

EVALUATION OF MIMOSINE TOXICITY IN GOAT AND BUFFALO ERYTHROCYTE SYSTEM *IN VITRO*

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ABSTRACT: Dose and time dependent effect of mimosine in goat and buffalo erythrocyte system *in vitro* have been evaluated in the present study. The results obtained exhibit that mimosine induces hemolysis in both mode of studies with concomitant increase in cellular methemoglobin and malondialdehyde(MDA) level and depletion of cellular reduced glutathione(GSH) level. Such changes in RBC metabolic profiles are indicative of oxidative stress caused by mimosine. No major species variation was observed in effect of mimosine on erythrocytes *in vitro*.

Key Words: Mimosine, Erythrocytes, *In vitro*, Toxicity.

INTRODUCTION

Mimosine or leucenol is an alkaloid, β 3-hydroxy-4 pyridone amino acid. It is a toxic non-protein free amino acid otherwise chemically similar to tyrosine, and was first isolated from *Mimosa pudica*. It occurs in a few other *Mimosa spp.* and all members of the closely related genus *Leucaena*. This compound, also known as leucenol, was first isolated from the seeds of *Leucaena glauca* Benth (Adams *et al.* 1945). Subabul (*Leucaena leucocephala*) is considered as a miracle tree due to its protein rich foliage (20-30% CP), fast growing and drought tolerant habits. Use

of its foliage as animal feed is being limited by presence of a toxic amino acid-mimosine and its metabolites 3, 4 DHP and 2, 3 DHP. Inclusion of subabul leaves on ration of farm animals results in varied response to severe adverse effects (Paul 2000). A number of *in vivo* toxicity studies have been done by *Leucaena* feeding which contains other antinutritional factors including mimosine. A very few *in vitro* studies to evaluate the toxic effect of mimosine as a pure compound have been undertaken. The present study was carried out to explore the interaction of mimosine with RBC components and its metabolism *in vitro*, in addition to study the feasibility and efficacy

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of such model to evaluate toxicity of mimosine and related plant toxins *in vitro*.

MATERIALS AND METHODS

Blood samples from healthy adult goats and buffaloes were collected aseptically from local slaughter house and animal shed, Division of Biochemistry, Indian Veterinary Research Institute, Izatnagar, U.P., India using ACD as anticoagulant. The containers are capped, shaken gently to avoid any clot formation and transported to the laboratory. The blood samples were centrifuged at 5000g for 10-15 minutes at 4°C, plasma and buffy coats were removed and resulting erythrocyte pellet was washed thrice with PBS pH 7.4. Erythrocytes were incubated in 15 ml tubes in a medium as described by Dershwitz *et al.* (1985) at 37°C in a temperature controlled roller incubator with shaking and exposure to atmospheric oxygen upto 20 hours. The optimum incubation time was selected on the basis of hemoglobin leakage into medium measured as absorbance at 540nm (Table 1). The incubation medium contained 0.5 mM mimosine while time course (0.5, 10, 20 hours) was studied and 0.1-0.5mM in dose response study. At the end of incubation, the incubation tubes were centrifuged at 5000g for 10 minutes, upper medium was aspirated out and resulting erythrocyte pellet was used for biochemical estimations of hemoglobin (Richterich 1969) MDA production (Jain 1988), reduced glutathione (Prins and Loos 1969) and met hemoglobin (Evelyn and Malloy 1938).

RESULTS AND DISCUSSION

Erythrocyte toxicity in peripheral circulation is mediated by an effect either on the cell membrane, hemoglobin, red cell antioxidant

protective mechanism or a combination of all three resulting in loss of erythrocyte integrity. The useful parameters to check the above include hemolysis, membrane lipid peroxidation, methemoglobin, GSH level, alteration in protein aggregated in membrane and membrane fluidity (Link *et al.* 1985). Mimosine produces a variety of physiological effects when ingested by animals (Owen 1958, Thompson *et al.* 1969, Hegarty and Peterson 1973). A very few *in vivo* studies using chemically pure mimosine by i.v. infusion method had been carried out in sheep and goat to successfully explore its toxic effect like alopecia, hepatotoxicity, nephrotoxicity etc. by a number of scientists (Reis *et al.* 1999, Reis 1975, Shalu *et al.* 1995) and in most of the cases the circulating plasma mimosine concentration had been found to be in between 89-108 µmol/L. Literature survey to our best possible extent did not reveal any earlier report of mimosine toxicity in goat and buffalo erythrocyte system *in vitro*. The results of the present study has been given in Tables 1-5. The hemolysis did not differ significantly between the different periods of incubation. A period extending upto 20 hours was selected on the basis of this result. In buffalo erythrocyte system, hemoglobin level decreased significantly at 0.3-0.5mM dose level. The overall mean values of hemoglobin observed with mimosine treatment however decreased nonsignificantly in time response study. In goat erythrocyte system, hemoglobin level decreased nonsignificantly with increasing dose and incubation period. This may be attributed to dose and time dependant leakage of hemoglobin in the incubation medium. Methemoglobin level of buffalo erythrocytes increased significantly at all dose levels of

mimosine as compared to control. The incubation period also revealed its significant effect with highest increment being met at 20 hours of incubation. Methemoglobin level increased significantly with increasing dose and incubation period in goat erythrocyte system. Methemoglobin is a component of oxidative injury to RBC and thus mimosine may induce oxidative injury to RBC. Reduced glutathione was depleted from buffalo and goat erythrocytes significantly at all dose levels of mimosine and also at different periods of incubations. Reduced glutathione is an important constituent of RBC and its depletion is suggestive of mimosine induced toxicity. GSH protects the red cell against the oxidative injury through the activity of the enzyme glutathione peroxidase by endogenously formed hydroperoxides of unsaturated fatty acids to hydroxy derivatives (Christopherson 1969). The lipid peroxidation increased significantly at all incubation periods whereas at 0.4 and 0.5mM dose level in dose response study with buffalo erythrocytes. A significant rise in lipid peroxidation at 0.5mM dose level was observed in goat erythrocytes and it also increased significantly with increasing period of incubation. Lipid peroxidation precedes exposure of cell lipid to oxidizing free radicals. Living cells like RBC are rich in polyunsaturated fatty acids and therefore intrinsically vulnerable. Lipid peroxides and peroxidation products are powerful enzyme inhibitors (Slater 1968) and peroxidised membrane cease to be competent intercompartmental barriers (Hunter *et al.* 1964). In present study mimosine by its iron chelation and cytotoxic effect (Restivo *et al.* 2005) may generate free radicals inside the cell leading to lipid peroxidation. GSH depletion

as observed in the study may curb the cell's potential to detoxify peroxides thus enhancing attack of cell lipids by ROS. No major species variation in response of RBC to mimosine exposure was revealed in the present experiment.

CONCLUSION

The results obtained reveal that mimosine induces oxidative stress in goat and buffalo erythrocyte system *in vitro*. However further research work is needed in this field. Toxic potential of mimosine on erythrocytes can be evaluated *in vitro* in the present model of study.

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