

Full Length Research Paper

Effectiveness of carnosine on disturbed electrolytes homeostasis induced by cisplatin

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We aimed to assess the effect of well known antioxidant carnosine on disturbed plasma and intra-erythrocytes electrolytes and Na⁺-K⁺-ATPase activity by cisplatin. 24 male albino Wistar rats were selected and divided into 4 groups: Group I = untreated control; Group II = cisplatin control (received cisplatin at a dose of 3 mg/ kg body weight; i.p. for 13 alternate days); Group III = carnosine alone (treated group at a dose of 10 mg/kg body weight; i.p. for 13 consecutive days); Group IV = carnosine + cisplatin pretreated group. Carnosine was administered 30 min prior to cisplatin. Carnosine significantly restored the intra – erythrocytes Na⁺, K⁺ and Na⁺-K⁺-ATPase level which consequently affect the plasma Na⁺, Ca⁺⁺, and Mg⁺⁺ level. Effective role conferred by carnosine exhibits its protective activity.

Key words: Cisplatin, carnosine, electrolytes, Na⁺-K⁺-ATPase.

INTRODUCTION

Cisplatin and other platinum derivatives are among the most effective antineoplastic drugs used for the treatment of solid tumors. Cisplatin DNA cross links, cause cytotoxic lesions in tumors, and other dividing cells. DNA damaging agents usually have less toxicity in non - proliferating cell, yet the quiescent proximal tubule cells are selectively damaged by cisplatin (Schrier et al., 2004). A potential mechanism influencing the sensitivity of the cells to cisplatin may result from the interaction of specific proteins with cisplatin–damaged DNA (Arany and Safirstein, 2003; Kuriakose and Kurup, 2008). RNA and protein also interact with cisplatin. Cisplatin protein interactions are the determining step for the therapeutic efficacy of antitumor agents. These interactions alter the rate of clearance of the drug from circulations, thus, are responsible for toxicity to the kidneys and gastrointestinal tract dysmotility (Cabezos et al., 2010). Moreover, cisplatin changed the liver enzymes activities (Ray et al., 2006). Decreased body weight, haemoglobin levels and leucocyte counts were observed with cisplatin administration (Bogin et al., 1994). Electrolytes disturbances are also associated with cisplatin therapy (Blachley and Hill,

1981).

Carnosine is a naturally occurring histidine containing dipeptide also known as β – alanyl – L- histidine (Hipkiss et al., 1998), has number of roles such as buffering, quenching free radicals, enzyme regulation and sarcoplasmic reticulum calcium regulation (Gulshanara et al., 2005; Suer et al., 2011).

Carnosine regulated calcium has been previously reported. This study was conducted to evaluate the possible role of carnosine, if any, on depressed Na⁺ K⁺ ATPase activity related to ion homeostasis induced by cisplatin.

MATERIALS AND METHODS

Animals and diet

24 Wistar albino rats, of male sex (200 to 260 g body weight), purchased from the animal house of ICCBS (International center for Chemical and Biological Center Karachi, Pakistan), were used for the study. Animals were acclimatized to the laboratory conditions one week before the start of experiment and caged in a quite temperature controlled room (23±4°C). Rats had free access to water and standard rat diet. The experiments were conducted in accordance with ethical guidelines of internationally accepted principles for Laboratory use and care in animal research (Health research extension Act of 1985).

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Study design

The animals were randomly divided into four experimental groups (n = 6). Each group consists of six rats and received the following treatment:

Group I: Control group remains untreated

Group II: (positive cisplatin control) received cisplatin i.p. (3 mg/kg body weight) for 5 alternate days

Group III: Received carnosine i.p. (10 mg/kg body weight) for 10 consecutive days

Group IV: Received cisplatin i.p. (3 mg/kg body weight) for 5 alternate days + Carnosine i.p. (10 mg/kg body weight) 30 min prior to cisplatin, for 10 consecutive days

Sample collection

After 48 h of last dose of treated groups, animals were decapitated and blood was sampled from head wound in the lithium heparin coated tubes. The collected blood was mixed gently. A portion of blood was used to collect plasma. The blood was then transferred to the clean glass tube and centrifuged at 2000 rpm for 20 min. The plasma was separated and collected in disposable Eppendorf tubes and stored at -70°C till analysis.

