



## PROTECTIVE EFFECTS OF COMMELINA BENGHALENSIS LINN (ROOT) EXTRACT ON ETHANOL INDUCED ACUTE HEPATOTOXICITY IN RATS

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### ABSTRACT

The present study was undertaken to investigate the protective effect and possible mechanism of alcoholic (AIE) and aqueous extract (AqE) from *Commelina benghalensis* root (CB) on EtOH-induced hepatic injury in Wistar rat. Hepatotoxic parameters studied *in vivo* include serum transaminases (AST, and ALT), ALP, bilirubin, protein, lipid profile (Cholesterol, triglyceride, VLDL and HDL) and level of antioxidants together with histopathological examination. Liv 52<sup>®</sup> was used as a reference hepatoprotective agent (5ml/kg<sup>-1</sup>b.w.). AIE and AqE (200 mg/kg<sup>-1</sup>b.w.) on oral administration decreased the level of AST, ALP, ALT, bilirubin, cholesterol, triglyceride, VLDL, MDA and increased the level of protein, HDL and antioxidants (SOD, GSH and CAT) in rats being treated with ethanol (EtOH). Pentobarbitone -induced sleeping time study was carried out to verify the effect on microsomal enzymes Histopathological observations confirmed the beneficial roles of MF against EtOH-induced liver injury in rats. Possible mechanism may involve their antioxidant activity.

**Keywords:** Commelina benghalensis, hepatoprotective ethanol, Liv 52<sup>®</sup>, sleeping time

## INTRODUCTION

Alcohol affects the liver in both the way; nutritional disturbances and damaging cells, that leads to alcoholic liver disease (ALD): fatty liver, alcoholic hepatitis and cirrhosis.<sup>1</sup> These potential damage elicits by complex mechanisms involving metabolite of EtOH that has direct cytotoxicity and inability to form protein adduct with several protein of hepatocytes,<sup>2,3</sup> the increase in reduced form of nicotinamide adenine dinucleotide (NADH) causing fat accumulation,<sup>3</sup> free radicals inducing oxidative stress; leading to peroxidation and inflammatory response<sup>4</sup> and EtOH-induced elevation of endotoxin pass to the liver. Endotoxin stimulates Kupffer cells to produce free radicals and pro-inflammatory cytokines such as TNF $\alpha$  and IL-1 $\beta$ , the two important mediators of inflammation and cell death<sup>5,6,7</sup>

Hepatitis, a high incidence ailment around the world, is induced by viruses, alcohol, lipid peroxidative products and various drugs<sup>8</sup> Chronic liver injury leads to liver fibrosis and end stage cirrhosis. This is a major public health problem, owing to life-threatening complications of portal hypertension, liver failure and increased incidence of hepatocellular carcinoma<sup>9</sup>.

In spite of tremendous advances in modern medicine no effective drugs are available, which stimulate liver functions and offers protection to the liver from the damage or help to regenerate hepatic cells<sup>10</sup> In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders<sup>11</sup> and quite often claimed to offer significant relief. Attempts are being made globally to get scientific evidences for these traditionally reported herbal drugs. *Commelina benghalensis* is a widely distributed plant throughout the India, and is a popular folk medicine<sup>12</sup> extensively used by traditional healers in the treatment of jaundice.

Indian subcontinent; uses this plant as a folk medicine for the treatment of leprosy, headache, fever, constipation, jaundice and snake bite.<sup>13, 14</sup> The plant is also used for mouth thrush, inflammation of the conjunctiva, psychosis, epilepsy, nose blockage in children,<sup>15</sup> insanity and exophthalmia.<sup>16</sup> It is also used as diuretic, febrifuge and anti-inflammatory.<sup>17, 18</sup> It is used as an animal fodder, eaten by humans as a vegetable in Pakistan including as a laxative and to cure inflammations of the skin as well as leprosy. The plant is also reported to have antitumor, anticancer and antioxidant activity.<sup>13, 19, 20.</sup>

## MATERIALS AND METHODS

### Preparation of *Commelina benghalensis* root Extract:

Roots of *Commelina benghalensis* were collected from open field around the Belgaum city in the month of September and were authenticated by the taxonomist Dr. Harsha Hegde and the herbarium (voucher No. RMRC 486) has been preserved at RMRC, Belgaum. Shade dried roots were powdered to moderately coarse grade and subjected for the various extractions. Petroleum ether, chloroform, alcohol extracts of leaves were obtained by using soxhlet extractor and aqueous extract by maceration process. After evaporating the solvent; the dark brown semisolid extract was kept in an air tight container at 4<sup>o</sup> C for

future use. Suspensions of each extract were freshly prepared using 0.1% Tween 80, for experimental use.

