total protein were determined by using commercially available kits (Span Diagnostic Limited, Surat, India).

Lipid peroxidation

Liver tissues were homogenized in 1.15% KCl and the homogenate were centrifuged at 10000 g at 4 °C for 20 min. From this microsomal fraction, lipid peroxidation in liver was ascertained by the formation of malondialdehyde (MDA) and measured by the thiobarbituric acid reactive substance method according to Ohkawa et al (24). The levels of lipid peroxides were expressed as 'n' moles of thiobarbituric acid substances (MDA)/gm of tissue using extinction co-efficient of 1.56×10^5 $M^{-1}cm^{-1}$.

Glutathione content (GSH)

Tissue fragments was thawed and homogenized on ice in 1 mL of homogenizing buffer (250 mM sucrose, 20 mM Tris-HCl, 1mM dithiothreitol, pH 7.4), using glass-teflon homogenizers. The homogenates were centrifuged at 15,000 rpm at 4 °C for 2 h. The reduced glutathione was determined by the method of Ellman (25).

Catalase activity (CAT)

Liver tissue was homogenized in M/150 phosphate buffers in ice and centrifuged at 2000 rpm for 10 min at 4 °C. From the supernatant, Catalase activity was assayed by the method of Aebi (26).

Histological study

Liver tissues were fixed in 10% buffered formaldehyde and processed for histological examination by conventional methods (27) and stained with hematoxylin and eosin dye and finally, observed under a photomicroscope and morphological changes such as cell necrosis, ballooning degeneration, fatty changes or inflammation of lymphocytes were observed.

Statistical analysis

The results were expressed as mean ± S.D. The statistical significance of differences between groups was determined by one way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons among groups by using GraphPad Prism4, La Jolla, CA 92037, USA. Differences of $p<0.05$ were considered statistically significant.

RESULT AND DISCUSSION

Preliminary phytochemical analysis of methanol extract of *C. infortunatum* showed the presence of saponins, terpenoids and flavonoids. The acute toxicity of MECI was found to have the LD_{50} value of 450 mg/kg.

Biochemical estimations

The effect of methanol extract of *C. infortunatum* on serum biochemical parameters was shown on Table 1. Administration of CCl_4 alone resulted in significantly (*p<0.01*) increase of all the serum biochemical parameters like aspartate aminotransferase (GOT), alanine aminotransferase (GPT), alkaline phosphatatse (ALP) and total bilirubin. Treatment with MECI at a dose of 100 mg/kg significantly decreased ALT (63.41 IU/L, *p<0.01*), ALP (293.4 IU/L, *p<0.01*) and bilirubin (3.12 IU/L, *p<0.01*) where as AST (304.4 IU/L) was not significantly reduced. Treatment with MECI at 200 mg/kg significantly (*p<0.01*) reduced ALT, ALP, bilirubin and AST (*p<0.05*). Treatment with MECI restored the decreased level of protein caused by CCl_4. The increase of all the serum enzymes and bilirubin indicates the cellular leakages and loss of functional integrity of cell membrane in liver. Decreased level of GOT, GPT, ALP and bilirubin suggested that MECI preserved the structural integrity of the hepatocellular membrane and liver cell architecture damage caused by CCl_4.

MDA levels

Effect of MECI on lipid peroxidation has been shown on Table 2. MDA contents in liver homogenate of CCl_4 treated group (65.35 nM/mg tissue) was significantly (*p<0.01*) increased than the normal rats (25.61 nM/mg tissue). Lipid peroxidation was inhibited with the treatment of MECI

Table 1. Effects of methanol extract *C. infortunatum* (MECI) and silymarin on serum biochemical parameters#.

Biochemical parameters	Normal	CCl_4 1 ml/kg	MECI 100 mg/kg	MECI 200 mg/kg	Silymarin 25 mg/kg	F value
ALT	33.84 ± 4.1*a*	91.76 ± 18.37	63.41 ± 5.02*a*	61.17 ± 4.72*a*	41.34 ± 12.15*a*	27.88
AST	109.94 ± 18.05*a*	372.72 ± 72.95	304.4 ± 18.38^{ns}	297.9 ± 13.95*b*	187.0 ± 53.38*a*	30.32
ALP	120.6 ± 25.53*a*	400.74 ± 34.07	293.4 ± 5.84*a*	278.6 ± 10.16*a*	161.70 ± 21.79*a*	128.8
Total protein	8.39 ± 1.19*a*	4.72 ± 0.54	6.08 ± 0.48*b*	6.12 ± 0.24*b*	7.63 ± 0.47*a*	19.89
Total bilirubin	1.29 ± 0.19*a*	4.66 ± 0.24	3.12 ± 0.16*a*	2.9 ± 0.27*a*	2.12 ± 0.13*a*	180.3

#Values are mean ± s.d. (n = 6)

a $p<0.01$,

b*p<0.05, ns* = not significant

Table 2: Effects of methanol extract of *C. infortunatum* on lipid peroxidation, GSH content and CAT activity*.

