

## 1 RESEARCH ARTICLE

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

 5 POMBYA BOSE, MALAYA GUPTA, UPAL KANTI MAZUMDER, RAMANATHAN SAMBATH KUMAR,  
 6 THANGAVEL SIVAKUMAR and RAMAN SURESH KUMAR

7 For author affiliations, see end of text.

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## 10 ABSTRACT

 11 The present study was carried out to evaluate the hepatoprotective and antioxidant effect of the methanol  
 12 extract of *Eupatorium ayapana* (MEEA) (Family- Asteraceae) leaves in Wistar albino rats. The different  
 13 groups of animals were administered with carbon tetrachloride (CCl<sub>4</sub>) at 72 h interval. The MEEA 100,  
 14 200 and 300 mg/kg and silymarin 25 mg/kg were administered to the CCl<sub>4</sub> treated rats. The effect of  
 15 MEEA and silymarin on serum transaminase (SGOT, SGPT), serum alkaline phosphates (SALP),  
 16 bilirubin, uric acid and total protein were measured. CCl<sub>4</sub> induced hepatotoxicity in rats. Further, the effects  
 17 of the extract on lipid peroxidation (LPO), enzymatic antioxidant such as superoxide dismutase (SOD)  
 18 and catalase (CAT), and nonenzymatic antioxidant like glutathione (GSH) were estimated. The MEEA  
 19 and silymarin produced significant ( $p < 0.05$ ) hepatoprotective effect by decreasing the activity of serum  
 20 enzymes, bilirubin, uric acid, and lipid peroxidation and significantly ( $p < 0.05$ ) increased the levels of  
 21 SOD, CAT, GSH and protein in a dose dependent manner. From these results, it was suggested that  
 22 MEEA possess potent hepatoprotective and antioxidant properties.

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

 24 Free radical initiated autoxidation of cellular mem- 46 such as metabolism, secretion and storage. Furthermore,  
 25 brane lipids can lead to cellular necrosis and is now ac- 47 detoxification of a variety of drugs and xenobiotics oc-  
 26 cepted to be important in connection with a variety of 48 curred in liver. Liver diseases are mainly caused by toxic  
 27 pathological conditions [1]. Living cell evolved de- 49 chemicals (antibiotics, chemotherapeutics, peroxidised  
 28 fences against free radicals and reduction of these per- 50 oil, aflatoxin, carbon tetrachloride, chlorinated hydro-  
 29 oxy radicals by antioxidant molecules is crucial to the 51 carbons, etc.), excess consumption of alcohol, infections  
 30 protection of cells against the development of prooxi- 52 and autoimmune disorder. Most of the hepatotoxic  
 31 dant state. Antioxidants may protect against reactive 53 chemicals damage liver cells mainly by inducing lipid  
 32 oxygen species (ROS) toxicity by the prevention of 54 peroxidation and other oxidative damages in liver [4,  
 33 ROS formation, by the interruption of ROS attack, by 55 5]. Plants have proven to be used effectively as hepato-  
 34 scavenging the reactive metabolites and converting 56 protective agents [6].

 35 them to less reactive molecules and/or by enhancing the 57 *Eupatorium ayapana* Vent (Family: Asteraceae) is  
 36 resistance of sensitive biological targets to ROS attack, 58 commonly known as Visalyakarani- Hindi. The Eupato-  
 37 by facilitating the repair caused by ROS and by provid- 59 rium genus is one of the most important plants used in  
 38 ing co-factors for the effective functioning of other anti- 60 herbal medicine. *Eupatorium ayapana* is a native of  
 39 oxidants [2]. Developments of life threatening diseases 61 Brazil and has long been naturalized in India. It is an  
 40 like cancer are linked to the availability of these anti- 62 aromatic under shrub. Chemical constitutions like 7-  
 41 oxidants [3]. Low levels of antioxidants, which further 63 Methoxy coumarin [ayapanin], 6, 7-dimethoxy cou-  
 42 increases the free radical activity, are clearly associated 64 marin [ayapin]; carotene, vitamin-C and stigmaterol  
 43 with cancer conditions. 65 were isolated from its leaves [7]. Five additional cou-