Analytical method

Assessment of electrolytes homeostasis

Plasma separation for electrolytes estimation: Plasma was separated from heparinized blood by centrifugation and analyzed for the estimation of sodium and potassium by flame photometry (Corning 410), and estimation of plasma calcium ion by ion selective electrode method jenway (Ion Meter 3345)

Estimation of intra - erythrocyte sodium and potassium: Washed erythrocytes used for the estimation of intra - erythrocytes sodium and potassium were mixed with gentle inversion and a microhaematocrit capillary sample for packed cell volume determination was taken. Suspension of cells was then lysed by adding 0.01 ml of saponin solution (20% in MgCl₂ 112 mM). 0.3 ml of well mixed lysate was added to 10 ml of lithium nitrate diluent (15 mM) with an automatic pipette. Concentration of erythrocyte sodium and potassium was calculated as mM.

Estimation of plasma sodium and potassium: Plasma was diluted 1:100 with 0.1 NHCl and was used for simultaneous determination of sodium and potassium. The emission intensities of standards and sample were recorded against the respective blank solutions. The emission intensities of sodium, potassium were recorded at 589 and 768 nm respectively.

Erythrocyte membrane preparation: Heparinized blood was centrifuged and plasma was separated. Buffy coat was aspirated and discarded. Erythrocytes were washed three times at room temperature by suspension in the magnesium chloride solution (112 mM), centrifugation at 450 × g at 4°C for 5 min. Final supernatant was retained for the estimation of intra erythrocyte sodium and potassium concentration. Neither electrolyte was detectable in the final wash. The packed red cells extracted by centrifugation at 4°C, 450 × g for 15 min were resuspended and diluted in 25 volumes of 0.011 M Tris-HCl buffer at pH = 7.4. The hemolyzed cells were then centrifuged for 30 min at 12,000 rpm at 4°C and the membrane pellet was resuspended in 30 ml of 0.011 M Tris-HCl buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was ~4 mg protein/ml

of Tris buffer. The concentration of protein was estimated by Biuret Method (Savory et al., 1968). The membrane suspension was stored at -80°C until the assay was performed.

Erythrocyte Na⁺-K⁺-ATPase activity measurement (Denis et al., 1996): ATPase activity was measured in a final volume of 1 ml as follows: membrane (400 µg) were pre-incubated for 10 min at 37°C in a mixture containing 92 mM Tris-HCl (pH = 7.4), 100 mM NaCl, 20 mM KCl, 5 mM MgSO₄.H₂O and 1 mM EDTA. Assays were performed with or without 1 mM ouabain, a specific inhibitor of Na-K-ATPase activity was calculated as the difference between inorganic phosphates released during the 10 min incubation with and without ouabain. Activity was corrected to a nanomolar concentration of inorganic phosphate released mg protein/hour. All assays were performed in duplicate, and blanks for substrate, membrane and incubation time were included to compensate for endogenous phosphate and non - enzyme related breakdown of ATP. Under these experimental conditions, the coefficient of variation was 7.5%.

Estimation of plasma magnesium (Hallry and Sky Peck, 1964): Concentration of magnesium in plasma was estimated by the method described earlier by Hallry and Sky Peck (1964). A protein free filtrate was prepared by mixing 0.2 ml of plasma with 1.8 ml TCA (5% w / v). Standard magnesium solution (5 mg %) was also treated in the same manner. 1 ml of filtrate was taken in separate tubes. 1.5 ml of titan yellow (0.05%) and 0.5 ml of NaOH (4 N) was added. A blank was prepared by taking 1 ml of deionized water and treated similarly as test and standards. The color intensity was measured against blank at 540 nm after 15 min on Schimdzuspectrophotometer UV-120-01.

