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IJPT
1735-2657/07/61-27-33
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS
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IJPT 6:27-33, 2007

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RESEARCH ARTICLE

Hepatoprotective and Antioxidant Effects of *Eupatorium ayapana* against Carbon Tetrachloride Induced Hepatotoxicity in Rats

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Received May 6, 2006; Revised May 29, 2006; Accepted June 5, 2006

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

The present study was carried out to evaluate the hepatoprotective and antioxidant effect of the methanol extract of *Eupatorium ayapana* (MEEA) (Family- Asteraceae) leaves in Wistar albino rats. The different groups of animals were administered with carbon tetrachloride (CCl_4) at 72 h interval. The MEEA 100, 200 and 300 mg/kg and silymarin 25 mg/kg were administered to the CCl_4 treated rats. The effect of MEEA and silymarin on serum transaminase (SGOT, SGPT), serum alkaline phosphates (SALP), bilirubin, uric acid and total protein were measured CCl_4 induced hepatotoxicity in rats. Further, the effects of the extract on lipid peroxidation (LPO), enzymatic antioxidant such as superoxide dismutase (SOD) and catalase (CAT), and nonenzymatic antioxidant like glutathione (GSH) were estimated. The MEEA and silymarin produced significant ($p < 0.05$) hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin, uric acid, and lipid peroxidation and significantly ($p < 0.05$) increased the levels of SOD, CAT, GSH and protein in a dose dependent manner. From these results, it was suggested that MEEA possess potent hepatoprotective and antioxidant properties.

Keywords: *Eupatorium ayapana, Hepatoprotective effects, Antioxidants, Carbon tetrachloride*

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Free radical initiated autoxidation of cellular membrane lipids can lead to cellular necrosis and is now accepted to be important in connection with a variety of pathological conditions [1]. Living cell evolved defences against free radicals and reduction of these peroxyl radicals by antioxidant molecules is crucial to the protection of cells against the development of prooxidant state. Antioxidants may protect against reactive oxygen species (ROS) toxicity by the prevention of ROS formation, by the interruption of ROS attack, by scavenging the reactive metabolites and converting them to less reactive molecules and/or by enhancing the resistance of sensitive biological targets to ROS attack, by facilitating the repair caused by ROS and by providing co-factors for the effective functioning of other antioxidants [2]. Developments of life threatening diseases like cancer are linked to the availability of these antioxidants [3]. Low levels of antioxidants, which further increases the free radical activity, are clearly associated with cancer conditions.

Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. Liver diseases are mainly caused by toxic chemicals (antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune disorder. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver [4, 5]. Plants have proven to be used effectively as hepatoprotective agents [6].

Eupatorium ayapana Vent (Family: Asteraceae) is commonly known as Visalyakarani- Hindi. The Eupatorium genus is one of the most important plants used in herbal medicine. *Eupatorium ayapana* is a native of Brazil and has long been naturalized in India. It is an aromatic under shrub. Chemical constitutions like 7-Methoxy coumarin [ayapanin], 6, 7-dimethoxy coumarin [ayapin]; carotene, vitamin-C and stigmasterol were isolated from its leaves [7]. Five additional coumarins, *viz*. daphnetin, daphnetin dimethyl ether, hydrangetin, daphnetin –7-methyl ether and umbelliferone

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have also been isolated [8]. The leaves possess a coumarin like odour and yield a pale green essential oil on steam distillation. The principal constituent of the oil is thymohydroquinone, dimethyl ether, sesquiterpene, and traces of coumarin.