 44 Liver has a pivotal role in regulation of physiologi- 66 marins, viz. daphnetin, daphnetin dimethyl ether, hy-  
 45 cal processes. It is involved in several vital functions 67 drangetin, daphnetin -7-methyl ether and umbelliferone

68 have also been isolated [8]. The leaves possess a cou-124 to standard dry pellet diet (Hindustan Lever, Kolkata,  
69 marin like odour and yield a pale green essential oil on 125 India) and water *ad libitum*. The rats were acclimatized  
70 steam distillation. The principal constituent of the oil is 126 to laboratory condition for 10 days before commence-  
71 thymohydroquinone, dimethyl ether, sesquiterpene, and 127 ment of experiment. All procedures described were re-  
72 traces of coumarin. 128 viewed and approved by the University Animal Ethical

73 The plant is used as antiperiodic, cardiac stimulant, 129 Committee.

74 diaphoretic, diuretic, emetic, expectorant and tonic. In-

75 fusion maybe useful in ague, cough, and dyspepsia. The

76 leaves (fresh bruised) are applied to scores, snakebite

77 and foul ulcers. The juice of fresh leaves is a digestive

78 agent and haemostatic [9]. *Eupatorium ayapana* is con-

79 sidered as the most powerful therapeutic agent. A de-

80 coction of the leaves has been used as a popular remedy

81 against various kinds of hemorrhage. It is given inter-

82 nally as an antidote to snake bites. In Europe, the dried

83 leaves of the plant were used as a tonic under the name

84 of 'Ayapana tea'. The coagulation time of rabbit's blood

85 was diminished when traces of finely divided ayapanin

86 or ayapin were added *in vitro* [10]. Both ayapanin and

87 ayapin are non-toxic and are effective when applied

88 locally or when administered by subcutaneous injections

89 or by mouth. They have no effect on respiration or on

90 blood pressure [11]. Previous report from our laboratory

91 on *Eupatorium ayapana* showed antimicrobial activity

92 [12]. The tribal peoples of Kolli Hills of Tamilnadu,

93 India, used the leaves for the treatment of liver disor-

94 ders.

95 Based on the pervious report, traditional usage and

96 chemical constituents we selected the plant for the

97 study. The purpose of the present study was to evaluate

98 the hepatoprotective and antioxidant activities of metha-

99 nol extract of *Eupatorium ayapana* (MEEA) in rats.

## 100 MATERIALS AND METHODS

### 101 Plant materials and Extraction

102 The plant *Eupatorium ayapana* (Family: Asteraceae)

103 leaves were collected in the month of March 2004 from

104 the Kolli Hills, Tamil Nadu, India. The plant material

105 was taxonomically identified by Botanical Survey of

106 India, Kolkata, India, and the Voucher specimen

107 (No.GMP-12) was retained in our laboratory for future

108 reference. The dried powder material of the *Eupatorium*

109 *ayapana* leaves was extracted with methanol (Yield

110 8.53 %) in a soxhlet apparatus. The methanol extract

111 was then distilled, evaporated and dried in vacuum. The

112 chemical constituents of the extract were identified by

113 qualitative analysis followed by their confirmation by

114 thin layer chromatography.