Statistical analysis

Results were presented as mean ± SD. Statistical significance and difference from control and test values were evaluated by Student's t-test. P-values of P < 0.001, P < 0.01 and P < 0.05 were considered significant.

Significant difference between control with cisplatin, carnosine and cisplatin + carnosine - pretreated rats by t-test were **P < 0.05, *P < 0.01, ***P < 0.001 respectively. Significant difference between positive control, cisplatin with carnosine and cisplatin + carnosine - pretreated rats by t-test were +++P < 0.001, ++P < 0.05, +P < 0.01 respectively.

RESULTS

Intra - erythrocyte sodium level in control, CDDP, carnosine and CDDP + carnosine - pretreated rats

Intra - erythrocyte sodium level was increased (P < 0.01) in cisplatin treated rats (Figure 1). In cisplatin + carnosine group, the increased sodium level was prevented (P < 0.001) while carnosine alone showed decreased sodium level (P < 0.01) as compared with the positive control.

Intra - erythrocyte potassium level in control, CDDP, carnosine and CDDP + carnosine - pretreated rats

Figure 2 shows a marked decreased erythrocyte potassium level (P < 0.05) in cisplatin treated rats as compared with the control group while carnosine alone

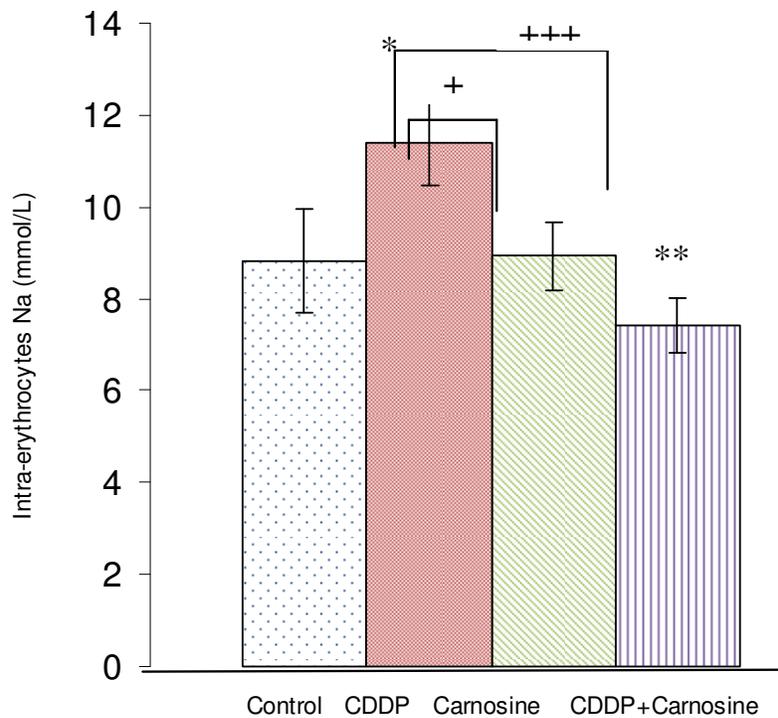


Figure 1. Intra - erythrocytes Na⁺ level in control, Cisplatin, Carnosine and Cisplatin + Carnosine - pretreated rats. *P < 0.01, **P < 0.05 compared with control, +P<0.01,+++P<0.001 compared with cisplatin.