Biochemical parameters	Normal	CCl_4 1 ml/kg	MECI 100 mg/kg	MECI 200 mg/kg	Silymarin 25 mg/kg	F value
MDA content (nM/gm tissue)	25.61 ±1.77*a*	65.35 ±1.95	57.02 ±10.69*b*	49.12 ±5.17*a*	44.15 ±2.09*a*	44.24
GSH level (µM/mg tissue)	11.01 ±2.08*a*	7.67 ±1.27	9.08 ±1.33^{ns}	10.04 ±0.55*b*	10.20 ±0.53*a*	4.913
CAT activity (U/mg tissue)	274.4 ±7.81*a*	152.1 ±9.71	201.6 ±11.25*a*	209.9 ±20.38*a*	251.4 ±8.32*a*	73.13

*values are mean ± s.d. (n=5)

[a]*p<0.01,*

[b]*p<0.05, ns* not significant

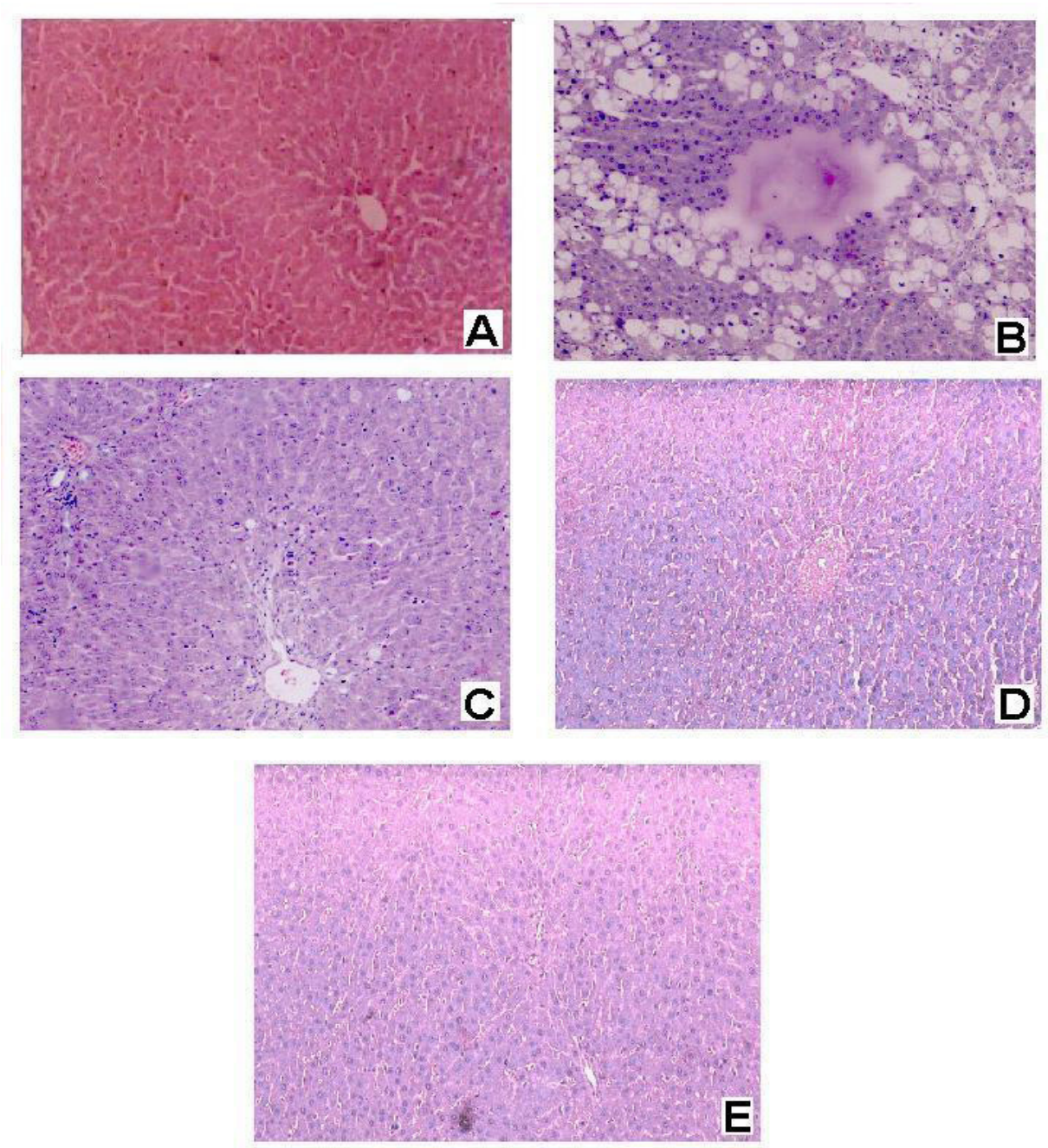

Figure 1: Histopathological examination of liver tissues of different experimental groups (A) normal rats (B) CCl_4 treated rats (C) rats treated with MECI (100 mg/kg) along with CCl_4 (D) rats treated with MECI (200 mg/kg) along with CCl_4 (E) rats treated with silymarin (25 mg/kg) along with CCl_4.

at 100 mg/kg (57.02 nM/mg tissue, $p<0.05$) and MECI at 200 mg/kg (49.12 nM/mg tissue, $p<0.01$). Metabolism of CCl_4 leads to formation of reactive intermediates which induce lipid peroxidation and increase MDA content. It has been shown that MDA is mutagenic to human cells (28) and play a significant role in DNA damage, sister-chromatid exchanges (SCEs) and carcinogenesis (29). The significant reduction in MDA content in animals treated with MECI suggests the protection of liver through its inhibitory action on lipid peroxidation.

GSH content

Table 2 showed the effect of MECI on the GSH content of liver homogenate. Treatment with CCl_4 alone was significantly ($p<0.01$) reduced the total GSH content of liver homogenate (7.67 µM/mg tissue) compared to normal rats (11.01 µM/mg tissue). GSH content was significantly ($p<0.05$) increased on treatment with MECI at 200 mg/kg (10.04 µM/mg tissue). Glutathione can be considered as an important reductant and protects cells against free radicals, peroxides and other toxic compounds. Liver injury by consuming alcohol (30) or by taking drugs like acetaminophen (31), lung injury by smoking (32) and muscle injury by intense physical activities (33) are all known to be correlated with low tissue levels of GSH. The GSH reduction in control group may be explained by increased utilization of GSH for removal of ROS. In our experiment MECI, prevents the depletion of GSH which caused detoxification of free radicals produced following carbon tetrachloride intoxification.

