

## CISPLATIN OR CARBOPLATIN CAUSED CHANGES IN THE ACTIVITY LEVELS OF AMINO ACID METABOLIC ENZYMES IN LIVER OF ALBINO RATS

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### ABSTRACT

The present study was aimed to investigate the possible interference of cisplatin or carboplatin on amino acid metabolic enzymes in liver of albino rats. Rats were divided into three groups consisting of eight animals in each group. The rats in the first group were served as control and received 0.9% of normal saline only. The rats in the second group were received cisplatin (3 mg/kg body wt) only. The rats in the third group were received carboplatin (10 mg/kg body wt) respectively. Injections were given intra-peritoneally to rats on 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> day of experimentation. On 45<sup>th</sup> day of experiment, animals were sacrificed by cervical dislocation. Aspartate aminotransferase (AAT), Alanine aminotransferase (ALAT), Glutamate dehydrogenase (GDH) activities were assayed in the cytosol fraction of the liver tissue. Platinum compounds caused significant changes in activity levels of transaminase enzymes. Since these enzymes can be used as biomarkers to assess the stress in the tissues, elevated levels of transaminase enzymes activity levels in the liver of rats treated with platinum-based anticancer drugs indicates hepatic damage after treatment.

**Keywords:** Cisplatin, Carboplatin, AAT, ALAT, GDH, Liver, Rats.

### INTRODUCTION

Enzymes make life on earth possible and all metabolic activities are under the control of enzymes. The myriad chemical reactions going on continuously in living matter would not be possible without enzymes, which are the tools of the living cells. Moreover, a biological system with a balanced metabolic pattern is characterized by a dynamic equilibrium between different enzymatic activities. Any abnormality in the enzyme system and their co-ordination may lead to an inhibition or hyper function of the organ concerned, which ultimately manifests as disease<sup>1</sup>.

Transamination is an important wing of the amino acid metabolism which is mainly involved in transferring amino group of one amino acid to another keto acid thus forming another amino acid, through the enzymes aminotransferases. In addition aspartate aminotransferase (AAT), alanine aminotransferase (AIAT) were studied during several pathological conditions. GDH catalyzes the reversible oxidative deamination of L-glutamate to  $\alpha$ -ketoglutarate and ammonia. An alteration in the GDH activity invariably indicates an alteration in the production of ammonia<sup>2</sup>.

The metabolism of amino acid gained an importance in the overall physiology of an animal and this forms an evidence to understand the biochemical state of the cell. So the present study was designed to assess the activity of selected enzymes which belongs to amino acid metabolism in liver of albino rats which was exposed to platinum compounds. Despite its excellent anticancer activity, the clinical use of cisplatin or carboplatin is often limited by its undesirable side effect such as severe hepatotoxicity. Since, the liver is an important organ actively involved in many metabolic functions and is the

frequent target of number of toxicants<sup>3</sup>. Hence, an attempt was made in the present study to elucidate the influence of platinum-compounds on activity levels of aspartate (AAT), alanine (AIAT) aminotransferases, and Glutamate dehydrogenase (GDH) activities in the cytosol fraction of the liver of albino rats.

### MATERIALS AND METHODS

#### Animals

Healthy adult male albino rats of same age group (70±5 Days) were selected for the present study. Animals were housed in an air conditioned animal house facility at 26±1°C, with a relative humidity of 75%, under a controlled 12 h light/dark cycle. The rats were reared on a standard pellet diet (HLL Animal Feed, Bangalore, India) and tap water ad libitum.

#### Test chemicals

Cisplatin, Carboplatin, was purchased from Sigma chemicals, St.Louis Co., MO and USA. These compounds were dissolved in 0.9% normal saline separately to obtain the final concentration of the 3mg/kg, 10mg/kg body wt. of the animal respectively.

#### Experimental Design

The rats were divided into three groups consisting of eight animals in each group. The rats in the first group were served as control and received 0.9% of normal saline only. The rats in the second group were received cisplatin (3 mg/kg body wt) only. The rats in the third group were received carboplatin (10 mg/kg body wt). Injections were given intra-peritoneally to rats on 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> day of experimentation. On 45<sup>th</sup> day of experiment animals were sacrificed by cervical dislocation.



