Hepato and Renal protective effect of Bacosides against Aluminium insult in rats: A Proton magnetic resonance study on serum and urine

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INTRODUCTION:

Substantial increase of Al in the body results in oxidative damage of liver, kidney and brain. Evidences suggest that Bacosides obtained from Bacopa monnieri extract has potential to protect the brain from Al induced oxidative damage[1]. Donepezil is a drug used to treat Alzheimer's disease patients and also possesses antioxidative property. Further, 1H NMR quantitative metabolic assessment of serum and urine often shows perturbations in response to toxic or disease-induced stress. Recently, 1HNMR study has been carried out which shows that Al causes oxidative damage in liver and kidney[2]. In view of the aforementioned informations, we aimed to evaluate the protective effect of Bacosides and Donepezil against Al-induced hepato and renal oxidative stress using ^{1}H NMR studies. The models rats' serum and urine were analysed in order to assess the time dependent alternations induced by the two drugs against Al toxicity.

MATERIALS AND METHODS

The urine and blood samples (2 mL) were collected for all the rats (n=28) after 45 and 90 days of treatment and urine samples were centrifuged at 3000 rpm for 5 min at 4◦C to remove particulate contaminates. Immediately after the collection, the blood samples were placed in a sterile stoppered test tube and were allowed to coagulate for 30 min and centrifuged to separate the sera. Samples were stored at −80◦C until NMR spectroscopic analysis. 500 µL of samples were taken in 5-mm NMR tubes and sealed coaxial capillary tube containing 0.375% TSP in 35 µL D_2O served as a chemical shift reference. The ^{1}H NMR spectra of these samples were obtained on a Bruker Biospin Avance 400 MHz spectrometer using 5-mm broad band inverse probehead. One-dimensional ^{1}H NMR spectra were obtained using one-pulse sequence with suppression of water resonance by presaturation. For serum samples, additional one dimensional ^{1}H NMR spectra were also obtained using standard CPMG pulse sequence.

RESULTS:

Typical ^{1}H NMR serum and urine spectra of 90 days of treated groups and that of a control are shown in Figure 1. The concentrations of ten serum metabolites and six urine metabolites of 90 days of treated and control rats along with statistical evaluation using one way ANOVA are shown in Table 1 & 2 respectively. A significant increase in Alanine on 45 and glutamine on 90 days of Aluminium treatment was found to be significantly reduced near to control in BR treated group of rats. Whereas significant reduced level of acetone and pyruvate in Al treated group were found to be increased in BR (Bacoside) and DP (Donepezil) treated groups. Significant increased level of acetoacetate and BHBT in Al treated group of rats were also found to be insignificant in BR and DP treated group of rats. In urine, significant decrease of succinate and citrate on 45 and 90 days of Al treatment was found to be significantly increased in both BR and DP treated groups of rat. However, significant decreased levels of creatinine, allantoin and trans-aconitate as observed in Al treated group was found to be significantly increased in BR treated group compared to DP treated group.

Table :1 90 Days Serum (Mean ± SD) (mg/dL)

Metablites	Control	Al treated	BR treated	DP treated
Latate	65.2 ± 16.7	66.9 ± 12.1	59.1 ± 4.6	73.7 ± 17.5
Alanine	8.2 ± 2.4	7.1 ± 1.1	9.2 ± 1.1	7.9 ± 1.7
Acetone	2.3 ± 0.7	0.4 ± 0.3[a]	1.3 ± 0.5[b]	1.1 ± 0.5[b]
Acetate	0.9 ± 0.2	1.1 ± 0.2	0.9 ± 0.3	0.8 ± 0.2
Acetoacetate	ND	1.5 ± 0.9[a]	ND[b]	ND[b]
Glutamine	4.3 ± 0.9	6.3 ± 0.8[a]	4.2 ± 1.1[b]	7.1 ± 2.0
Creatine	2.4 ± 0.5	2.1 ± 0.4	2.9 ± 0.3	3.9 ± 1.1
Glucose	89.6 ± 14.5	94.3 ± 10.7	98.7 ± 20.6	88.6 ± 15.4
BHBT	ND	1.7 ± 0.6[a]	ND[b]	ND[b]
Pyruvate	2.2 ± 0.5	1.2 ± 0.7[a]	1.9 ± 0.2[b]	1.9 ± 0.2[b]