CAT activity

CAT activities of liver homogenate of rats for experimental groups are shown on table 2. The CAT activity in the liver tissue homogenates of CCl_4 treated rats (152.1 U/mg tissue) was significantly ($p<0.01$) lower than that of normal rats (274.4 U/mg tissue). In the MECI and silymarin treated rats the CAT activity was significantly ($p<0.01$) higher than the CCl_4 treated rats. CAT is the key component of antioxidant defense system, which decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Increase in CAT activity by MECI suggesting the improvement of enzymatic defense system against free radicals.

Histological studies

The hepatoprotective activity of MECI was supported by histological assessment, in which the normal rats did not showed any abnormal state in the liver architecture (Fig. 1A). Rats treated with CCl_4 showed excess fatty generation and nodules formation (Fig. 1B), whereas, the MECI and silymarin treated group showed reduction in all the abnormal architecture (Fig. 1C, 1D,1E). So, hepatoprotective activity also can be explained on the basis of histological examination.

CONCLUSION

On the basis of the experimental results, it can be concluded that *Clerodendrum infortunatum* have moderate hepatoprotective activity. The hepatoprotective effect may be due to inhibition of lipid peroxidation and increasing the activity the content of enzymatic defense system, which causes the recuperation of biological parameters and the integrity of the tissue. The hepatoprotective activity of methanol extract of *C. infortunatum* may be due to presence of flavonoids, terpenoids and saponins in the extract. The present results can be accounted for the traditional uses of the plant in treating some common diseases.

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