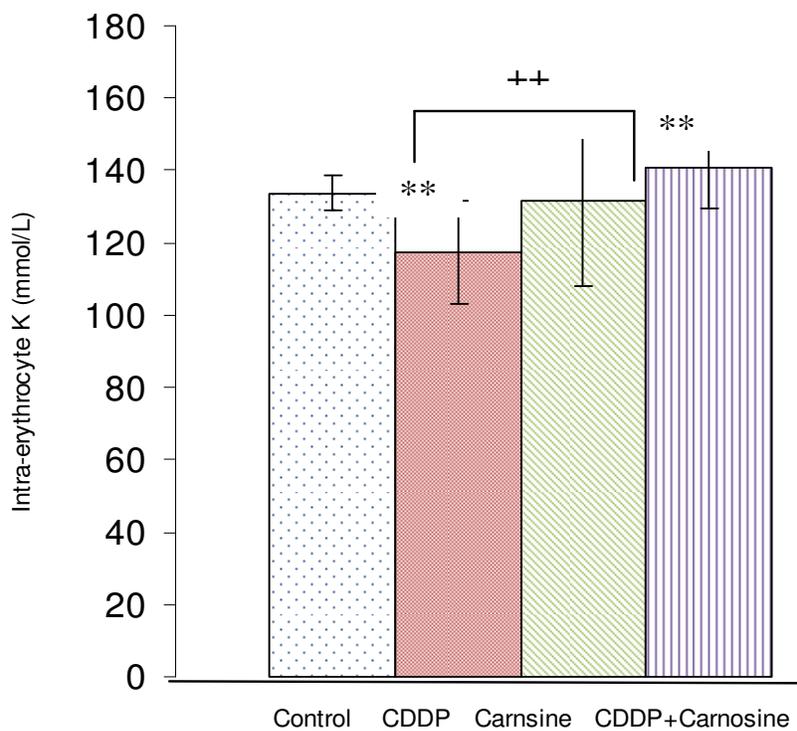


Figure 2. Intra-erythrocytes K⁺ level in control, cisplatin, carnosine and cisplatin + carnosine - pretreated rats. **P < 0.05 compared with control,++P<0.05 compared with cisplatin.

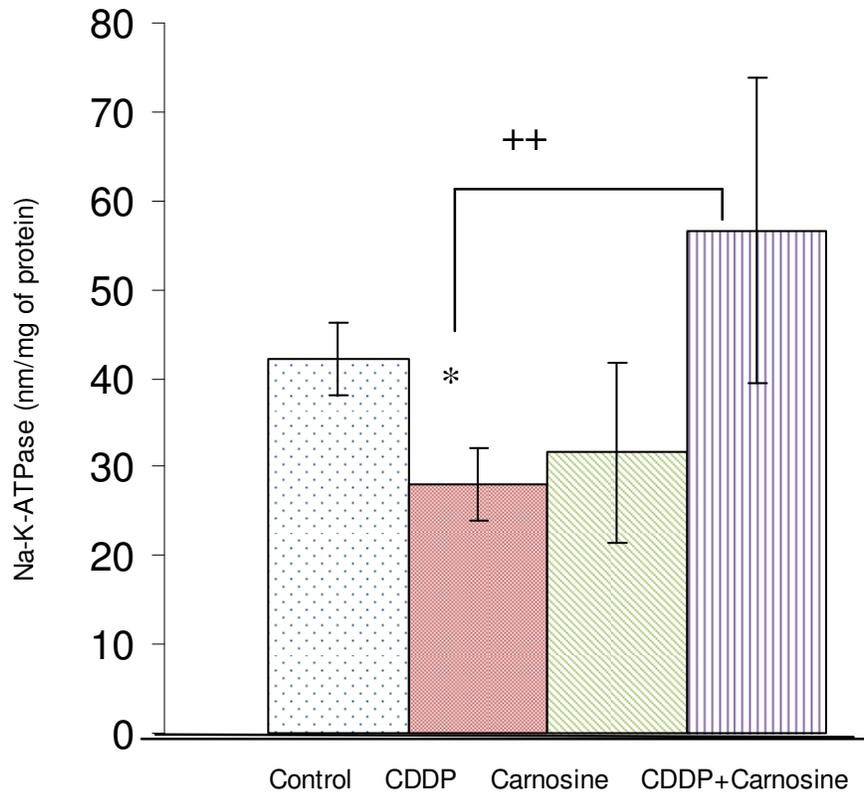


Figure 3. Intra - erythrocytes $\text{Na}^+\text{-K}^+\text{-ATPase}$ level in control, cisplatin, carnosine and cisplatin + carnosine - pretreated rats. * $P < 0.01$ compared with control, ++ $P < 0.05$ compared with cisplatin.

treatment showed no significant results. Pretreatment of carnosine partially prevented the decreased levels of potassium ($P < 0.05$) as compared to positive control.