### **Animals:**

The complete course of the experiment was carried out using healthy adult male Wistar rats obtained from registered breeders (Venkateshwara Enterprises, Bangalore) and was maintained at animal house of the institution. They were fed on commercial laboratory animal feed (Amrut brand, Sangli) and tap water ad lib. The rats weighing between 120-150g were housed in laboratory for about a week for acclimatization with natural 12:12 hr light-dark cycle. The animals were starved overnight with tap water ad lib prior to the day of experimentation. Ethical clearance was obtained from Institutional Animal Ethics Committee constituted as per CPCSEA guidelines.

### **Acute Toxicity Study:**

Acute toxicity studies were carried out for all the extracts as per OECD guideline 425<sup>21</sup> in Wistar rats weighing 80 to 120g by administering a dose 2000 mg/kg orally. The groups were almost continuously observed for mortality and behavioral changes during first 24hr and then daily for a fortnight. The oral LD<sub>50</sub> was found to be more than 2000mg/kg. Therefore 1/10<sup>th</sup> of LD<sub>50</sub> was used as effective dose in the further study.

### **Drugs used and their Doses:**

The rats weighing between 120-150g were divided into five groups (n=6). Group I (normal control) received 0.1% tween80, Group II (positive control) received EtOH 20%, 25 ml/kg<sup>-1</sup>, Group III received Liv52® 5ml/ kg<sup>-1</sup>, Group IV and V received alcoholic and aqueous extract 200mg/kg<sup>-1</sup>. All the treatments were administered orally for 90 days.

## **METHODOLOGY**

All the treatments and administration of EtOH was done orally with the gap of one hour for a total period of 90 days, on the 91<sup>st</sup> day all the rats were anaesthetized by halothane; blood was withdrawn by cardiac puncture and animals were sacrificed by over anesthesia to dissect out liver for histopathological studies and oxidative stress markers. Blood was allowed to coagulate for 30 min and serum was separated by centrifugation at 2500 rpm, to estimate alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein and bilirubin (total and direct) content,<sup>22</sup> Lipid profile (Cholesterol, triglyceride, VLDL and HDL).<sup>23</sup> Liver was kept in cold conditions. It was cross chopped with surgical scalpel into fine slices in chilled 0.25M sucrose solution, quickly blotted on a filter paper. The tissue was minced and homogenized in 10mM Tris-HCl buffer, pH 7.4 (10%w/v) with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for oxidative stress markers assays like lipid peroxidation,<sup>24</sup> reduced Glutathione,<sup>25</sup> Superoxide dismutase<sup>26</sup> and Catalase.<sup>27</sup>

### **Pentobarbitone induced sleeping time test:<sup>28</sup>**

The rats were kept on standard diet. Experiments were set as mentioned above for paracetamol, isoniazid, carbon tetrachloride and ethanol. Twenty four hours after the last treatment by standard and alcoholic extracts of *C. benghalensis* and pentobarbitone sodium in water for injection (75mg/kg b.w.) was administered intraperitoneally. Food was withdrawn and water given ad libitum 12hr before, pentobarbitone injection. All the experiments were conducted between 09.00 am to 5.00 pm. in temperature controlled room. The animals were placed on table after loss of righting reflex. The time interval between loss and regain of righting reflex was measured as pentobarbitone sleeping time. This functional parameter was used to determine the metabolic activity of the liver.

### **HISTOPATHOLOGICAL STUDIES**

Five mm thick piece of the liver was fixed in Bouin's solution (mixture of 75ml of saturated picric acid, 25ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 hr and then embedded in paraffin by conventional method and cut into 5µm thick sections. The sections stained with haematoxylin and eosin were observed under microscope (20X) for histopathological changes.

### **STATISTICAL ANALYSIS**

The results were analysed by ANOVA followed by Tukey's multiple comparison test and  $P \leq 0.05$  was considered as significant.

