dose 1	Dose 1	Dose 1	Dose 1 + CCl_4	Dose 1
Dose 2 500 mg/kg)	Dose 2	Dose 2	Dose 2	Dose 2	Dose 2	Dose 2	Dose 2 + CCl_4	Dose 2
Standard 25 mg/ kg)	Std	Std	Std	Std	Std	Std	Std + CCl_4	Std

Table 2. Effect of *Piper cubeba* fruit extract against CCl_4 induced hepatotoxicity

Groups	ALT	AST	ALP	TBil	DBil	STG	TP
		(IU/L)			(mg/dL)		(g/dL)
CCl_4 control	160.2 ± 2.023	277.4 ± 3.934	246.03 ± 4.379	0.925 ± 0.036	0.443 ± 0.034	65.767 ± 1.236	5.634 ± 0.122
Vehicle control	53.78 ± 1.458*	136.8 ± 1.756*	84.07 ± 0.810*	0.146 ± 0.040*	0.027 ± 0.006*	27.612 ± 0.636*	7.644 ± 0.291*
Dose 1 (250 mg/kg)	124.20 ± 2.268*	236.9 ± 3.535*	208.53 ± 2.042*	0.472 ± 0.038*	0.170 ± 0.022*	57.915 ± 0.725*	6.834 ± 0.183*
Dose 2 (500 mg/kg)	86.30 ± 1.374*	210.7 ± 2.359*	184.81 ± 1.663*	0.363 ± 0.026*	0.078 ± 0.006*	48.531 ± 0.810*	7.233 ± 0.250*
Standard (25 mg/ kg)	75.58 ± 0.926*	153.2 ± 3.003*	137.85 ± 1.587*	0.220 ± 0.023*	0.115 ± 0.013*	30.436 ± 0.700*	7.694 ± 0.316*

For each test, n=6 animals in each group. Values are expressed as mean ± SEM. One way ANOVA followed by Dunnett's test when compared with CCl_4 control *p<0.0001

Table 3. Effect of *Piper cubeba* fruit extract against CCl_4 induced elevation of LPO, depletion of Reduced GSH and depletion of CAT in rats

Groups	LPO (nMol /g wt)	GSH (mMol/g wt)	CAT (MU/L)
CCl_4 control	68.084 ± 0.306	3.273 ± 0.195	32.53 ± 1.535
Vehicle control	24.334 ± 0.509*	5.772 ± 0.199*	85.40 ± 2.387*
Dose 1 (250 mg/kg)	51.517 ± 0.777*	4.176 ± 0.173*	47.23 ± 0.957*
Dose 2 (500 mg/kg)	41.120 ± 0.530*	4.530 ± 0.267*	59.34 ± 1.172*
Standard (25 mg/ kg)	28.378 ± 0.478*	4.719 ± 0.264*	75.06 ± 0.505*

For each test, n=6 animals in each group. Values are expressed as mean ± SEM. One way ANOVA followed by Dunnett's test when compared with CCl_4 control *p<0.0001

In the CCl_4-intoxicated rats, serum values of ALT, AST, ALP, TB, DB and TG were increased when compared to control. These elevations in serum levels of ALT, AST, ALP, TB, DB and TG were significantly reduced in the *Piper cubeba* ethanolic extract treated animals. Treatment with ethanolic extract showed more significant activity (P <0.001) with maximum inhibition as shown in **Table 2**.

Moreover, in CCl_4-intoxicated rats, there is significant rise in the level of LPO was determined while GSH and CAT levels were significantly decreased. Treatment of ethanolic extract attenuated these CCl_4 induced changes. Ethanolic extract has shown maximum protection (P <0.001). The results are shown in **Table 3.**

Histopathological Observations

Histopathological study was done using light microscopy, 100X resolution employing hematoxylin-eosin stain.

Histopathological observation of liver sections of CCl_4 intoxicated rats showed severe hepatotoxicity as indicated by the appearance of size enlarged, friable, centrilobular necrosis, ballooning deterioration, permeation of the lymphocytes and the liver cells were lossed. Histopathological observation of the liver sections of control animals showed apparently normal cytoplasm, prominent nucleus, nucleolus and visible central veins. The histological architecture of sections of liver in the control and ethanolic extract group showed normal lobular pattern but not observed as effective as the silymarin treated group (**Figure 1**) and observations were shown in **Table 4.**

Discussion

Proton radical scavenging action shows the mechanism of oxidation. Due to proton donating ability the capacity of DPPH radicals was decreased by decrease in its absorbance at 517 nm, hence the *Piper cubeba* fruit extract shows the antioxidant activity (Ganapathy et. al., 2007; Adesegun et. al., 2009). The dose required of *Piper cubeba* fruit extract is 1.5-fold greater than ascorbic acid to scavenge DPPH, hence the extract was less active for scavenging radicals than ascorbic acid.