The plant is used as antiperiodic, cardiac stimulant, diaphoretic, diuretic, emetic, expectorant and tonic. Infusion maybe useful in ague, cough, and dyspepsia. The leaves (fresh bruised) are applied to scores, snakebite and foul ulcers. The juice of fresh leaves is a digestive agent and haemostatic [9]. *Eupatorium ayapana* is considered as the most powerful therapeutic agent. A decoction of the leaves has been used as a popular remedy against various kinds of hemorrhage. It is given internally as an antidote to snake bites. In Europe, the dried leaves of the plant were used as a tonic under the name of 'Ayapana tea'. The coagulation time of rabbit's blood was diminished when traces of finely divided ayapanin or ayapin were added *in vitro* [10]. Both ayapanin and ayapin are non-toxic and are effective when applied locally or when administered by subcutaneous injections or by mouth. They have no effect on respiration or on blood pressure [11]. Previous report from our laboratory on *Eupatorium ayapana* showed antimicrobial activity [12]**.** The tribal peoples of Kolli Hills of Tamilnadu, India, used the leaves for the treatment of liver disorders.

Based on the pervious report, traditional usage and chemical constituents we selected the plant for the study. The purpose of the present study was to evaluate the hepatoprotective and antioxidant activities of methanol extract of *Eupatorium ayapana* (MEEA) in rats.

MATERIALS AND METHODS

Plant materials and Extraction

The plant *Eupatorium ayapana (*Family: Asteraceae) leaves were collected in the month of March 2004 from the Kolli Hills, Tamil Nadu, India. The plant material was taxonomically identified by Botanical Survey of India, Kolkata, India, and the Voucher specimen (No.GMP-12) was retained in our laboratory for future reference. The dried powder material of the *Eupatorium ayapana* leaves was extracted with methanol (Yield 8.53 %) in a soxhlet apparatus. The methanol extract was then distilled, evaporated and dried in vacuum. The chemical constituents of the extract were identified by qualitative analysis followed by their confirmation by thin layer chromatography.

Animals

Studies were carried out using male Wistar albino rats (150–180 g). They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 $\pm$ 2^{o}C) with dark and light cycle (14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Drugs and Chemicals

Silymarin was purchased from Micro labs Hosur Tamilnadu India, 1-Chloro-2, 4-dinitrobenzene [CDNB], Bovine serum albumin was from Sigma chemical St. Louis, MO, USA, Thiobarbituric acid, and Nitrobluetetrazolium chloride (NBT) were from Loba Chemie, Bombay, India, and 5,5'-dithio *bis*-2-nitrobenzoic acid (DTNB) and Carbon tetrachloride were from SICCO research laboratory, Bombay. The solvent and / or reagent obtained were used as received.

Toxicity study

For toxicity studies groups of 10 mice were administered (p.o.) with test extracts in the range 100-1600 mg/kg and mortality rates were observed after 24 hours. LD_{50} was determined using the graphical methods of Litchfield and Wilcoxon [13].

Carbon tetrachloride-induced liver damage in rats

Healthy male albino rats were divided into 6 groups each containing 6 animals. Group 1 Normal (Liquid paraffin 1ml/kg body weight, p.o.) Group 2 (Control) received 30% CCl_4 in liquid paraffin (1 ml/kg body weight, i.p.). Group 3, 4 and 5 received MEEA 100, 200 and 300 mg/kg p.o. respectively and Group 6 received standard drug Silymarin (25 mg/kg p.o) once in a day and CCl_4 as mentioned above. Treatment duration was 10 days and the dose of CCl_4 was administered every 72-h [14]. Animals were sacrificed 24 h after the last injection. Blood was collected, allowed to clot and serum separated. The liver was dissected out and used for biochemical and histopathology studies.

Biochemical Studies

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 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters namely Serum glutamyl pyruvate transaminase (SGPT), Serum glutamyl oxalacetic acid transaminase (SGOT) [15], Serum alkaline phosphatase (SALP) [16], serum bilirubin [17], protein content [18] and plasma uric acid [19].