### 115 Animals

116 Studies were carried out using male Wistar albino

117 rats (150–180 g). They were obtained from the animal

118 house, Indian Institute of Chemical Biology (ICB),

119 Kolkata, India. The animals were grouped and housed in

120 polyacrylic cages (38 x 23 x 10 cm) with not more than

121 six animals per cage and maintained under standard

122 laboratory conditions (temperature  $25 \pm 2^\circ\text{C}$ ) with dark

123 and light cycle (14/10 h). They were allowed free access

124 to standard dry pellet diet (Hindustan Lever, Kolkata,  
125 India) and water *ad libitum*. The rats were acclimatized  
126 to laboratory condition for 10 days before commence-  
127 ment of experiment. All procedures described were re-  
128 viewed and approved by the University Animal Ethical

129 Committee.

### 130 Drugs and Chemicals

131 Silymarin was purchased from Micro labs Hosur

132 Tamilnadu India, 1-Chloro-2, 4-dinitrobenzene

133 [CDNB], Bovine serum albumin was from Sigma

134 chemical St. Louis, MO, USA, Thiobarbituric acid, and

135 Nitrobluetetrazolium chloride (NBT) were from Loba

136 Chemie, Bombay, India, and 5,5'-dithio *bis*-2-

137 nitrobenzoic acid (DTNB) and Carbon tetrachloride

138 were from SICCO research laboratory, Bombay. The

139 solvent and / or reagent obtained were used as received.

### 140 Toxicity study

141 For toxicity studies groups of 10 mice were adminis-

142 tered (p.o.) with test extracts in the range 100-1600

143 mg/kg and mortality rates were observed after 24 hours.

144 LD<sub>50</sub> was determined using the graphical methods of

145 Litchfield and Wilcoxon [13].

### 146 Carbon tetrachloride-induced liver damage in rats

147 Healthy male albino rats were divided into 6 groups

148 each containing 6 animals. Group 1 Normal (Liquid

149 paraffin 1ml/kg body weight, p.o.) Group 2 (Control)

150 received 30% CCl<sub>4</sub> in liquid paraffin (1 ml/kg body

151 weight, i.p.). Group 3, 4 and 5 received MEEA 100, 200

152 and 300 mg/kg p.o. respectively and Group 6 received

153 standard drug Silymarin (25 mg/kg p.o) once in a day

154 and CCl<sub>4</sub> as mentioned above. Treatment duration was

155 10 days and the dose of CCl<sub>4</sub> was administered every

156 72-h [14]. Animals were sacrificed 24 h after the last

157 injection. Blood was collected, allowed to clot and se-

158 rum separated. The liver was dissected out and used for

159 biochemical and histopathology studies.

### 160 Biochemical Studies

161 Blood was obtained from all animals by puncturing

162 retro-orbital plexus. The blood samples were allowed to

163 clot for 45 min at room temperature. Serum was sepa-

164 rated by centrifugation at 2500 rpm at 30°C for 15 min

165 and utilized for the estimation of various biochemical

166 parameters namely Serum glutamyl pyruvate transami-

167 nase (SGPT), Serum glutamyl oxalacetic acid transami-

168 nase (SGOT) [15], Serum alkaline phosphatase (SALP)

169 [16], serum bilirubin [17], protein content [18] and

170 plasma uric acid [19].

171 After collection of blood samples rats were sacri-

172 ficed and their livers excised, rinsed in ice cold normal

173 saline followed by 0.15 M Tris-HCl (pH 7.4) blotted dry

174 and weighed. A 10 % w/v of homogenate was prepared

175 in 0.15 M Tris-HCl buffer and processed for the estima-

176 tion of lipid peroxidation [20]. A part of homogenate

177 after precipitating proteins with Trichloro acetic acid

178 (TCA) was used for estimation of glutathione [21]. The

179 rest of the homogenate was centrifuged at 15000 rpm

180 for 15 min at 4° C. The supernatant thus obtained was

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<sub>4</sub> induced hepatic damage in rats

Parameters	Normal (liquid paraffin 1ml/kg, b.wt)	Control (CCl <sub>4</sub> 1ml/kg, b.wt)	Silymarin (25 mg/kg) + CCl <sub>4</sub>	MEEA (100 mg/kg) + CCl <sub>4</sub>	MEEA (200 mg/kg) + CCl <sub>4</sub>	MEEA (300mg/kg) + CCl <sub>4</sub>
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** (95.65)	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<sub>4</sub>. The % of protection is calculated as 100 X (values of CCl<sub>4</sub> control - values of sample) / (values of CCl<sub>4</sub> 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<sub>4</sub> control group \*\*  $p < 0.05$

