THE HEPATOTOXICITY OF MIMOSINE IN PRECISION CUT GOAT LIVER SLICE SYSTEM

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ABSTRACT : Mimosine, a non protein toxic amino acid, induced toxicity in precision cut goat liver slice system had been studied in term of its effect on certain enzymatic and metabolic parameters in vitro. Significant depletion of tissue sulfhydryl groups (TSH, PBSH and NPSH), rise in lipid peroxidation and altered membrane bound ATPases activity were observed during the study suggestive of hepatotoxic effects of mimosine.

INTRODUCTION:

Mimosine, a non protein toxic amino acid is present in high concentration (8-10% of dry weight in growing tips) in Leucaena leucocephala, a leguminous shrub. Presence of mimosine along with other antinutritional factors like tannins, saponins, haemagglutinins, trypsin inhibitors etc has restricted its large scale use as fodder. Mimosine toxicity has been studied in respect of variable clinical, pathological and biochemical changes in vivo in a variety of species at length (Bhatnagar et al, 1999, Prasad and Paliwal, 1989). However, most of the findings reported are on the basis of leucaena feeding experiment. In Vitro studies using chemically pure mimosine to evaluate its toxicity are very few. The present study was carried out to evaluate the effect of pure mimosine on liver at cellular level and its interaction with certain enzymatic and metabolic profiles in vitro.

MATERIALS AND METHODS:

Chemically pure mimosine(extrapure for biochemistry) was procured from SISCO Research Pvt. Ltd, Bombay.The liver tissue was collected in ice cold normal saline solution(NSS) immediately after slaughter of healthy goats. Frozen liver slices (100-150μm thick) were prepared in at -2°C in a minetome according to standard procedure (Azri-Meehan et al 1992). Slices were incubated in Dulbecco's Modified Eagle Medium (100mg slices/ 4 ml) at pH 7.4 in closed vials at 37°C with shaking in a roller incubator (temperature controlled)for 3,6 and 9 hours. Prior to incubation, aliquots from freshly prepared stock solution of mimosine were added to the incubation medium to obtain a final mimosine concentration of 0.1(S1), 0.5(S2),2(S3), 5(S4) and 10mM(S5).The viability of tissue slices up to nine hours was assayed by mitochondrial viability test(Mossman,1983).At the end of the each incubation period the tissue slices were homogenized(10% w/v) in ice cold NSS and used for assay of total protein(Lowry et al, 1951) , ATPases (Yohtalau,1973), lipid peroxidation (Placer et al 1966) and tissue sulfhydryl groups (Sedlak and Lindsay, 1968) with certain modifications in assay procedures whenever necessary.

RESULTS AND DISCUSSIONS:

Hepatocytes of liver is the site of metabolism

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(Abhraham et al, 1983) and provides an opportunity to study both the nature and the extent of metabolism of a new compound at an early stage. Toxicity of many chemicals results from reactive metabolites following conversion in the liver which causes cellular damage and altered metabolic activity. The results of our study has been elaborated in tables I-III. The tissue total(TSH), protein bound(PBSH)and nonprotein(NPSH/ GSH) sulfhydryl levels were significantly depleted in a dose and time dependant manner,the lowest level being recorded at 9th hour of incubation. Loss of cellular glutathione is indicative of cytotoxicity (Docks and Krishna, 1976). Glutathione is present in all types of living cells and is able to conjugate with a variety of chemically active compounds including reactive intermediates formed during the metabolism of xenobiotics (Mitchell et al, 1976).Sufficient depletion of cellular glutathione content allows some reactive metabolites to react with cellular macromolecules leading to cellular injury and death as reported in a number of studies (Docks and Krishna, 1976; Olson et al, 1980; Azri-Meehan et al, 1992).). Burk et al (1980) suggested that protective effect of GSH against LP in rat liver preparation may be mediated by GST(Glutathione -S- Transferase). Anundi and coworkers (1979) reported that depletion of GSH alone can evoke cell damage. A significant decrease in tissue total protein in liver slice system with increasing dose and time is indicatve of cellular injury. In our present study lipid peroxidation(LP) increased in a dose and time dependent fashion that may be correlated with reduced GSH level and increased glutathione -S- transferase activity induced by mimosine in goat liver slice system reported earlier(Brahmachari et al 2005). The specific activity of membrane bound ATPases decreased significantly in our experiment. The ATPases are integral membrane proteins and require membrane phospholipids for their activity (Murray et al, 1993). A significantly higher LP observed in this study may reduce the access of these enzymes to phospholipid leading to alteration of enzyme activity. The Mg2+-ATPase and pyrophosphatase activities of chick epiphyseal cartilage matrix vesicles originate from one enzyme, namely, alkaline phosphatase(Robert et al 1975). As mimosine is a strong inhibitor of alkaline phosphatase activity(Chang ,1960) it may be a contributory factor for altered ATPases activity observed in our study. On the basis of the observations in the present experiment it may be concluded that mimosine induces toxic injury to goat hepatocytes in vitro.