Intra - erythrocyte $\text{Na}^+\text{-K}^+\text{-ATPase}$ level in control, CDDP, carnosine and CDDP + carnosine - pretreated rats

Cisplatin treated group showed a marked decreased in $\text{Na}^+\text{-K}^+\text{-ATPase}$ level ($P < 0.01$) as compared with the control (Figure 3). CDDP + carnosine treated rats prevented decrease $\text{Na}^+\text{-K}^+\text{-ATPase}$ level ($P < 0.05$), while alone carnosine showed no significant results as compared to positive control.

Plasma magnesium level in control, CDDP, carnosine and CDDP + carnosine - pretreated rats

Figure 4 shows that a significant decreased plasma magnesium level ($P < 0.01$) was observed in cisplatin treated rats as compared with the control. The decreased magnesium level was partially prevented in carnosine + CDDP group ($P < 0.01$). Alone carnosine treatment

showed significant increased level ($P < 0.01$) as compared to positive control.

Plasma calcium level in control, cisplatin treated, carnosine treated and cisplatin + carnosine - pretreated rats

Figure 5 shows a significant effect on plasma calcium level in cisplatin - treated rats. Calcium level was significantly decreased ($P < 0.01$) in cisplatin treated rats as compared with the control. Pretreatment with carnosine partially prevented increase in plasma calcium level in CDDP + carnosine ($P < 0.05$) group and alone carnosine group showed slight increased calcium level ($P < 0.05$) as compared with the positive control.

Plasma sodium level in control, CDDP, carnosine and CDDP + carnosine - pretreated rats

Figure 6 shows the significant effect on plasma sodium level in cisplatin treated rats. Sodium level was decreased in cisplatin treated ($P < 0.05$) rats as compared to the control group. Pretreatment with carnosine prevented

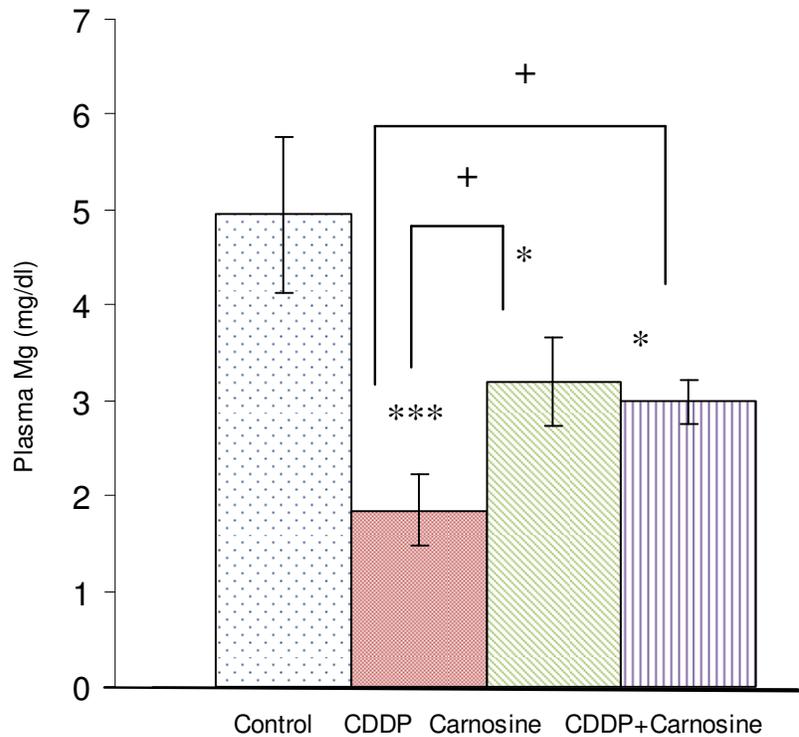


Figure 4. Plasma Mg⁺⁺ level in control, cisplatin, carnosine and cisplatin + carnosine - pretreated rats. *P<0.01, ***P<0.001 compared with control,+P<0.01 compared with cisplatin