### **RESULTS**

#### **Effect on serum enzymes (ALT, AST and ALP):**

The serum activities of ALT, AST and ALP were used as biochemical markers for the early acute hepatic damage. The group of animals that received only EtOH showed significant ( $P < 0.001$ ) increase in serum AST, ALT and ALP with the mean values of  $85.00 \pm 0.44$ ,  $137.3 \pm 2.70$ , and  $120.50 \pm 1.73$  respectively, as compared to the corresponding values of  $47.83 \pm 1.30$ ,  $35.92 \pm 1.61$  and  $67.17 \pm 0.75$  in normal control group. The animals treated with Liv52<sup>®</sup>AIE and AqE showed significant decrease in AST, ALT and ALP compared to the control (Table-1). Response shown by AIE was nearly equal to the Liv52<sup>®</sup>.

#### **Effect on serum total protein:**

The group of animals that received only EtOH showed significant ( $P < 0.001$ ) reduction in the level of total protein with the mean value of  $5.25 \pm 0.17$  as compared to  $7.55 \pm 0.10$  of normal control group.

The animals treated with Liv52<sup>®</sup> showed significant ( $P < 0.001$ ) increase in serum protein with the mean value of  $6.68 \pm 0.08$ . Similarly, animal treated with 200mg/kg-1 of AIE and AqE showed significant ( $P < 0.001$ ) increase in serum protein with the mean values of  $6.34 \pm 0.09$  and  $6.06 \pm 0.12$ .

### Effect on serum bilirubin:

The animals that received only EtOH showed significant ( $P < 0.001$ ) increase in serum bilirubin (total and direct) with the mean values  $1.94 \pm 0.02$  and  $0.34 \pm 0.01$  respectively, as compared to the corresponding values of  $0.73 \pm 0.01$  and  $0.15 \pm 0.01$  in normal control group.

The group treated with Liv52® showed significant ( $P < 0.001$ ) reduction with the mean value of  $0.85 \pm 0.03$  and  $0.25 \pm 0.01$  respectively. The animals treated with 200mg/kg-1 of AIE and AqE showed significant decrease in total and direct bilirubin compared to the control (Table-1).

Biochemical Parameters						
Treatment/ groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total protein (g/dl)	Bilirubin (mg/dl)	
					Total	Direct
Mean $\pm$ SEM						
Normal	$47.83 \pm 1.30$	$35.92 \pm 1.61$	$67.17 \pm 0.75$	$7.55 \pm 0.10$	$0.73 \pm 0.01$	$0.15 \pm 0.01$
EtOH	$85.00 \pm 0.44$ #	$137.3 \pm 2.70$ #	$120.50 \pm 1.73$ #	$5.25 \pm 0.17$ #	$1.94 \pm 0.02$ #	$0.34 \pm 0.01$ #
Liv52®	$56.00 \pm 0.58$ ***	$76.58 \pm 0.87$ ***	$81.83 \pm 2.23$ ***	$6.68 \pm 0.08$ ***	$0.85 \pm 0.03$ ***	$0.25 \pm 0.01$ ***
Alcoholic Extract	$58.50 \pm 1.03$ ***	$78.39 \pm 0.47$ ***	$91.50 \pm 1.43$ ***	$6.34 \pm 0.09$ ***	$0.89 \pm 0.01$ ***	$0.26 \pm 0.01$ ***
Aqueous Extract	$68.53 \pm 1.04$ ***	$122.50 \pm 2.75$ **	$100.30 \pm 1.43$ **	$6.06 \pm 0.12$ **	$0.94 \pm 0.01$ ***	$0.29 \pm 0.01$ **

**Table 1:** Effect of Commelina benghalensis in ethanol induced hepatotoxicity

One way ANOVA followed by Turkey's multiple comparison tests.

#  $P < 0.001$  when compared with Normal control group. \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ .

### Effect on the lipid profile (Cholesterol, triglyceride, VLDL and HDL):

The group of animals that received only EtOH showed significantly ( $P < 0.001$ ) increased cholesterol, triglycerides, VLDL and decreased level of HDL as compared to the corresponding values of normal control group.

The animals treated with Liv52® showed significant ( $P < 0.001$ ) decrease in cholesterol, triglycerides, VLDL and increase in HDL.