In the reducing power assay, Fe^{3+}/ ferricyanide complex to the ferrous form is reduced in presence of antioxidants. Hence the reducing capacity of drug shows the antioxidant property and increase in absorbance indicates increase in reducing power (Umamaheswari et. al., 2008; Adesegun et. al., 2009). The dose required for *Piper cubeba* fruit extract is 2.0 fold greater than dose of ascorbic acid to produce same reducing power.

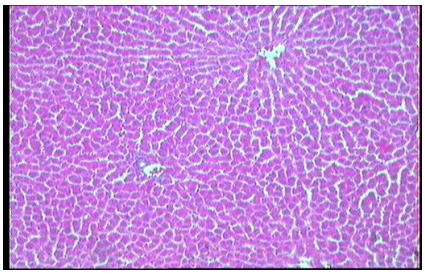
Hydrogen peroxide is intracellular precursor of hydroxyl radicals produce very toxic effect to the cell (Ganapathy et. al., 2007; Adesegun et. al., 2009). Piper *cubeba* fruit extract scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water (Jafri et. al., 1999). The dose required for *Piper cubeba* fruit extract is 1.5-fold greater than dose of ascorbic acid to scavenge hydrogen peroxide.

The results of in vitro antioxidant tests of *Piper cubeba* fruit

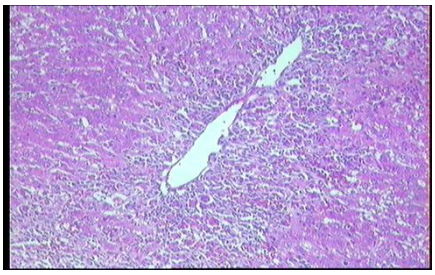
Table 4. Observations of histological changes in liver tissues by light microscopy

Groups	Histological Changes In Liver
CCl_4 control	Centilobular areas of necrosis are infiltrated by mononuclear cells and macrophages. Erythrophagocytosis is seen in some macrophages. Large focal fatty changes are seen.
Vehicle control	No abnormality was detected.
Dose 1 (250 mg/kg)	Centrilobular fatty change and foamy cells.
Dose 2 (500 mg/kg)	Scanty periportal mononuclear cells and congestion.
Standard (25 mg/ kg)	Vacuolated hepatocytes around centrilobular veins are seen.

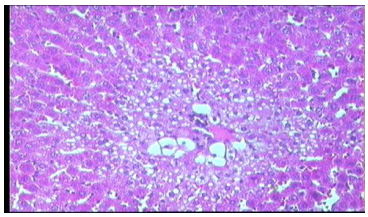
Figure 1. The histological architecture of sections of liver in the control and ethanolic extract group.



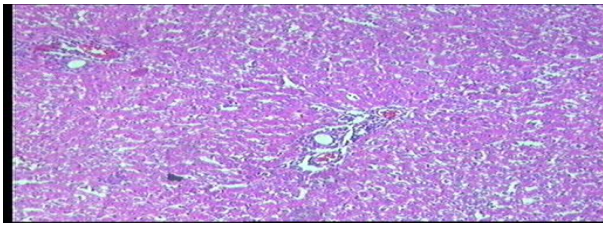
Vehicle control



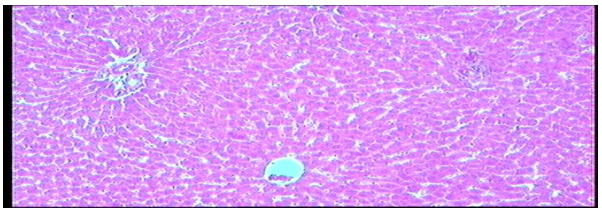
CCl_4 control



Ethanolic extract of *Piper cubeba* (Dose 250 mg/kg)



Ethanolic extract of *Piper cubeba* (Dose 500 mg/kg)



Silymarin (25 mg/kg)

shows strong free radical scavenging activity, which also produces useful activity against hepatotoxicity produced by CCl_4. The *Piper cubeba* fruits extract showed the reduction in levels of SGPT and SGOT, stabilized and repaired plasma membrane and hepatic tissue damaged by CCl_4. The serum levels of transaminases, healing of hepatic parenchyma and restoration of hepatocytes goes to normal (Thabrew et al., 1987). The *Piper cubeba* fruits extract restrain the increased level of cholesterol and triglyceride, also produced inhibition of increased ALP activity with reduction of increased bilirubin shows the possibility of prevention of biliary dysfunction in rat liver during hepatic damage with CCl_4.