### Aspartate aminotransaminase (AAT) activity

AAT activity in the liver tissue of rats was assayed by the method of Reitman and Frankel (1957) as described by Bergmeyer and Burns (1965)<sup>8,10</sup>.

The tissue was homogenized (10% W/V) in 0.25 M ice-cold sucrose solution. The homogenate was centrifuged at 5000 rpm for 30 minutes to remove cell debris and nuclei. The supernatant was centrifuged at 35,000 rpm for 60 minutes at 4°C. The clear supernatant thus obtained was used to assay the enzyme activity.

The reaction mixture in a final volume of 1.5 ml contained: 100  $\mu$  moles of sodium phosphate buffer (pH 7.4), 2  $\mu$  moles of 2-keto glutarate, and 50  $\mu$  moles of L-aspartic acid. The reaction was initiated by the addition of appropriate amount of enzyme protein and the reaction mixture was incubated at 37°C for 30 minutes, in a thermostatic water bath. The reaction was then arrested by the addition of 1.0 ml of 2, 4-dinitro phenyl hydrazine (0.01 M) solution (ketone reagent). The contents of the tubes were allowed to stand at room temperature for 20 minutes. Zero time controls were maintained for all the samples by the addition of 1.0 ml of ketone reagent prior to the addition of the enzyme source. To all the tubes 10 ml of 0.4 N sodium hydroxide (NaOH) solution was added and the intensity of the colour developed was read at 545 nm in a spectrophotometer (Hitachi model U, 2001) against blank. The enzyme activity was expressed as  $\mu$  moles of pyruvate formed/mg protein/h.

### Alanine aminotransaminase (AIAT)

AIAT activity in the liver tissue of rats was assayed by the method of Reitman and Frankel (1957) as described by Bergmeyer and Burns (1965).

The tissues were homogenized (10% W/V) in 0.25 M ice-cold sucrose solution. The homogenate was centrifuged at 5000 rpm for 30 minutes. The supernatant was centrifuged at 35,000 rpm for 60 minutes. The clear supernatant thus obtained was used to assay the enzyme activity.

The reaction mixture in a final volume of 1.5 ml contained: 100  $\mu$  moles of sodium phosphate buffer (pH 7.4), 2  $\mu$  moles of 2-keto glutarate, and 100  $\mu$  moles of DL-alanine. The reaction was initiated by the addition of appropriate amount of enzyme source and the reaction mixture was incubated at 37°C for 30 minutes, in a thermostatic water bath. The reaction was then arrested by the addition of 1.0 ml of 2, 4-dinitro phenyl hydrazine (0.01 M) solution (ketone reagent). The contents of the tubes were allowed to stand at room temperature for 20 minutes. Zero time controls were maintained for all the samples by the addition of 1.0 ml of ketone reagent prior to the addition of the enzyme source. To all the tubes, 10.0 ml of 0.4 N NaOH solution was added and the intensity of the colour developed was read at 545 nm in a spectrophotometer (Hitachi model U, 2001) against blank.

The enzyme activity was expressed as  $\mu$  moles of pyruvate formed/mg protein/h.

### Glutamate dehydrogenase (GDH) activity

The GDH activity was assayed in the tissue (liver) by the method of Lee and Lardy (1965). The tissue was homogenized (10% W/V) in 0.25 M ice-cold sucrose solution. The homogenate was centrifuged at 5000 rpm for 30 minutes to remove cell debris and nuclei. The supernatant was centrifuged at 35,000 rpm for 60 minutes. The clear supernatant (cytosol) fraction was used for enzyme assay.

The reaction mixture in a final volume of 2.0 ml contained: 40  $\mu$  moles of sodium glutamate, 100  $\mu$  moles of phosphate buffer (pH 7.4), 0.1  $\mu$  moles of NAD and 4  $\mu$  moles of INT. The reaction was initiated by the addition of 0.5 ml of the enzyme source. The mixture was incubated at 37°C for 30 minutes in a thermostatic water bath, and the reaction was stopped by the addition of 5.0 ml of glacial acetic acid. The formazan formed was extracted overnight into 5.0 ml of toluene at 4°C and measured at 495 nm in spectrophotometer (Hitachi model U, 2001) against a zero time control. The enzyme activity was expressed as  $\mu$  moles of formazan formed/mg protein/h.