After collection of blood samples rats were sacrificed and their livers excised, rinsed 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 [20]. A part of homogenate after precipitating proteins with Trichloro acetic acid (TCA) was used for estimation of glutathione [21]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4° C. The supernatant thus obtained was

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Table 1. Effect of methanol extract of Eupatorium ayapana leaves (MEEA) on serum enzymes (GPT, GOT and ALP), bilirubin, protein and uric acid in CCl_4 induced hepatic damage in rats

Parameters	Normal (liquid paraffin 1ml/kg, b.wt)	Control (CCl_4 1ml/ kg, b.wt)	Silymarin (25 mg/kg) + CCl_4	MEEA (100 mg/kg) + CCl_4	MEEA (200 mg/kg) + CCl_4	MEEA (300mg/kg) + CCl_4
SGOT (U/l)	61.32 ± 4.25	175.41 ± 7.82*	62.36 ± 5.62** (99.08)	145.79± 8.53** (25.96)	106.32 ± 6.11** (60.55)	66.21± 5..54** (95.11)
SGPT (U/l)	51.35 ± 4.61	125.91± 8.42*	54.21 ± .4.30** (96.16)	101.95 ± 7.41** (32.16)	78.83 ± 6.42** (63.14)	57.84 ± 4.12** (91.29)
SALP (U/l)	65.32 ± 5.38	116.31± 7.22*	67.54 ± .5.64** (9565)	100.81± 9.61** (30.39)	86.55± 8.10** (58.36)	71.34 ± 6.84** (88.19)
Bilirubin (mg/dl)	0.91 ± .0.52	2.42 ± 0.45*	0.94 ± 0.11** (98.01)	2.02 ± 0.32** (26.49)	1.08 ± 0.22** (88.74)	0.98 ± 0.16** (95.36)
Protein (mg/dl)	7.05 ± 0.51	5.35 ± 0.32*	6.98± 0.62** (95.88)	5.73 ± 0.42 (22.35)	6.51± 0.53** (68.23)	6.86 ± 0.45** (88.82)
Uric acid (mg/dl)	2.77 ± 0.22	1.36 ± 0.14*	2.75 ± 0.28** (98.58)	2.31 ± 0.29** (67.37)	2.40 ± 0.21** (73.76)	2.76 ± 0.22** (99.29)

The data in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the CCl_4. The % of protection is calculated as 100 X (values of CCl_4 control -values of sample) / (values of CCl_4 control - values of vehicle control) Values are mean ± S.E.M. number of rats=6. Control group compared with normal group * $p < 0.001$ Experimental groups compared with CCl_4 control group * * $p < 0.05$

used for the estimation of SOD [22] and CAT activities [23].

Lipid peroxidation

The tissues were then homogenized in 0.1M buffer (pH 7.4) with a Teflon-glass homogenizer. Lipid peroxidation in this homogenate was determined by measuring the amounts of malondialdehyde (MDA) produced primarily, according to the method of Ohkawa et al. (1979). 0.2 mL of tissue homogenate, 0.2 ml of 8.1% Sodium dodecyl sulphate (SDS), 1.5 ml 20% acetic acid and 1.5 ml 8% TBA were added. The volume of the mixture was made upto 4 ml with distilled water and then heated at 95^0 C on a water bath for 60 min using glass balls as condenser. After incubation the tubes were cooled to room temperature and final volume was made to 5 ml in each tube. 5.0 ml of butanol:pyridine (15:1) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken and its OD read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of $1.56 \times 10^5 M^{-1}cm^{-1}$.

Estimation of GSH

To measure the reduced glutathione (GSH) level, the tissue homogenate (in 0.1 M phosphate buffer pH 7.4) was taken. The procedure was followed initially as described by Ellman 1959. The homogenate was added an equal volume of 20% tetrachloroacetic acid (TBA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 200 rpm. The supernatant (200µl) was then transferred to a new set of test tubes and added 1.8 ml of the Ellman's reagent (5, 5'-dithio bis-2-nitrobenzoic acid) (0.1mM) which was prepared in 0.3M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes were made upto the volume of 2 ml. After completion of the total reaction, solutions were measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from standard curve from known GSH.