A major defense mechanism involves the antioxidant enzymes like SOD, CAT and GSH which convert active oxygen molecules into non-toxic compounds. Enhanced lipid peroxidation results when free radicals are overwhelmingly formed. Results revealed reduced LPO as indicated by significant decrease in MDA level in extracts treated groups. Simultaneously, significant increase in GSH, SOD and CAT content of liver suggested antioxidant activity of *Piper cubeba* fruits extracts and silymarin. Scavenging of free radicals is known to be one of the major antioxidation mechanisms to inhibit the chain reaction of LPO (Constantin et al., 1990).

The acute toxicity studies were carried out according to OECD guidelines. *Piper cubeba* is nontoxic up to 2000 mg/kg as indicated by observations and hence, the doses were selected i.e. 250 mg/kg and 500 mg/kg for the hepatoprotective evaluation against carbon tetrachloride (CCl_4) induced hepatotoxic effect.

Further, the hepatoprotective effect of *Piper cubeba* fruits extract was confirmed by histological examination of the liver tissue of control and treated animals. The histological architecture of liver sections of carbon tetrachloride treated group showed fatty degeneration of hepatocytes, however administration of *Piper cubeba* fruits extract treated group almost normalized to the level of the Silymarin treated groups, showing its potent hepatoprotective effects. The administration of ethanolic extract of *Piper cubeba* fruits exposed significant protection in hepatocyte regeneration against the toxic effect of carbon tetrachloride. Hence, the histological examination of *Piper cubeba* fruits extract treated group showing hepatoprotective effects and it supported to biochemical investigations.

Carbon tetrachloride induced hepatic injuries are commonly used models for the screening of hepatoprotective drug and the extent of hepatic damage is assessed by the level of released cytoplasmic alkaline phosphatase, transaminases, triglycerides and bilirubin in circulation (Adesegun et. al., 2009). It is well documented that CCl_4 are biotransformed under the action of microsomal cytochrome P-450 of liver to reactive metabolites (Larrey et. al., 2000). These free

radicals bind covalently to unsaturated lipid membrane, provoking a sharp increase of lipid peroxides followed by pathological changes such as elevated levels of serum marker enzymes like ALT, AST and ALP; decreased total protein, increased levels of total and direct bilirubin, serum triglycerides accumulation, depletion of GSH, increased LPO, and finally hepatocyte damage (Sies, 1999). This suggests that, CCl_4 induces liver injury by sharing a common property of free radical mechanism. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by hepatotoxin is the index of its protective effect (Suja et. al., 2004).

In hepatotoxicity, a depression in total protein is observed due to the defect in protein biosynthesis similar to our results. This is due to the disruption and disassociation of polyribosomes from endoplasmic reticulum following CCl_4 administration (Das et. al., 2005). Administration of fruit extract at a dose of 250 mg/kg and 500 mg/kg body weight prevented this change. This indicates that *Piper cubeba* possibly promotes the assembly of ribosomes on endoplasmic reticulum to facilitate uninterrupted protein biosynthesis.

The results indicate that triglycerides (TG) increase in CCl_4 induced fatty liver. It is well known that CCl_4 administration induce an increased synthesis of fatty acids as well as decreased release of hepatic lipoproteins due to impair in ß-oxidation of fatty acids (Sun et. al., 2009). The accumulation of TG in liver of CCl_4 treated rats is not due to the interference with the TG formation by the liver, but due to the inhibition or destruction of TG secreting mechanism (Sun et. al., 2009). Administration of fruit extract at a dose of 250 mg/kg and 500 mg/kg body weight prevented this change, suggesting that *Piper cubeba* probably improve ß-oxidation of fatty acids.

Serum activities of catalase (CAT) are the most sensitive enzymatic index in liver injury caused by Reactive Oxygen Species (ROS) and oxidative stress (Sanmugapriya et. al., 2006). 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 (Oh SI, et. al., 1997). A reduction in the activity of this enzyme is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes (Bhakta et. al., 1999). Administration of CCl_4 leads to generation of peroxy radical, O_2^- which is associated with inactivation of CAT enzyme. This probably explains the significantly reduced activities of CAT observed by us in rats challenged with CCl_4. In rats receiving CCl_4 and *Piper cubeba* extract the activity of CAT is significantly higher than in CCl_4 control rats, and very similar to the values noted in normal rats. This suggests that *Piper cubeba* can reduce ROS that may lessen the oxidative damage to the hepatocytes and improve the activities of the liver antioxidant enzymes, thus protecting the liver from CCl_4.