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Table I: Effect of mimosine on Membrane bound ATPases(µM Pi released/hr/mg protein)

Incubation	TREATMENTS						
Period(Hours)	Control	S1	S2	S3	S4	S5	Overall mean ± SE
Total ATPase							
3	11.82	11.20	10.66	5.86	5.72	5.16	**8.40± 0.73** [a]
	±1.07	±0.57	±0.57	±0.61	±0.19	±0.28	
6	11.56	9.82	8.50	4.90	3.94	3.80	**7.09± 0.77** [b]
	±1.18	±0.58	±0.59	±0.50	±0.28	±0.26	
9	11.20	9.48	7.76	4.70	3.84	3.96	**6.82± 0.73** [b]
	±1.19	±0.76	±0.81	±0.44	±0.38	±0.34	
Overall Mean±SE	**11.50** [a]	**10.17** [b]	**8.97** [c]	**5.15** [d]	**4.50** [d]	**4.30** [d]	
	±0.58	**±0.49**	**±0.55**	**±0.32**	**±0.34**	**±0.26**	
Mg^{2+} ATPase							
3	6.30	6.06	6.22	3.20	3.08	3.04	**4.65± 0.0.39** [a]
	±0.37	±0.46	±0.40	±0.18	±0.1	±0.17	
6	5.94	3.74	4.58	2.62	2.66	2.14	**3.61± 0.0.35** [b]
	±0.32	±0.82	±0.40	±0.32	±0.31	±0.10	
9	5.94	3.84	4.34	2.58	2.22	1.95	**3.47± 0.36** [b]
	±0.27	±0.44	±0.50	±0.32	±0.25	±0.40	
Overall Mean±SE	**6.06** [a]	**4.55** [b]	**5.50** [b]	**2.80** [c]	**2.65** [c]	**2.38** [c]	
	±0.77	±0.48	±0.36	±0.17	±0.18	±0.21	

Na^+K^+- ATPase							
3	5.52 ±1.06	5.14 ±1.30	4.46 ±0.99	2.82 ±0.68	2.64 ±0.18	2.18 ±0.34	**3.78± 0.43 ^{a}**
6	5.62 ±1.12	6.82 ±2.29	3.88 ±0.84	2.62 ±0.65	0.93 ±0.23	1.66 ±0.31	**3.58± 0.64^a**
9	5.26 ±1.16	5.44 ±0.76	3.92 ±0.57	1.95 ±0.67	1.62 ±0.28	1.82 ±0.16	**3.34± 0.46^a**
Overall Mean± SE	**5.47^{ab} ±0.56**	**5.80^a ±0.84**	**4.08^b ±0.42**	**2.46^c ±0.36**	**1.73 ^{c} ±0.27**	**1.86^c ±0.16**	

1. All values are Mean ± SE (n=3)

2. Means bearing common superscripts in a column/row do not differ significantly(P> 0

Table II: **Effect of mimosine on Lipid peroxidation (nM MDA/gm tissue) and tissue total protein content (1**