Estimation of SOD

SOD activity of the liver tissue was analyzed by the method described by Kakkar et al. (1984). Assay mixture contained 0.1 ml of sample, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml phenazine methosulphate (186 µM), 0.3 ml of 300 µM nitroblue tetrazolium and 0.2 ml NADH (750 µM). Reaction was started by addition of NADH. After incubation at 30^0 C for 90 s, the reaction was stopped by the addition of 0.1 ml glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 ml of n-butanol. Mixture was allowed to stand for 10 min, centrifuged and butanol layer was separated. Color intensity of the chromogen in the butanol layer was measured at 560 nm spectrophotometrically and concentration of SOD was expressed as units/mg protein.

Estimation of CAT

Catalase activity was measured by the method of Aebi (1974). 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 77.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein.

Histological investigation

Liver slices fixed for 48hr in 10% formalin were processed for paraffin embedding following the standard micro technique [24]. Sections (5µm) of livers were stained with haematoxylin and eosin was evaluated for histopathological changes under a light microscope.

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Table 2. Effect of the methanol extract of Eupatorium ayapana leaves (MEEA) on lipid peroxidation (LPO) antioxidant enzymes (SOD and CAT) and non enzymatic antioxidant (GSH,) in the liver of CCl_4 intoxicated rats.

Parameters	Normal (liquid paraffin 1ml/kg, b.wt)	Control (CCl_4 1ml/ kg, b.wt)	Silymarin (25 mg/kg) + CCl_4	MEEA (100 mg/kg) + CCl_4	MEEA (200 mg/kg) + CCl_4	MEEA (300mg/kg) + CCl_4
Lipid peroxidation (n mole of MDA/mg protein)	0.90 ± 0.08	7.06 ± 0.51*	1.01 ± 0.05** (98.21)	5.67 ± 0.51** (22.56)	4.22 ± 0.48** (46.10)	1.27 ± 0.11** (93.99)
Glutathione content (µg/mg protein)	5.23 ± 0.49	0.63 ± 0.07*	5.17 ± 0.34** (98.69)	1.26 ± 0.26** (13.69)	3.67 ± 0.29** (66.08)	4.97 ± 0.25** (94.34)
Superoxide dismutase (U/mg protein)	92.56 ± 6.35	55.33 ± 5.76*	89.56 ± 7.53** (92.43)	59.22 ± 6.23** (10.50)	66.41 ± 5.29** (29.92)	85.44 ± 7.72** (81.31)
Catalase (U/mg protein)	350.51± 20.07	264.52 ± 22.07*	351.43 ± 41.35** (95.25)	278.34 ± 31.13** (16.07)	308.27 ± 14.29** (50.87)	340.51± 22.05** (88.37)

The data in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the CCl_4. The % of protection is calculated as 100 X (values of CCl_4 control -values of sample) / (values of CCl_4 control - values of vehicle control) Values are mean ± S.E.M. number of rats=6. Control group compared with normal group * $p < 0.001$. Experimental groups compared with CCl_4 control group * * $p < 0.05$

Statistical analysis

Results are reported as means ± S.E.M. ANOVA was used to evaluate differences between groups. If significance was observed between groups, the Student's *t*-test was used to compare the means of specific groups, with $p < 0.05$ considered as significant.

RESULTS

Acute toxicity

The methanol extract of leaves of *Eupatorium ayapana* was found to be non-toxic up to doses of 1.6 g/kg and did not cause any death of the animals tested.

Effect of MEEA on serum enzymes, bilirubin, uric acid and protein

Activities of serum enzymes (SGPT, SGOT and SALP), concentrations of bilirubin, uric acid and total protein content of the serum of CCl_4 induced liver damage rats are presented in Table 1. The level of serum marker enzymes SGPT, SGOT, SALP, bilirubin and uric acid were found to be significantly increased and protein content significantly decreased in CCl_4-induced liver damage rats when compared with the normal group (**$p < 0.001$). Where as treatment with MEEA at the dose of 100, 200 and 300 mg/kg showed significantly decreased activity of serum transaminase, SALP, uric acid, bilirubin and increased protein content in CCl_4-induced liver damage in rats compared to that of control groups (*$p < 0.05$). Silymarin (25 mg/kg) also significantly decreased the levels of serum enzymes, bilirubin, uric acid and increased the protein content in CCl_4 treated groups as compared with the respective control group (*$p < 0.05$).