Conclusion

In conclusion, the ethanolic extract of *Piper cubeba* fruits possesses the significant antioxidant and hepatoprotective activity. Therefore, the present study support the traditional believes of this plant and highlighted profound potential of *Piper cubeba* to be investigated for bioactive compounds responsible for hepatoprotective and antioxidant effect.

REFERENCES

1. Aboul-Enein, H, Kładna A, Kruk I. (2011). Radical scavenging ability of some compounds isolated from Piper cubeba towards free radicals. *Luminescence* 26 (3), 202–207.
2. Adesegun S, Fajana A, Orabueze C, Cocker A. (2009). Evaluation of antioxidant activity of Phaulopsis fascisepala C. B. Cl. (Acanthaceae). *eCAM* 6, 227-231.
3. Aebi H. (1984). Catalase in Vitro. *Methods Enzymol* 105, 121- 126.
4. Anderson M. (1985). Determination of Glutathione and Glutathione disulfide in biological sample. *Methods Enzymol* 113, 548-555.
5. Badami S, Omprakash, Dongre S, Suresh B. (2005). In vitro antioxidant properties of Solanum pseudocapsicum leaf extracts. *Indian J. Pharmacology* 37(4), 251-252.
6. Bhakta T, Pulok KM, Kakali M, Subhash C, Tapan K, Pal M, Saha B.(1999). Evaluation of hepatoprotective activity of Cassia fistulata leaf extract. *J Ethnopharmacol* 66, 277-282.
7. Bowers G, McCommb R. (1972). Recommendations of German Society for Clinical Chemistry. *Z. Clin. Chem,* 18:97
8. Bucolo G, David H. (1973). Quantitative determination of serum triglycerides by the use of enzymes *Clin. Chem* 19 (5), 476-482.
9. Buege J, Aust S. (1978). Microsomal lipid peroxidation. *Methods Enzymol* 52, 302-310.