181 used for the estimation of SOD [22] and CAT activities 219 volume of 2 ml. After completion of the total reaction,  
182 [23]. 220 solutions were measured at 412 nm against blank. Ab-

### 183 Lipid peroxidation

184 The tissues were then homogenized in 0.1M buffer 223 Estimation of SOD  
185 (pH 7.4) with a Teflon-glass homogenizer. Lipid per-  
186 oxidation in this homogenate was determined by meas-224 SOD activity of the liver tissue was analyzed by the  
187 uring the amounts of malondialdehyde (MDA) produced 225 method described by Kakkar et al. (1984). Assay mix-  
188 primarily, according to the method of Ohkawa et al. 226 ture contained 0.1 ml of sample, 1.2 ml of sodium pyro-  
189 (1979). 0.2 mL of tissue homogenate, 0.2 ml of 8.1% 227 phosphate buffer (pH 8.3, 0.052 M), 0.1 ml phenazine  
190 Sodium dodecyl sulphate (SDS), 1.5 ml 20% acetic acid 228 methosulphate (186 µM), 0.3 ml of 300 µM nitroblue  
191 and 1.5 ml 8% TBA were added. The volume of the 229 tetrazolium and 0.2 ml NADH (750 µM). Reaction was  
192 mixture was made upto 4 ml with distilled water and 230 started by addition of NADH. After incubation at 30° C  
193 then heated at 95° C on a water bath for 60 min using 231 for 90 s, the reaction was stopped by the addition of 0.1  
194 glass balls as condenser. After incubation the tubes were 232 ml glacial acetic acid. Reaction mixture was stirred vig-  
195 cooled to room temperature and final volume was made 233 orously with 4.0 ml of n-butanol. Mixture was allowed  
196 to 5 ml in each tube. 5.0 ml of butanol:pyridine (15:1) 234 to stand for 10 min, centrifuged and butanol layer was  
197 mixture was added and the contents were vortexed thor- 235 separated. Color intensity of the chromogen in the bu-  
198 oughly for 2 min. After centrifugation at 3000 rpm for 236 tanol layer was measured at 560 nm spectrophotometri-  
199 10 min, the upper organic layer was taken and its OD 237 cally and concentration of SOD was expressed as  
200 read at 532 nm against an appropriate blank without the 238 units/mg protein.

201 sample. The levels of lipid peroxides were expressed as  
202 n moles of thiobarbituric acid reactive substances 239 Estimation of CAT  
203 (TBARS)/mg protein using an extinction coefficient of  
204  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

### 205 Estimation of GSH

206 To measure the reduced glutathione (GSH) level, the 244 prepared 30 mM H<sub>2</sub>O<sub>2</sub>. The rate of decomposition of  
207 tissue homogenate (in 0.1 M phosphate buffer pH 7.4) 245 H<sub>2</sub>O<sub>2</sub> was measured spectrophotometrically from  
208 was taken. The procedure was followed initially as de- 246 changes in absorbance at 240 nm. Activity of catalase  
209 scribed by Ellman 1959. The homogenate was added an 247 was expressed as units/mg protein.

### 212 The mixture was allowed to stand for 5 min prior to 248 Histological investigation

213 centrifugation for 10 min at 200 rpm. The supernatant 249 Liver slices fixed for 48hr in 10% formalin were  
214 (200µl) was then transferred to a new set of test tubes 250 processed for paraffin embedding following the stan-  
215 and added 1.8 ml of the Ellman's reagent (5, 5'-dithio 251 dard micro technique [24]. Sections (5µm) of livers  
216 bis-2-nitrobenzoic acid) (0.1mM) which was prepared 252 were stained with haematoxylin and eosin was evalu-  
217 in 0.3M phosphate buffer with 1% of sodium citrate 253 ated for histopathological changes under a light micro-  
218 solution). Then all the test tubes were made upto the 254 scope.