Similarly, animals treated with 200mg/kg-1 of AIE and AqE showed significant decreased in cholesterol, triglycerides VLDL and increased in HDL compared to the control (Table-2). Response shown by AIE was nearly equal to the Liv52.®

Treatment/ Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Normal	101.2 ± 1.74	67.83 ± 0.70	13.57 ± 0.14	29.17 ± 1.10
EtOH	177.50 ± 1.26#	103.70 ± 1.43#	20.73 ± 0.29#	11.67 ± 0.76#
Liv 52®	125.80 ± 1.30***	76.33 ± 1.38***	15.37 ± 0.28***	24.50 ± 0.42***
Alc.Extract	132.30 ± 2.69***	74.00 ± 0.57***	14.80 ± 0.11***	23.17 ± 0.54***
Aq.Extract	149.50 ± 1.33**	86.67 ± 0.84***	17.33 ± 0.16***	15.17 ± 0.30*

**Table 2:** Effect of *Commelina benghalensis* on lipid profile

One way ANOVA followed by Turkey's multiple comparison tests.

#P<0.001 when compared with Normal control group. \*\*\*P<0.001, \*\*P<0.01, \*p<0.05

### Effect on hepatic antioxidants (GSH, SOD and CAT) in EtOH induced hepatic injury:

The animals that received only EtOH showed significant (P<0.001) decrease in SOD, GSH and CAT with the mean values of 56.20 ± 1.11, 20.01 ± 0.64 and 105.10 ± 1.72 respectively; as compared to the corresponding values of normal control group.

The animals treated with Liv52.® AIE and AqE showed significant increase in GSH, SOD and CAT compared to the corresponding values of normal control group as shown in Table-3.

Treatment/ Group	GSH (µg/ mg protein)	SOD (U/ mg protein)	CAT (U/ mg protein)	MDA (nm/mg protein)
Normal	106.70 ± 1.22	47.14 ± 0.52	246.90 ± 1.53	6.54 ± 0.31
EtOH	56.20 ± 1.11#	20.01 ± 0.64#	105.10 ± 1.72#	31.17 ± 1.05#
Liv 52®	83.37 ± 1.52***	33.48 ± 1.31***	153.50 ± 3.08***	14.37 ± 0.29***
Alc.Extract	82.62 ± 1.41***	33.26 ± 0.89***	148.70 ± 2.77***	14.76 ± 0.57***
Aq.Extract	75.91 ± 1.01***	26.23 ± 1.44**	146.50 ± 1.80***	16.23 ± 0.23***

**Table 3:** Effect of *Commelina benghalensis* in ethanol induced changes on antioxidants and oxidant

One way ANOVA followed by Turkey's multiple comparison tests.

#P <0.001 when compared with Normal control group. \*\*\*P<0.001, \*\*p<0.01

### Effect on hepatic oxidant MDA:

The animals that received only EtOH showed significant ( $P < 0.001$ ) increase in MDA level with the mean value of  $31.17 \pm 1.05$  as compared to the corresponding value  $6.54 \pm 0.31$  of normal control group.

The group of animals treated with Liv52,<sup>®</sup> AIE and AqE showed significantly ( $P < 0.001$ ) decreased in MDA level with the mean value of  $14.37 \pm 0.29$ ,  $14.97 \pm 0.42$  and  $16.39 \pm 0.42$  respectively as compared to the normal. Response shown by AIE was nearly equal to the Liv52<sup>®</sup>

### Effects on sleeping time:

Effect of hepatotoxicant EtOH prolonged pentobarbitone induced sleeping time. Sleep duration with pentobarbitone at a dose of 75mg/kg i.p., was  $117.20 \pm 0.88$  min. whereas treatment with EtOH significantly ( $P < 0.001$ ) prolonged the pentobarbitone sleeping time with the respective mean duration of  $235.40 \pm 0.90$ .

However, prior treatment of animals with Liv52<sup>®</sup> restored the duration almost to normal with mean duration of  $138.30 \pm 1.48$  min. Similarly, prior treatment of animals with alcoholic extracts of CB significantly ( $P < 0.001$ ) reduced with mean duration of  $143.40 \pm 0.82$ . (Table- 4).

