PROTECTIVE ACTIVITY OF *COMMELINA BENGHALENSIS*- ROOT EXTRACTS AGAINST PARACETAMOL INDUCED HEPATIC DAMAGE IN WISTAR RATS

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Summary

The objective of the present investigation was to study hepatoprotective activity of various root extract of *Commelina benghalensis*- Linn in paracetamol induced liver damage model in Wistar rats. Liver damage was produced by paracetamol (2gm/kg,p.o.) in 1% CMC. The Plant extracts (200mg/kg, p.o.) were administered every 24 hrs for seven days, while standard group received N-acetyl l-cystine. At the end of the study the marker enzymes in serum and histopathological analysis were carried out. The aqueous as well as alcoholic extract showed significant hepatoprotective activity and efficacy of alcoholic extract was almost comparable to that of N-acetyl l-cystine.

Keywords: *Commelina benghalensis;* Hepatoprotective; N-acetyl l-cystine; Paracetamol

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Introduction

The liver, because of its strategic anatomical location, is exposed to many kinds of xenobiotics and therapeutic agents. Moreover, the rapidly increasing morbidity and mortality rates from liver diseases are largely attributable to the repeated chemical insult either from drug abuse or from environmental pollution. Unfortunately so far, in the modern era of medicine there is no specific treatment to counter the life threatening impact of these dreaded conditions[1, 2], though N-acetyl l-cystine can reverse the pathology due to paracetamol induced injury. Several plants have been investigated and reported to possess antioxidant property and hepatoprotective activity eg. *Baliospermum montanum*[3], *Ocium sanctum*[4], *Tamarindus indica*[5] etc. Similarly *Commelina benghaensis* is a widely distributed plant throughout India, and is a popular folk medicine[6]. Whole plant is used as antileprosy, emollient, laxative[7], leaf is used in diarrhea while roots are used in fever and liver complaints[8].

The survey revealed that cold water extract of *Commelina benghalensis* root is used by the traditional healers for the treatment of jaundice and patient feedback is quite encouraging. However hepatoprotective activity of *Commelina benghalensis* has not been scientifically investigated. Therefore, the present study is planned to investigate the effect of aqueous as well as other extracts of *Commelina benghalensis* root in paracetamol induced liver damage in Wistar rats.

Materials and methods

Preparation of *Commelina benghalensis* Extract: *Commelina benghalensis* roots collected from open field around the Belgaum city in the month of September were identified and authenticated by the taxonomist Dr. Harsha Hegde and the herbarium (voucher No. RMRC 486) has been preserved at Regional Medical Research Centre (Belgaum). Shade dried roots were powdered to moderately coarse grade. Petroleum ether, chloroform, alcohol & aqueous extracts of roots were obtained by using soxhlet extractor. The extraction was continued for 12 cycles or until the solvent in the thimble was clear. After evaporating the solvent, the dark brown semisolid extract was kept in an air tight

container at 4^0c 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 were maintained at the 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 120-150 g were housed for about a week for acclimatization with natural 12:12hr 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[9] in swiss mice weighing 25 to30gms 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_{50} was found to be more than 2000mg/kg.

Drugs used and their Doses: In four groups (n=6, in each) of animals Alcoholic, aqueous, petroleum ether & chloroform extracts of roots were administered with the dose of 200mg/kg b.w. Fifth group received Liv52 5ml/kg b.w[10]. while sixth group recived N-acetyl L-cystine (Lobe chem.)100mg/kg b.w., seventh group and eighth groups recived equivalent volume of 1%CMC., Paracetamol 2gm/kg b.w in 1% CMC was administered to all seven groups on fifth day[11]. All the treatments were administered orally.

Methodology: All the treatments were given for a total period of 7days, on the eighth day all the rats were anaesthetized by halothane to withdraw cardiac blood and the animals were sacrificed by overanesthesia to descect out liver for histopathological studies. 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), total protein and billirubin content[12] .

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 5nm thick sections.The sections stained with haematoxylin and eosin were observed under microscope (20 X) for histopathological changes.

Statistical analysis: The results were analysed by ANOVA followed by Dunnet's posthoc test and $p \leq 0.05$ was considered as significant.