10. Clawson G. (1989). A mechanism of carbon tetrachloride hepatotoxicity. *Pathology and Immunopathology Research* 8, 104–112.
11. Constantin M, Bromont C, Fickat R, Massingham R. (1990). Studies on the activity of Bepridil as a scavenger of free radicals. *Biochemical Pharmacology* 40, 1615-1622.
12. Das D, Mukherjee S, Mukherjee M, Das AS, Mitra C. (2005). Aqueous extract of black tea (Camellia sinensis) prevents chronic ethanol toxicity. *Current Sci* 88, 952-961.
13. De Groot H, Noll T. (1986). The crucial role of low steady state oxygen partial pressure in halloalkan; free-radical mediated lipid peroxidations possible implications in haloalkane liver injury. *Biochemical Pharmacology* 35,15–19.
14. Doumas B. (1975). Standards for total serum protein assays-a collaborative study. *Clin Chem* 21, 1159-1166
15. Eun-Mi Choi, Jae-Kwan Hwang, (2003). Investigations of anti-inflammatory and antinociceptive activities of Piper cubeba, Physalis angulata and Rosa hybrid, *JOETD* 89 (1), 171–175.
16. Ganapathy S, Chandrashekhar V. Chitme H, Lakshmi N. (2007). Free radical scavenging activity of gossypin and nevadensin: An in vitro evaluation. *Ind J Pharmacol* 39, 281-283.
17. Gayatri N, Sahu R. (2011). Phytochemical Evaluation and Antioxidant activity of Piper cubeba and Piper nigrum. *Journal of Applied Pharmaceutical Science* 01 (08), 153-157
18. Ghosh, M. (1996). Toxicity Studies Fundamentals of Experimental Pharmacology, 3rd Edition, Hilton & Company, Kolkata. pp 190-197.
19. Herencia F, Lopez-Garcia M, Ubeda A, Ferrandiz M. (2002). Nitric oxide- scavenging properties of some chalcone derivatives. *Biol chem* 6, 242-246
20. Hiramoto K, Sekguchi K, Aso-o R, Ayuha K, Hi-iyama H, Kato T, Kikugawa K. (1995). DNA strand breaks induced through active oxygen radical by fragrant component 4-hydroxy-2-hydroxymethyl-5-methyl-3(2H)-furanone in Maillard reaction of Hexose/amio acid. *Fd Chem Toxic* 33, 803- 814
21. IFCC Methods for the measurement of catalytic concentrations of enzymes (1986). *J. Clin. Chem. Clin. Biochem* 24, 481, 497
22. Jafri M, Subhani M, Javed K, Singh S. (1999). Hepatoprotective activity of leaves of Cassia occidentalis against paracetamol and ethyl alcohol intoxication in rats. *J Ethnopharmacol* 66, 355-361.
23. Jendrassik, L, Grof, P (1938). Colorimetric Method of Determination of bilirubin. *Biochem* Z 297,81-82
24. Joaquin A, Sofia S, Mercedes P, Pablo J, Tetris A, Manuel N, Aldolfo S. (2002) Preliminary assay on radical scavenging activity of olive wood extracts, *Fitoterapia*, 76, 348-351.
25. Kokate, C. K. (1997). Practical Pharmacognosy, 4th Edition, Vallabh Prakashan, Delhi, pp 107 - 111.
26. Kokate, C, Purohit, A, Gokhale S. (2007). Pharmacognosy, 39th Edition, Nirali Prakashan, Pune, pp 607-611.
27. Larrey D. (2000). Drug induced liver diseases. *J Hepatol* 32, 77-88.
28. Lloyd R, Hanna P, Mason R. (1997). The origin of hydroxyl radical oxygen in the Fenton reaction. *Free Rad Biol Med* 22, 885-888
29. Marcocci L, Maguire J, Droy- Lefaix M, Packer L. (1974). The nitric oxide scavenging properties of Ginko biloba extract EGB 761. *Biochem Biophys Res Comm*, 201: 748.
30. Mukherjee P. (2002). Quality Control of Herbal Drugs. 1st Edition. Business Horizons Pharmaceutical Publishers, New Delhi, pp 131-219.
31. Nadkarni A. (2007). Indian Materia Medica. 2nd Edition. Vol I. Bombay Popular Prakashan, Mumbai, 629.
32. OECD, Guidelines for testing of chemicals, Acute oral toxicity, Environmental Health and Safety Monograph Series on Testing and Adjustment No. 425, 2001.
33. Oh SI, Kim C, Chun H, Lee M, Park S. (1997). Glutathione recycling in attenuated by acute ethanol feeding in rat liver. *JKMS* 12, 316-321.
34. Recnagal R. (1983). A new direction in the study of carbon tetrachloride hepatotoxicity. *Life Sciences* 33, 401–408.
35. Sanmugapriya E, Venkataraman S. (2006). Studies on hepatoprotective and antioxidant actions of Strychnos potatorum Linn. seeds on CCl_4-induced acute hepatic injury in experimental rats. *J Ethnopharmacol* 105, 154-160.
36. Satyanarayana T, Devi K, Mathews A. (2009). Hepatoprotective activity of Capparis sepiaria stem against carbon tertrachloride induced hepatotoxicity in rats, *The Journal of Pharmaceutical research and health care* 1, 34- 45.
37. Shanmugasundaram P, Venkataraman S. (2006). Hepatoprotective and antioxidant effects of Hygrophila auriculata (k. Schum) Heine Acanthaceae root extract. *J. Ethanopharmacol* 104, 124-128
38. Sies H. (1999). Glutathione and its role in cellular functions. *Free Rad Biol Med* 27, 916-921.
39. Sroks Z, Cisowski W. (2003). Hydrogen peroxide scavenging, antioxidant and antiradical activity of some phenolic acids. *Food Chem Toxicology* 41, 753-758
40. Sroks Z, Cisowski W. (2003). Hydrogen peroxide scavenging, antioxidant and antiradical activity of some phenolic acids. *Food Chem Toxicology* 41,: 753-758

41. Suja S, Latha P, Pushpandadan P, Rajasekharan S. (2004). Evaluation of hepatoprotective effects of Helminthostachys zelyanica (L.) Hook against carbon tetrachloride- induced liver damage in wistar rats. *J Ethnopharmacol* 92, 61-66.
42. Sun F, Xie M, Zhu L, Xue J, Gu Z. (2009). Inhibitory effect of osthole on ethanol-induced fatty liver in mice. *Digest Liv Dis* 41, 127-133.
43. Thabrew M, Joice P, Rajatissa W. (1987). Comparative study of efficacy of Paetta indica and Osbeckia octandra in the treatment of liver dysfunction. *Planta Medica* 53, 239–241.
44. Tomasi A, Albano E, Banni SB, Corongiu F. (1987). Free radical metabolism of carbon tetrachloride in rat liver mitochondria. *Biochemistry* 46, 313–317.
45. Trinder, P. (1969). Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor, *Ann. Clin. Biochem* 6, 24-25.
46. Turner R. Screening methods in Pharmacology. 1st Edition. *Academic press, New York*. 22-41.
47. Umamaheswari M, Chatterjee T. (2008). In vitro antioxidant activities of the fractions of Coccinia Grandis L. leaf extract. *Afr J Trad Comp Alter Med* 5, 61-73.