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<sub>4</sub> intoxicated rats.

Parameters	Normal (liquid paraffin 1ml/kg, b.wt)	Control (CCl <sub>4</sub> 1ml/kg, b.wt)	Silymarin (25 mg/kg) + CCl <sub>4</sub>	MEEA (100 mg/kg) + CCl <sub>4</sub>	MEEA (200 mg/kg) + CCl <sub>4</sub>	MEEA (300mg/kg) + CCl <sub>4</sub>
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<sub>4</sub>. The % of protection is calculated as 100 X (values of CCl<sub>4</sub> control - values of sample) / (values of CCl<sub>4</sub> 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<sub>4</sub> control group \*\*  $p < 0.05$

## 255 Statistical analysis

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

292 MDA level of MEEA 100, 200 and 300 mg/kg group,  
293 were significantly inhibited by 22.56, 46.10 and 93.99  
294 % compared to CCl<sub>4</sub> control (\* $p < 0.05$ ). At the same  
295 time, the effect of silymarin 25 mg/kg on MDA levels in  
296 CCl<sub>4</sub> was significantly inhibited by 98.22 % respec-  
297 tively (\* $p < 0.05$ ).

## 298 Effect of MEEA on Glutathione

299 The effect of MEEA on glutathione content in the  
300 liver is shown in Table 2. GSH level in normal group  
301 was measured to be significantly higher than in CCl<sub>4</sub>  
302 control group (\* $p < 0.001$ ). GSH level of MEEA 100,  
303 200 and 300 mg/kg groups were significantly increased  
304 by 13.69, 66.08, and 94.34% respectively as compared  
305 to CCl<sub>4</sub> control group (\*\* $p < 0.05$ ). Silymarin almost  
306 completely restored the glutathione level in CCl<sub>4</sub> treated  
307 groups to the normal level.

## 261 RESULTS

### 262 Acute toxicity

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

### 266 Effect of MEEA on serum enzymes, bilirubin, uric 267 acid and protein

268 Activities of serum enzymes (SGPT, SGOT and  
269 SALP), concentrations of bilirubin, uric acid and total  
270 protein content of the serum of CCl<sub>4</sub> induced liver dam-  
271 age rats are presented in Table 1. The level of serum  
272 marker enzymes SGPT, SGOT, SALP, bilirubin and  
273 uric acid were found to be significantly increased and  
274 protein content significantly decreased in CCl<sub>4</sub>-induced  
275 liver damage rats when compared with the normal group  
276 (\*\* $p < 0.001$ ). Where as treatment with MEEA at the  
277 dose of 100, 200 and 300 mg/kg showed significantly  
278 decreased activity of serum transaminase, SALP, uric  
279 acid, bilirubin and increased protein content in CCl<sub>4</sub>-  
280 induced liver damage in rats compared to that of control  
281 groups (\* $p < 0.05$ ). Silymarin (25 mg/kg) also signifi-  
282 cantly decreased the levels of serum enzymes, bilirubin,  
283 uric acid and increased the protein content in CCl<sub>4</sub>  
284 treated groups as compared with the respective control  
285 group (\* $p < 0.05$ ).

### 286 Effect of MEEA on In vivo Lipid peroxidation

287 The localization of radical formation resulting in  
288 lipid peroxidation, measured as MDA in rat liver ho-  
289 mogenate, is shown in Table 2. MDA content in the  
290 liver homogenate was significantly increased in CCl<sub>4</sub>  
291 control group compared to normal group (\*\* $p < 0.001$ ).