Effect of MEEA on In vivo Lipid peroxidation

The localization of radical formation resulting in lipid peroxidation, measured as MDA in rat liver homogenate, is shown in Table 2. MDA content in the liver homogenate was significantly increased in CCl_4 control group compared to normal group (**$p < 0.001$). MDA level of MEEA 100, 200 and 300 mg/kg group, were significantly inhibited by 22.56, 46.10 and 93.99 % compared to CCl_4 control (*$p < 0.05$). At the same time, the effect of silymarin 25 mg/kg on MDA levels in CCl_4 was significantly inhibited by 98.22 % respectively (*$p < 0.05$).

Effect of MEEA on Glutathione

The effect of MEEA on glutathione content in the liver is shown in Table 2. GSH level in normal group was measured to be significantly higher than in CCl_4 control group (*$p < 0.001$). GSH level of MEEA 100, 200 and 300 mg/kg groups were significantly increased by 13.69, 66.08, and 94.34% respectively as compared to CCl_4 control group (**$p < 0.05$). Silymarin almost completely restored the glutathione level in CCl_4 treated groups to the normal level.

Effect of MEEA on SOD and CAT activity in liver tissues

The effect of MECA on SOD and CAT activities in liver is shown in Table 2. SOD activity in CCl_4 control group was examined to be lower than in normal group (*$p< 0.001$). SOD activities in MECA 100, 200 and 300 mg/kg groups were observed to be significantly higher than in CCl_4 control group (*$p < 0.05$). SOD activities of MEEA 100, 200 and 300 mg/kg were significantly improved by 10.50, 29.92 and 81.31 % respectively. Silymarin 25 mg/kg also restored the SOD activity in CCl_4 treated groups. CAT activity of CCl_4 control group was measured to be strikingly lower than in normal group (*$p < 0.001$). Liver CAT activities in MEEA 100, 200 and 300 mg/kg groups were significantly increased by 16.07, 50.87 and 88.37 % respectively when compared with control group (**$p<0.05$). MEEA and silymarin completely restored the enzyme activity to the normal level at the respective doses of 300 mg/kg and 25 mg/kg.

Effect of MEEA on Histopathololgyof liver

Comparison of liver section of normal animals (Fig.1) with CCl_4 treated animals is presented in Fig 2.

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The liver cells of rats intoxicated with CCl_4 had high degree of damage. Treatment with MECA at the dose of 200 and 300 mg/kg body weight is shown in (Fig. 3 and 4 respectively.

DISCUSSION

In the assessment of liver damage by CCl_4 hepatotoxin, the determination of enzyme levels such as SGPT and SGOT is largely used. Necrosis or membrane damage releases the enzyme in to circulation; therefore, it can be measured in serum. High levels of SGOT indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury [25]. Our results using the model of CCl_4-induced hepatotoxicity in the rats demonstrated that MEEA at the different doses caused significant inhibition of SGPT and SGOT levels. Serum ALP and bilirubin levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [26]. Our results using the model of CCl_4-induced hepatotoxicity in rats demonstrated that MEEA at different doses caused significant inhibition of SGPT, SGOT, SALP and bilirubin levels.

The reduced level of uric acid in hepatotoxicity conditions may be due to the increased utilization of uric acid against increased production of the free radicals, which is a characteristic feature of cancer condition. The result from the study suggested that altered uric acid level to near normal in MEEA treated rats could be due to strong antioxidant property of MEEA.