## RESULTS

The activity levels of AAT, AIAT and GDH were increased significantly in the liver of rats exposed to cisplatin or carboplatin, when compared to that of the control rats (Table 1; Fig. 1).

## DISCUSSION

Transaminases namely aspartate amino transferase and alanine aminotransferase activities were increased significantly in liver of rats treated with platinum compounds. These transaminases play an important role in the synthesis and lysis of amino acids. Moreover, AAT and AIAT enzymes function at a strategic point as a link between the carbohydrate and protein metabolism. They serve this function by eliminating the nitrogen moieties from amino acids and at the same time provide ketoacids for Kreb's cycle and gluconeogenesis. Elevated activities of aminotransferases during stress would lead to affect oxidative metabolism<sup>4</sup>.

Increase in activity of AAT and AIAT in the present study are in consonance with previous reports. Ramadan *et al.* (2001) demonstrated that cisplatin induced a significant increase in serum AAT and AIAT activities in male rats<sup>5</sup>. This is due to cisplatin-induced liver damage with the consequent leakage of transaminases from hepatocytes. Increased GDH activity was observed in experimental rats treated with platinum-compounds, when compared to the control rats. Elevation in GDH activity in stressed rats indicates increased oxidation of glutamate. Increased GDH activity was reported in rats treated with anticancer drugs such as cyclophosphamide, methotrexate<sup>7</sup>.



**Table 1:** Effect of cisplatin or carboplatin on the changes in activity levels of AAT, AIAT and GDH in the liver of rats.

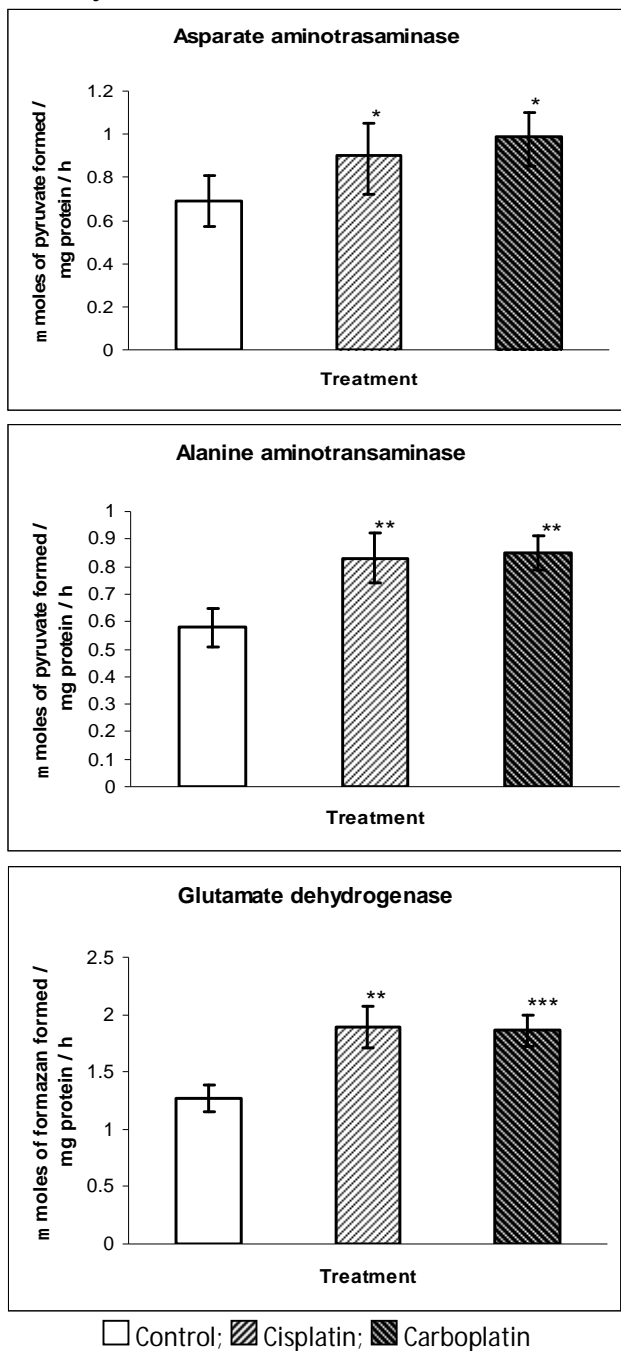
Enzyme	Control	Cisplatin	Carboplatin	ANOVA
AAT <sup>a</sup>	0.69±0.12	0.90*±0.15 (+30.43)	0.99**±0.11 (+43.47)	F <sub>2,21</sub> =11.608 [P=0.0004]
AIAT <sup>a</sup>	0.58±0.07	0.83**±0.09 (+43.10)	0.85**±0.06 (+46.55)	F <sub>2,21</sub> =32.723 [P<0.0001]
GDH <sup>b</sup>	1.27±0.12	1.89**±0.18 (+48.81)	1.86**±0.14 (+46.45)	F <sub>2,21</sub> =44.181 [P<0.0001]