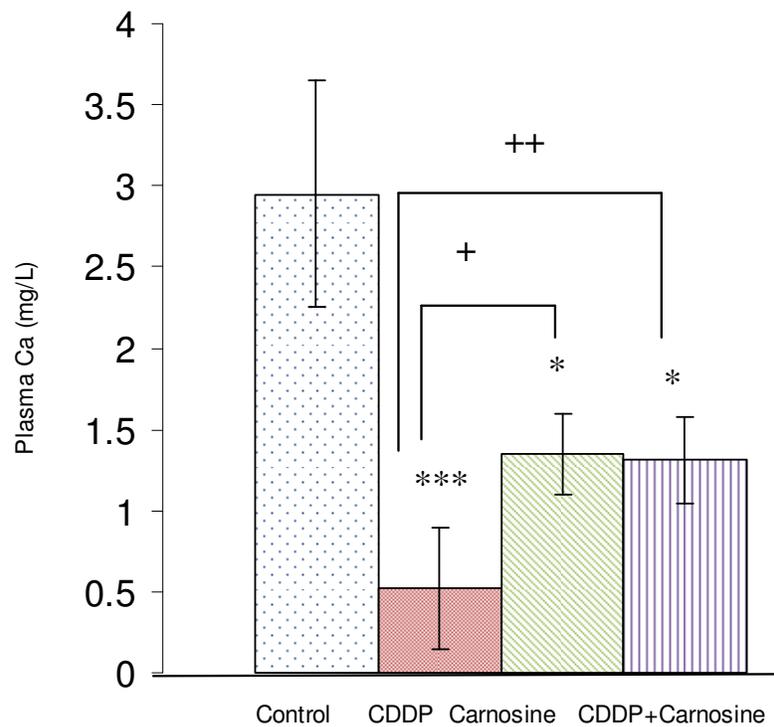


Figure 5. Plasma Ca⁺⁺ level in control, cisplatin, carnosine and cisplatin + carnosine - pretreated rats. *P<0.01,***P<0.001 compared with control,+P<0.01,++P<0.05 compared with Cisplatin