Treatment - DOSE/kg <sup>-1</sup>	MEAN $\pm$ SEM (in minutes)
Control /Pentobarbitone 75mg ( i.p.)	117.20 $\pm$ 0.88
EtOH 20% 25ml. + Pentobarbitone 75 mg ( i.p.)	235.40 $\pm$ 0.90
Liv52 <sup>®</sup> 5ml + EtOH 20% 25ml + Pentobarbitone 75mg ( i.p.)	138.30* $\pm$ 1.48
CB- EtOHE 200mg.+ EtOH 20% 25ml + Pentobarbitone 75mg (i.p.)	143.40* $\pm$ 0.82

**Table 4:** Effect of Alcoholic extract of *Commelina benghalensis* on pentobarbitone induced sleeping time (in minutes)

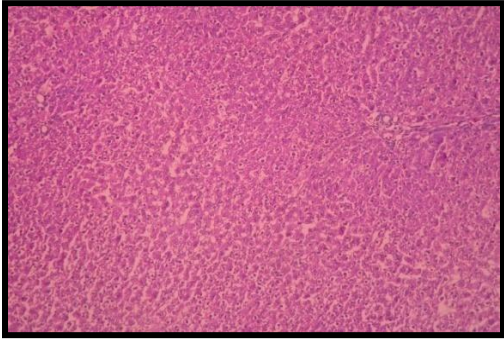
\* $P < 0.001$

### Effects of AIE and AqE on liver histology:

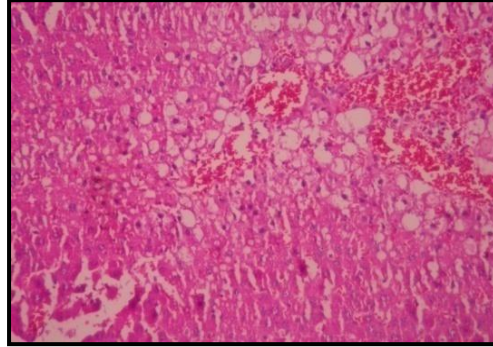
The histological features, as shown in Figure-a indicated a normal liver lobular architecture and cell structure of the livers in the control animals. There were no pathological changes in healthy control livers which showed normal lobular architecture. Figure-b shows (EtOH treated) moderate macrovesicular fatty changes and hepatocellular necrosis. Changes were improved in Liv52<sup>®</sup> AIE and AqE treated rats, which exhibited areas of normal liver architecture ( Fig-c, d and e)



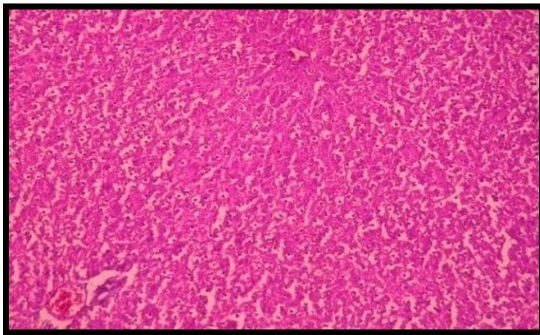
## HISTOPATHOLOGY



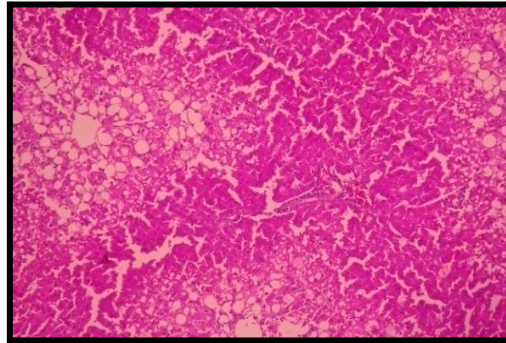
**Figure a:** Normal



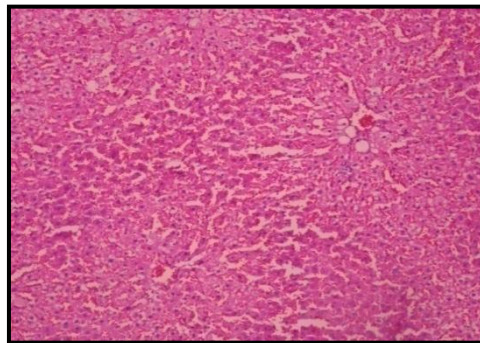
**Figure b:** EtOH



**Figure c:** EtOH + Liv 52®



**Figure d:** EtOH + Alcoholic extract



**Figure e:** EtOH + Aqueous extract

**Figure a:** Normal liver. **Figure b:** EtOH treated- showing histopathological evidence of moderate macrovesicular fatty changes. **Figure c, Figure d and Figure e:** Liv52®, ALE and AqE treated- Showing marked improvement towards normal hepatic architecture. Among all the treated groups Liv 52® and alcoholic extract showing better improvement.