Results

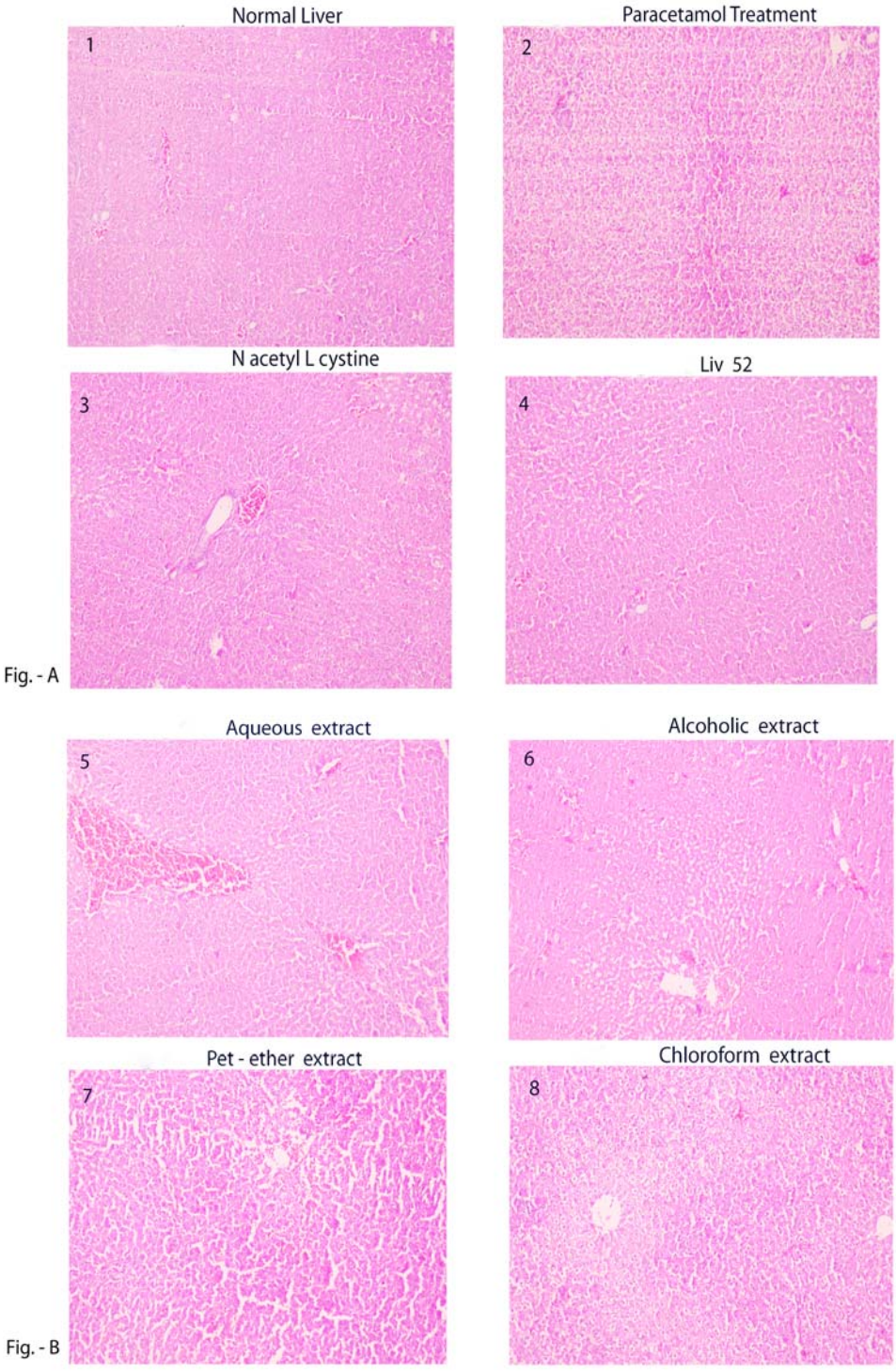
The groups treated with Paracetamol alone (positive control) showed significantly elevated level of ALT, AST, billirubin and significantly decreased total protein content as compared to negative control(not challenged with paracetamol) animals. The animals treated with aqueous, alcoholic extract, Liv52 and N-acetyl L-cystine showed significant reduction in all the biochemical parameters. Aqueous, alcoholic extract, Liv52 though significantly lowered all the biochemical parameters as compared to only paracetamol treated group but failed to restore them to the normal level. In contrast, N- acetyls L-cystine restored the biochemical parameter to the normal level. (Table 1)

Histopathological examination of liver from control group showed normal cellular architecture with distinct hepatic cells, sinusoidal space (Fig.A-1). The liver of the rats intoxicated with paracetamol there was sever central vein congestion ,sinus congestion focal haemorrhages, inflammation and centrilobular degeneration of hepatic cells with intense centrilobular necrosis(fig.-2). There was no change in the pathology after treatment with chloroform and pet ether extract (figB-7,8),while N- acetyl L-cystine improved histopathalogy almost to the normal(figA-3). Next to N- acetyl L-cystine (in the descending order of efficacy) alcoholic extract, Liv52and aqueous extract prevented the paracetamol induced hepatotoxicity.(fig.A-4,figB-5,6).