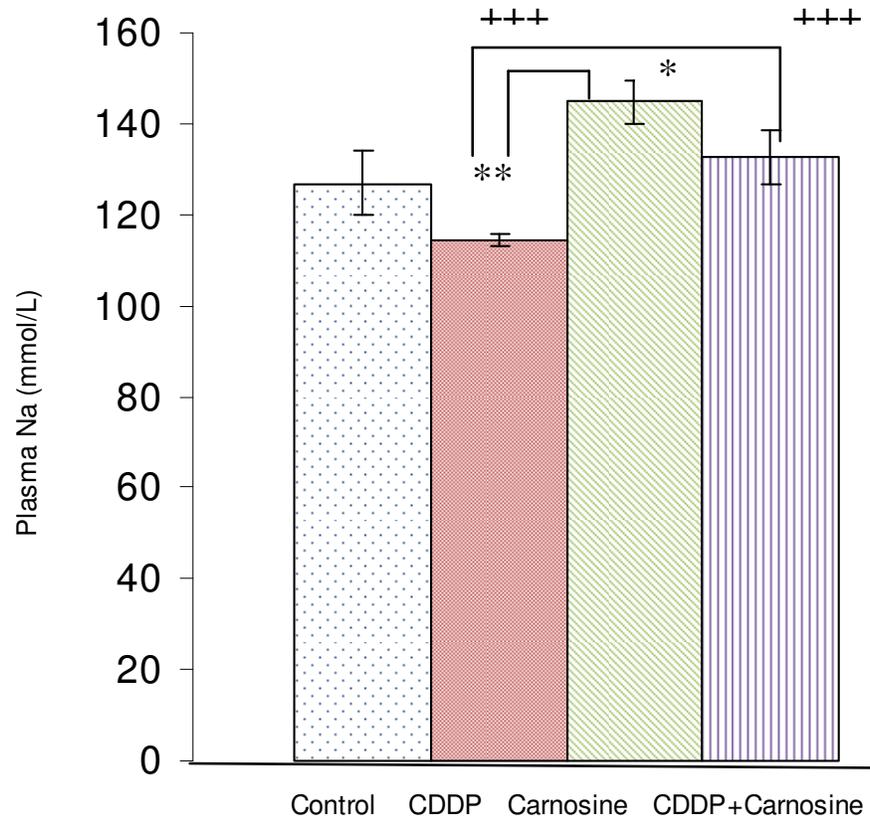


Figure 6. Plasma Na⁺ level in control, cisplatin, carnosine and cisplatin + carnosine - pretreated rats. **P < 0.05, *P<0.01 compared with control, +++P<0.001 compared with cisplatin.

the decrease in serum sodium level ($P < 0.001$). Alone carnosine treatment showed increased sodium level ($P < 0.001$).

Plasma potassium level in control, CDDP, carnosine and CDDP + carnosine - pretreated rats

Figure 7 shows non-significant increased potassium level in cisplatin treated rats as compared to the control. The results were not different in CDDP + carnosine group as compared to the control. While alone carnosine treatment showed no significant results.

DISCUSSION

In this study, we investigated the effect of cisplatin on plasma and membrane electrolytes because of their importance in the homeostasis of cell and its protection by carnosine. Our results demonstrated that plasma calcium, magnesium and sodium (Figures 4, 5 and 6), intra - erythrocytes potassium and enzyme Na⁺-K⁺-ATPase (Figures 2 and 3) significantly decreased after

cisplatin administration (3 mg/kg body weight). Similarly, intra-erythrocytes sodium, and plasma potassium (Figures 1 and 7) levels significantly increased. Previously, it has been reported that hypocalcaemia is a known side-effect in high-dose cisplatin chemotherapy of solid tumors (Arany and Safirstein, 2003). Hypocalcaemia is caused by excessive urinary loss and decreased renal up-take during high-dose cisplatin treatment. Proximal tubular damage leads to decreased reabsorption of cations. Calcium plays a key role in regulating cell metabolic processes by stimulating or inhibiting key enzymes (Zhang and Lindup, 1996). Cisplatin stimulate stage 4 respiration, which is similar to uncoupling of oxidative phosphorylation (Binet and Volfin, 1977), causing efflux Ca⁺² from the mitochondria. Cells must keep the intracellular Ca⁺⁺ concentration at a very low level to maintain Na⁺-K⁺-ATPase in an active state (Zhang and Lindup, 1996). Guarino et al. (1979) reported that increased levels of intracellular Ca⁺² are known to shut down Na⁺-K⁺-ATPase, which is an integral glycoprotein of the plasma membrane.