## DISCUSSION

The present study was planned to explore hepatoprotective activity of *Commelina benghalensis* - root extracts, since these plants are widely used by local traditional healers to treat jaundice, which is a major manifestation of liver injury.

The serum marker enzymes (AST, ALT and ALP) are cytoplasmic in nature, but upon liver injury these enzymes enter into the circulatory system due to altered permeability of membrane.<sup>29</sup> Our results showed EtOH caused a significant elevation of serum levels of ALT, AST and ALP in rats. These effects were markedly reduced if the rats were pre-treated with AIE and AqE of MF. Together these evidences suggest that the hepatoprotective effects of MF might be in part due to its ability to protect biomembrance against free radicals.

Total protein concentration of the EtOH treated rats was significantly reduced.. This suggests a reduction in the protein synthetic function of the liver, which could be as a result of possible damage to the hepatocytes induced by EtOH. Most protein found in the plasma are synthesized by the hepatocytes and secreted into circulation. Administration of AIE and AqE of MF lead to an increased in the total protein. Bilirubin has been attributed to the damaged structural integrity of the liver; decrease in the level of bilirubin by AIE and AqE indicates protective effect of MF.

The characteristic changes observed in the concentrations of plasma lipids, cholesterol <sup>30</sup> and triglycerides and lipoproteins VLDL and HDL in ethanol treated rats. Increase in the level of plasma lipids, cholesterol and triglycerides and lipoproteins VLDL is risk factor for ischemic heart disease. AIE and AqE of MF reduces these levels and increases level of HDL. HDL particles are responsible to remove cholesterol from within artery.

MDA is a major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid (PUFA).<sup>31</sup> MDA, a secondary product of lipid peroxidation, is a useful indicator of tissue damage involving a series of chain reactions.<sup>32</sup> Recent study also suggested that a reduction in the activity of SOD is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes.<sup>33</sup> GSH, an important protein thiol in living organisms plays a central role in coordinating the body's antioxidant defense process.<sup>34</sup> Reducing GSH constitutes the first line of defense against free radicals.<sup>35</sup> AIE and AqE of MF at tested doses 200mg/kg-1 prevented elevation of liver MDA content, reduction of liver SOD activity, and increase of GSH content resulted from rat liver intoxication with EtOH challenge. The hepatoprotective ability of CB might be due to its ability to stabilize liver cell membrane. Thus, the activity of SOD was commendably maintained, and the MDA production and the consumption of GSH were decreased.

CAT 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. Homogenated liver CAT activities in AIE and AqE groups were significantly higher than those in EtOH group. In this study, CAT was

increased by administration of ALE, suggesting that it can restore CAT enzyme.

Pentobarbitone -induced sleeping time study was carried out to verify the effect on microsomal enzymes. It was observed; the group received only hepatotoxicant; there was significant increased in the duration of sleep. Whereas on treatment duration of sleep was significantly reduced.

Liv-52® which contains the various herbal plants mainly Capparis spinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna, Cassia occidentalis and Achillea millefolium shows hepatoprotective activity by the virtue of their antioxidant property and this is due to the presence of flavanoids, cynogenic glycosides and triterpenes. Phytochemical investigation of the Commelina benghalensis showed it contains several types of compounds such of alkaloids, steroids, terpenoids, iridoids, flavonoids saponin, tannin etc.

Hepatoprotection offered by Commelina benghalensis extracts could be attributed to these constituents, since antioxidants have been reported to possess hepatoprotective activity.<sup>36</sup>

In order to confirm their antioxidant potential and to identify various enzymes involved in generating oxygen free radicals further studies are essential.

These shortcomings of the present studies open a new arena for the future research. Considering the efficacy of the plants, their phytoconstituents (fractions) need to be isolated in order to explore their hepatoprotective activity. Further activity guided chemical studies of the fractions may help in developing new leads that would be useful for the treatment of presently untreatable hepatotoxicities.

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