Values are mean ± S.D. of eight individuals. Values in parentheses are percent change from control.

Values are significantly different from control at \*P<0.05; \*\*P<0.001. <sup>a</sup>μ moles of pyruvate formed/mg protein/h

<sup>b</sup>μ moles of formazan formed/mg protein/h

**Figure 1:** Effect of cisplatin or carboplatin on the changes in activity levels of AAT, AIAT and GDH in the liver of rats.



**CONCLUSION**

From the present it was evident that significant increase in activity levels of aspartate aminotransaminase (AAT),

alanine aminotransaminase (AIAT) and glutamate dehydrogenase (GDH) were observed in experimental rats treated with platinum-based anticancer drugs when compared to control rats. Since, these enzymes can be used as biomarkers to assess the stress in the tissues. The elevation of transaminase activity levels in the liver of rats treated with platinum-based anticancer drugs would indicate hepatic damage after treatment.

**REFERENCES**

- Murray RK, Granner DK, Mayers PA, Rodwell VW. (1990). Harper's Biochemistry. Lange Medical Publications, Appleton and Lange, California.
- Dixon M, Webb EC. (1979). In: Enzymes. Academic Press, New York, 627.
- Shahjahan M, Vani G, Shyamala Devi CS. (2005). Protective Effect of Indigofera oblongifolia in CCl<sub>4</sub>-Induced Hepatotoxicity. J. Med. Food; 8: 261-265.
- Knox WE, Green Gard O. (1965). The regulation of some enzymes of nitrogen metabolism on introduction to enzyme physiology. 73 (Eds). G. Weber, In: Advance in enzyme regulation. Bergman Press, New York, 3: 247-248
- Ramadan LA, El-Habit OH, Arafa H, Sayed-Ahmed MM. (2001). Effect of cremophorel on cisplatin-induced organ toxicity in normal rat. J. Egyptian Nat. Cancer Inst. 13: 139-145.
- Cersosimo RJ. (1993). Hepatotoxicity associated with cisplatin chemotherapy. Annals Pharmacotherapy; 27: 438- 441.
- Capel ID, Jenner M, Dorrell HM, Williams DC. (1979). Hepatic function assessed (In rats) during chemotherapy with some Anti-cancer drugs. Clin. Chem. 25: 1381-1383.
- Bergmayer HU, Bruns E. (1965). "Methods of enzymatic analysis". Bergmayer HU(Ed) Academic Press, New York.
- Lee YL, Lardy HA. (1965). Influence of thyroid hormones on L-glycerophosphate and other hydrogenases in the rat. J. Biol. Chem; 240: 1427-1432.
- Reitman S, Fraenkal S. (1957). "A calorimetric method for the determination of glutamic oxaloacetic and glutamic pyruvate transaminases". Am. J. Clin. Pathol; 28: 56.

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