### 308 Effect of MEEA on SOD and CAT activity in liver 309 tissues

310 The effect of MECA on SOD and CAT activities in  
311 liver is shown in Table 2. SOD activity in CCl<sub>4</sub> control  
312 group was examined to be lower than in normal group  
313 (\* $p < 0.001$ ). SOD activities in MECA 100, 200 and 300  
314 mg/kg groups were observed to be significantly higher  
315 than in CCl<sub>4</sub> control group (\* $p < 0.05$ ). SOD activities  
316 of MEEA 100, 200 and 300 mg/kg were significantly  
317 improved by 10.50, 29.92 and 81.31 % respectively.  
318 Silymarin 25 mg/kg also restored the SOD activity in  
319 CCl<sub>4</sub> treated groups. CAT activity of CCl<sub>4</sub> control group  
320 was measured to be strikingly lower than in normal  
321 group (\* $p < 0.001$ ). Liver CAT activities in MEEA 100,  
322 200 and 300 mg/kg groups were significantly increased  
323 by 16.07, 50.87 and 88.37 % respectively when com-  
324 pared with control group (\*\* $p < 0.05$ ). MEEA and sily-  
325 marin completely restored the enzyme activity to the  
326 normal level at the respective doses of 300 mg/kg and  
327 25 mg/kg.

### 328 Effect of MEEA on Histopathology of liver

329 Comparison of liver section of normal animals  
330 (Fig.1) with CCl<sub>4</sub> treated animals is presented in Fig 2.

The liver cells of rats intoxicated with  $\text{CCl}_4$  had high degree of damage. Treatment with MEEA 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  $\text{CCl}_4$  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  $\text{CCl}_4$ -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  $\text{CCl}_4$ -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  $\text{CCl}_4$  involves initially the metabolism of  $\text{CCl}_4$  to trichloromethyl free radical by the mixed-function oxidase system of the endoplasmic reticulum. It is postulated that secondary mechanisms link  $\text{CCl}_4$  metabolism to the widespread disturbances in hepatocyte function. These secondary mechanisms could involve the generation of toxic products arising directly from  $\text{CCl}_4$  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  $\text{CCl}_4$  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  $\text{CCl}_4$  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  $\text{O}_2^-$  into  $\text{H}_2\text{O}_2$  plus  $\text{O}_2$ , 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  $\text{CCl}_4$  induced liver damage to prevent the accumulation of excessive free radicals and protects the liver from  $\text{CCl}_4$  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.  $\text{CCl}_4$  treated animals (Fig.2) shows that the liver cells of rats intoxicated with  $\text{CCl}_4$  have high degree of damage. In  $\text{CCl}_4$  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 neutrophilic 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  $\text{CCl}_4$  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

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  $\text{CCl}_4$  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  $\text{CCl}_4$  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.

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#### CURRENT AUTHOR ADDRESSES

POMBYA BOSE, 1Department of Pharmaceutical Technology, Division of Pharmacology and Pharmaceutical Chemistry, Jadavpur University, Kolkata, 700 032, India.

MALAYA GUPTA, 1Department of Pharmaceutical Technology, Division of Pharmacology and Pharmaceutical Chemistry, Jadavpur University, Kolkata, 700 032, India.

UPAL KANTI MAZUMDER, 1Department of Pharmaceutical Technology, Division of Pharmacology and Pharmaceutical Chemistry, Jadavpur University, Kolkata, 700 032, India.

RAMANATHAN SAMBATH KUMAR, Natural Products Research Laboratory, J. K. K. Nataraja College of Pharmacy, Komarpalayam 638 183, Namakkal, Tamilnadu, India. E-mail: sambathju2002@yahoo.co.in ( Corresponding author)

574 THANGAVEL SIVAKUMAR, Natural Products Research Labora-577 RAMAN SURESH KUMAR, Natural Products Research Laboratory,  
575 tory, J. K. K. Nataraja College of Pharmacy, Komarapalayam 638578 J. K. K. Nataraja College of Pharmacy, Komarapalayam 638 183,  
576 183, Namakkal, Tamilnadu, India. 579 Namakkal, Tamilnadu, India.  
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