Liver cell injury induced by CCl_4 involves initially the metabolism of CCl_4 to trichloromethyl free radical by the mixed-function oxidase system of the endoplasmic reticulum. It is postulated that secondary mechanisms link CCl_4 metabolism to the widespread disturbances in hepatocyte function. These secondary mechanisms could involve the generation of toxic products arising directly from CCl_4 metabolism or from peroxidative degeneration of membrane lipids [27]. In our study, elevations in the levels of end products of lipid peroxidation in liver of rats treated with CCl_4 were observed. The increase in MDA level in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatments with MEEA significantly decreased the level of lipid peroxidation. Hence it may be possible that the mechanism of hepatoprotection of MEEA is due to its antioxidant effect.

GSH is a naturally occurring substance that is abundant in many living creatures. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder and injury. Example, include liver injury induced by consuming alcohol or by taking drugs like acetaminophen, lung injury by smoking and muscle injury by intense physical activity [28]. All are known to be correlated with low tissue levels of GSH. From this result it was suggested that exogenous MEEA supplementation might provide a mean for recovery of reduced GSH levels and to prevent tissue disorders and injuries. In present study, we have demonstrated the effectiveness of MEEA by using CCl_4 induced rats.

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators; enzymes such as SOD, CAT and GPx system [29]. SOD dismutates superoxide radicals O_2- into H_2O_2 plus O_2, thus participating, with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. In this study, SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues. In present study it was observed that increased SOD activity due to MEEA treatment had an efficient protective mechanism in response to ROS. And also, these findings indicate that MEEA may be associated with decreased oxidative stress and free radical-mediated tissue damage.

Catalase is a key component of the antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. In rats administration of MEEA increases the activities of CAT in CCl_4 induced liver damage to prevent the accumulation of excessive free radicals and protects the liver from CCl_4 intoxication.

Histopathological Study

Examination of liver section of normal animals (Fig.1) showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and central vein. CCl_4 treated animals (Fig.2) shows that the liver cells of rats intoxicated with CCl_4 have high degree of damage. In CCl_4 treated animals the sections showed hydropic changes in centrilobular hepatocytes with single cell necrosis surrounded by neutrophils. Congestion of central vein and sinusoids were seen with acute and chronic inflammatory cells infiltrating sinusoids mainly in the central zone. The midzonal and periportal hepatocytes showed mild to moderate degree of fatty change along with neutrophillic abscess as characterized by the cell vacuolation, pyknotic and degenerated nuclei and the wall of bile capillaries were also damaged. The normal architecture of liver was completely damaged. The intralobular vein was badly damaged and their wall was broken at places. Cell lysis is visible around the intralobular vein. Wide spaces were formed at some sinusoids.

The hepatic cells of rat treated with MEEA and intoxicated with CCl_4 showed mild fatty change and mild sinusoidal congestion. The vacuolation is present, but is very much similar to the normal (Fig.3 Fig.4) The intralobular vein is almost normal in structure but damage

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at one or two places in the wall is visible showing space formation. The hepatic cells are mostly normal but with few vacuoles and some damaged cells but no pyknosis in the nucleus could be seen. MEEA pretreatment exhibited protection against liver damage caused by CCl_4 which is confirmed by the results of biochemical studies.

It has been reported that *Eupatorium ayapana* contain 7-Methoxy coumarin [ayapanin], 6, 7-dimethoxy coumarin [ayapin]; carotene, vitamin-C and stigmasterol [4, 5]. A number of scientific reports indicated certain coumarin carotene, vitamin-C and steroids have protective effect on liver due to its antioxidant properties [30, 31]. Presence of those compounds in MEEA may be responsible for the protective effect on CCl_4 induced liver damage in rats.

In conclusion, the results of this study demonstrate that MEEA has a potent hepatoprotective action upon carbon tetrachloride-induced hepatic damage in rats. Our results show that the hepatoprotective and antioxidant effects of MEEA may be due to its antioxidant and free radical scavenging properties. Further, investigation is underway to determine the exact phytoconstituents that is responsible for its hepatoprotective and antioxidant activity.

ACKNOWLEDGEMENT

One of the author Pombya Bose, grateful to AICTE, New Delhi, India, for providing financial support to this work.

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