Table : 2 90 Days Urine (Mean ± SD) (mg/dL)

Metablites	Control	Al treated	BR treated	DP treated
Citrate	294.1 ± 109.3	38.3 ± 16.3[a]	224.4 ± 80.7[b]	188.2 ± 102.3[b]
Creatinine	46.29 ± 33.7	11.66 ± 3.8[a]	37.73 ± 20.5[b]	30.9 ± 12.1
Allantoin	419.40 ± 205.5	105.14 ± 35.1[a]	149.19 ± 15.7[b]	178.2 ± 39.7
Acetate	ND	1.01 ± 0.5[a]	5.0 ± 3.5[ab]	3.6 ± 1.1[ab]
Trans - Aconitate	123.0 ± 83.8	16.83 ± 10.2[a]	43.78 ± 10.4[b]	42.6 ± 16.3
Succinate	18.97 ± 10.5	2.75 ± 1.7[a]	19.0 ± 5.2[b]	21.4 ± 8.7[b]

[a] $p < 0.05$ compared with control [b] $p < 0.05$ compared with Al treated group.

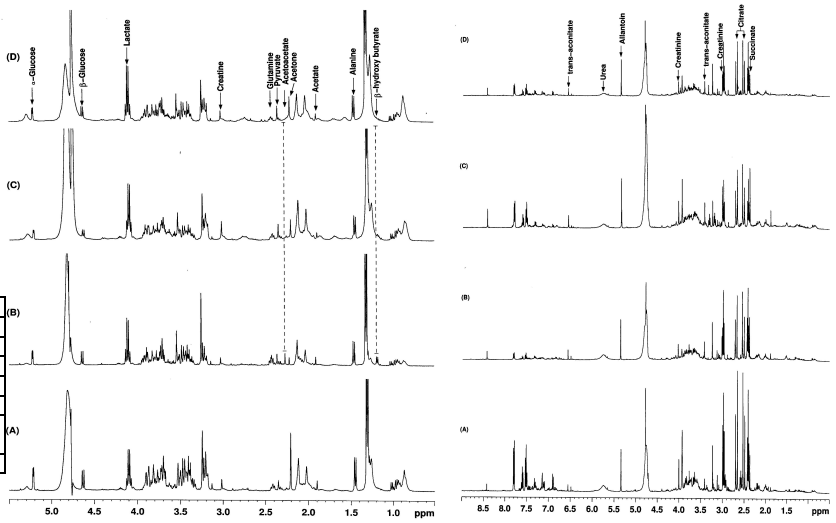

Fig 1: 1H NMR Spectra of serum and urine after 90 days of treatment A) control B) Al treated C) BR treated D) DP treated

DISCUSSION & CONCLUSIONS:

Altered metabolic profiling on 90 days Al administration indicates serious liver and kidney damage. Al also reduces the production of energy *via* oxidative phosphorylation due to interference with many key enzymatic processes of TCA cycle. Significant recovery of glutamine in serum & creatinine, allantoin and trans–aconitate levels in urine by 90 days administration of Bacoside indicate that Bacoside is more effective than Donepezil on prolonged Aluminium insults. Effectiveness of Bacoside could be attributed to its potent antioxidative property[3] as compared to Donepezil. Another possibility is that Bacosides may bind the Aluminium in GI tract and prevent its absorption and hence shows the hepatoprotective effects in rats. Histopathological finding also support our findings. Dilated intercellular spaces and necrosis of tissues as observed in Al treated rats were found to be absent only in Bacoside treated rats.The present metabolic profiling study using ^{1}H NMR showed that Bacoside causes a systemic recovery from the Al^{3+}-induced metabolic perturbation in rats and may be used as an animal model for the kidney and liver dysfunction. This study also demonstrates that in addition to imaging [4], metabolic profiling can also be utilized to study therapeutic effects of herbal medicines.

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