Incubation Period(Hours)	Control	S1	S2	S3	S4	S5	Overall mean ± SE
			TREATMENTS				
Lipid Peroxidation(nM MDA/gm tissue)							
3	1.373 ±0.113	3.087 ±0.337	4.040 ±0.436	10.270 ±0.239	11.153 ±0.80	10.510 ±0.663	6.734± 0.983 ^{c}
6	2.873 ±0.253	6.683 ±0.0.228	6.537 ±0.285	10.863 ±0.564	11.427 ±0.228	13.203 ±1.145	8.431± 0.904^b
9	5.750 ±0.365	6.667 ±0.302	7.700 ±0.408	13.293 ±0.098	12.417 ±0.361	15.253 ±0.072	10.18± 0.909^a
Overall Mean±SE	3.332^d ±0.655	5.146^c ±0.554	6.092^c ±0.673	11.476^b ±0.586	11.666 ^{b} ±0.324	12.989^a ±0.844	
Tissue Total Protein(mg/gm tissue)							
3	71.92 ±0.99	64.92 ±0.28	51.72 ±0.97	50.17 ±0.23	46.71 ±0.45	44.07 ±0.40	54.92± 2.44 ^{a}
6	64.92 ±0.45	57.21 ±2.06	49.62 ±0.70	46.97 ±0.54	47.22 ±5.44	42.63 ±0.16	51.43± 1.89 ^{b}
9	66.39 ±3.30	54.36 ±0.44	42.13 ±4.91	43.13 ±0.22	48.33 ±0.21	40.66 ±0.21	48.08± 0.40^c
Overall Mean±SE	67.74^a ±1.46	58.83^b ±1.69	47.82^c ±2.06	46.75^c ±1.03	45.25 ^{cd} ±1.32	42.46^d ±0.52	

1.All values are Mean ± SE (n=3)
2.Means bearing common superscripts in a column/row do not differ significantly(P> 0.05)

Table III : Effect of mimosine on tissue sulfhydryl groups(µM /g tissue)

Incubation Period(Hours)	Control	S1	S2	S3	S4	S5	Overall mean ± SE
	TREATMENTS						
Total sulfhydryl groups							
3	5.40	4.83	4.30	3.43	3.46	3.51	**4.09± 0.23** ^{a}
	±0.29	±0.44	±0.57	±0.20	±0.28	±0.81	
6	5.44	5.00	5.14	3.42	3.61	2.40	**4.01± 0.77** ^{a}
	±0.31	±0.28	±0.47	±0.11	±0.61	±0.46	
9	5.24	4.86	3.48	2.54	2.37	1.84	**3.39± 0.33** ^{b}
	±0.14	±0.13	±0.43	±0.44	±0.36	±0.51	
Overall Mean ± SE	**5.36** ^{a}	**4.90** ^{a}	**3.97** ^{b}	**3.13** ^{d}	**3.15** ^{c}	**2.45** ^{d}	
	±0.13	**±0.16**	**±0.23**	**±0.32**	**± 0.28**	**±0.26**	
Non Protein Sulfhydryl groups							
3	1.12	0.833	0.740	0.303	0.218	0.258	**0.578± 0.0.86** ^{a}
	±0.078	±0.068	±0.128	±0.021	±0.255	±0.329	
6	1.070	0.754	0.484	0.326	0.418	0.205	**0.543± 0.082** ^{a}
	±0.067	±0.103	±0.051	±0.042	±0.318	±0.372	
9	0.888	0.651	0.311	0.279	0.175	0.163	**0.411± 0.070** ^{b}
	±0.134	±0.084	±0.061	±0.058	±0.056	±0.029	
Overall Mean± SE	**1.025** ^{a}	**0.746** ^{b}	**0.512** ^{c}	**0.303** ^{d}	**0.269** ^{d}	**0.208** ^{d}	
	±0.061	**±0.051**	**±0.076**	**±0.076**	**±0.083**	**±0.024**	
Protein Bound Sulfhydryl groups							
3	4.280	4.014	3.557	3.125	3.215	2.828	3.503± **0.160** ^{a}
	±0.351	±0.398	±0.092	±0.265	±0.253	±0.331	
6	4.367	4.279	3.496	3.096	3.197	1.529	3.**327± 0.259** ^{ab}
	±0.314	±0.167	±0.378	±0.082	±0.232	±0.671	
9	4.353	4.209	3.172	2.261	2.198	1.682	2.979± **0.278** ^{b}
	±0.262	±0.097	±0.097	±0.458	±0.448	±0.383	
Overall Mean± SE	**4.333** ^{a}	**4.167** ^{a}	**3.408** ^{b}	**2.827** ^{b}	**2.870** ^{b}	**2.013** ^{c}	
	±0.156	**±0.134**	**±0.180**	**±0.209**	**±0.27**	**±0.318**	

1.All values are Mean ± SE (n=3)

2.Means bearing common superscripts in a column/row do not differ significantly(P> 0.05)