Table No.1: Effect of Commelina benghalensis in paracetamol induced Hepatotoxicity					
Biochemical Parameters					
Treatment/ groups	AST (IU/L)	ALT (IU/L)	Total protein (g/dl)	Bilirubin (mg/dl)	
				Total	Direct
	Mean ± SEM				
Normal	139.7 ± 1.58	89.83 ± 1.25	7.79 ± 0.17	0.49 ± 0.02	0.12 ± 0.07
Paracetamol Control	214.2 ± 4.43 #	168.7 ± 2.50 #	4.49 ± 0.16 #	0.90 ± 0.02 #	0.19 ± 0.01 #
Alcoholic Extract	152.2 ± 4.09 ***	120.5 ± 2.22 ***	5.48 ± 0.15 ***	0.65 ± 0.01 ***	0.12 ± 0.01 ***
Aqueous Extract	195.5 ± 1.23**	158.02 ± 1.62**	5.22 ± 0.12	0.90 ± 0.01**	0.15 ± 0.01**
Chloroform Extract	201.8 ± 1.62	161.08 ± 1.92	4.86 ± 0.16	0.94 ± 0.01	0.17 ± 0.01
Pet. Ether	207.02 ± 4.90	161.07 ± 2.60	4.47 ± 0.17	0.96 ± 0.01	0.18 ± 0.01
Liv 52	150.8 ± 1.35 ***	123.3 ± 1.25 ***	5.60 ± 0.17 ***	0.79 ± 0.01 ***	0.13 ± 0.01 ***
N-acetyl L-Cystine	133.0±2.42***	153.8±1.79***	5.54± 0.15***	0.61± 0.02***	0.15±0.01***

ANOVA: *** $p<0.001$ considered significant as compared to Paracetamol control group.

Students t test # $p<0.001$ considered significant as compared to Normal control group. *** $p<0.001$ ** $p<0.01$


Normal Liver
1
Paracetamol Treatment
2
N acetyl L cystine
3
Liv 52
4
Fig. - A
Aqueous extract
5
Alcoholic extract
6
Pet - ether extract
7
Chloroform extract
8
Fig. - B

Discussion

Findings of the present study clearly indicate that both water and alcoholic extracts of *Commelina benghalensis* showed significant Hepatoprotective activity against paracetamol induced hepatic injury. Alcoholic extract appears to be better than aqueous extract since it significantly elevated total serum protein in contrast to aqueous extract. No similar reports could be traced in available literature.

As expected N-acetyl L-cystine, a specific antidote for paracetamol hepatotoxicity totally restored the hepatic histology except sinus congestion. It is well known that N-acetyl L-cystine replenishes the glutathione stores of liver and prevents binding of the toxic metabolite to other cellular constituents, similarly Liv-52 which contains the various herbal plants mainly *Capparis spinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna, Cassia occidentalis and Achillea millefolium* shows the hepatoprotective activity by the virtue of their antioxidant property and this is due to the presence of flavanoids, cynogenic glycosides and triterpines.[13,14].*Commelina benghalensis* root have been reported to contain carotenoid (beta-carotene), flavocommelin, campesterol and n-octacosanol[7] in addition to alkaloids, tannins, saponnins etc. beta-carotene, flavocommelin and n-octacosanol have been reported to posses antioxidant activity,[15,16]while campesterol is reported to have anti-inflammatory and immunomodulatory activity.[17] Hepatoprotection offered by *Commelina benghalensis* extracts could be attributed to these constituents. Since antioxidants have been reported to posses Hepatoprotective activity[18].Phytochemical analysis of alcoholic extract had flavanoids, sterols, caratenoids ,aqueous extract showed the presence of caretenoids, while chloroform and pet.ether extract showed only presence of carbohydrates and glycosides and no flavanoids and sterols,probably extracts in providing hepatoprotection.

The present study was not aimed to elucidate hepatoprotective mechanisms of *Commelina benghalensis* extracts. In order to confirm their antioxidant potential and to identify

various enzymes involved in generating oxygen free radicals further studies are essential.

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