It is found that the loss of ATP leads to disruption of cellular ion homeostasis with decreased cellular K⁺ content, increased Na⁺ content and membrane

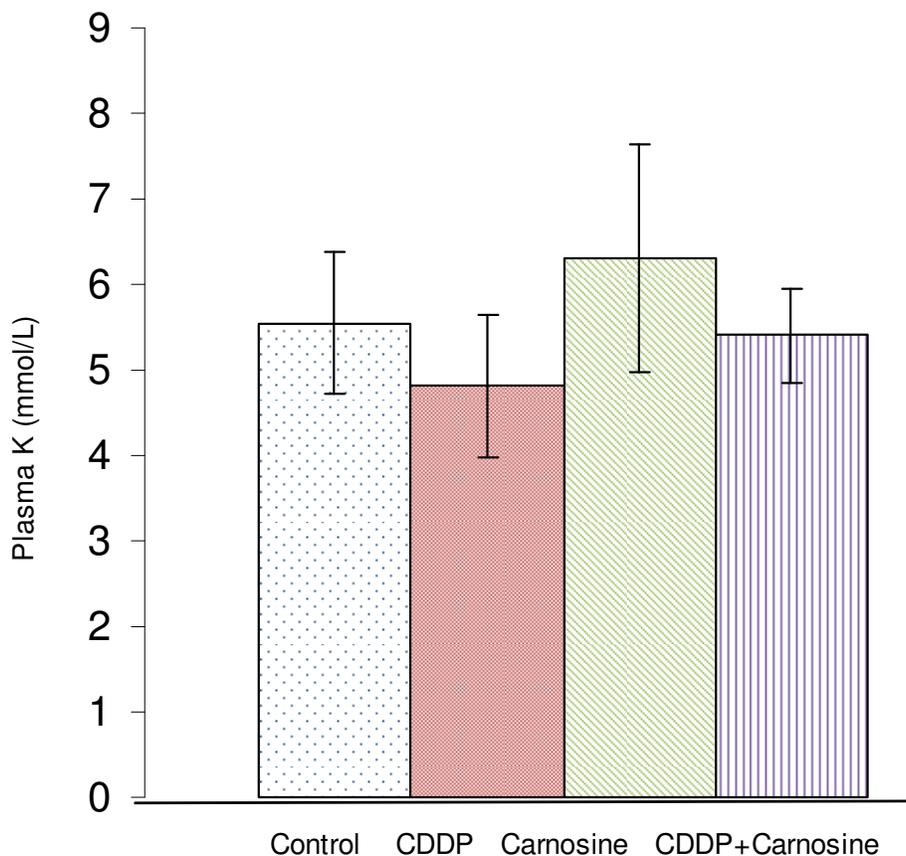


Figure 7. Plasma K⁺ level in Control, Cisplatin, Carnosine and Cisplatin + Carnosine - pretreated rats.

depolarization. Increased cytosolic free Ca²⁺ concentrations can occur in the early or late phase of cell injury and play a critical role leading to cell death (Goren, 2003). The Na⁺-K⁺-pump at the cellular membrane maintains a high cellular potassium concentration by active transport against a considerable gradient. Magnesium acts as a substrate for Na⁺-K⁺-ATPase, thus, hypomagnesium impaired the pump activity (Lajer et al., 2005). Cell permeability and potassium gradient increased, so potassium leaves the cell and in compensation an influx of sodium and hydrogen ions will take place passively. Also, magnesium leaves the cell, if not enough ATP is present for forming the ATP - Mg complex and calcium influx will follow (Lajer et al., 2003). Carnosine at a dose of 10 mg/ kg partially restored the calcium, magnesium and Na⁺-K⁺-ATPase level (Figures 3, 4 and 5) which implicates the Na⁺ and K⁺ level as shown in Figures 1 and 2.

Previously, was reported that cisplatin the increased ROS (reactive oxygen species) (Shafaq and Tabassum, 2010) which consequently altered the membrane permeability, thus, disturbed the electrolytes homeostasis as described earlier. ROS has high affinity towards the thiol group, which oxidized -SH (thiol) group

of Na⁺-K⁺-ATPase and decreased its activity. It is found from present results that carnosine treatment regulated the altered electrolytes homeostasis and activated Na⁺-K⁺-ATPase by scavenging the reactive oxygen species which subsequently restored the membrane permeability. Finally, electrolytes homeostasis of carnosine is also a Ca²⁺ regulator, known previously to maintain Na⁺-K⁺-ATPase activity.

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