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BIOCHEMICAL AND HAEMATOLOGICAL ASPECT OF HYPOPHOSPHATEMIA IN PREGNANT MURRAH BUFFALOES

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ABSTRACT

Present study was conducted on 40 pregnant Murrah buffaloes belonging to Malwa region of Madhya Pradesh, India. Animals were divided into two groups, Group I (n=10) consisting of clinically healthy animals and Group II (n=30) consisting of hypophosphataemic animals with a history of passing coffee colored urine, reduced appetite and decreased milk production. Serum biochemical parameters divulged high mean concentration of serum glucose, bilirubin, total bilirubin, ALT and AST besides serum alkaline phosphatase, creatinine and blood urea nitrogen while as notably decreased levels of serum albumin, enzyme glucose-6 phosphate dehydrogenase (G6PD) and total serum protein. The blood mineral picture divulged elevated levels of potassium, molybdenum and iron whereas remarkably reduced concentrations of copper and phosphorous were recorded. Hematological studies revealed decreased red blood cells (RBC) count, hemoglobin (Hb) and packed cell volume (PCV) with increased erythrocyte sedimentation rate (ESR), neutrophil and lymphocyte count.

Keywords: *Bubalus bubalis*, buffaloes, hypophosphatemia, haemoglobinurea, Murrah, PPH, G6PD

INTRODUCTION

Parturient haemoglobinuria (PHU) is one of the major, economically important and highly fatal diseases of dairy animals in India and other tropical countries (Pirzada and Hussain, 1998). It is an acute diseases characterized by hypophosphatemia, intravascular haemolysis, haemoglobinuria and anemia. Hypophosphataemia is a well-known metabolic disorder of cattle and buffaloes resulting in deficiency of phosphorus or imbalance in the Ca:P ratio leading to hypophosphataemia and haemoglobinurea by altering the process of glycolysis and ATP synthesis in RBCs (Sharma *et al*., 2014). Several metalloenzymes, which include glutathione peroxidase having Se as cofactor), catalase having Fe as cofactor, and superoxide dismutase having Cu, Zn and Mn as cofactors play a critical role in protecting the internal constituents from oxidative damage (Han *et al*., 2006). Buffaloes are particularly susceptible

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to this disease during advanced pregnancy or within first four weeks after parturition, usually between third and sixth lactation (Chugh *et al*., 1996). Therefor, present study was designed to investigate haematobiochemical parameters of hypophosphatemic pregnant Murrah buffaloes.

MATERIALS AND METHODS

The present study was conducted on 40 pregnant Murrah buffaloes of district Mhow (M.P, India). The animals were divided into two groups, Group I (n=10) consisted of apparently healthy animals while as Group II (n=30) consisted of hypophosphataemic animals. Clinical examination suspected animals having history of anorexia, passing of red coloured urine; revealed normal rectal temperature, increased respiration rate, pale mucous membrane and red, dark red to coffee colored and its pH was strongly alkaline. These animals were subjected to clinical and laboratory examination for further confirmation. Differential diagnosis was ruled out through laboratory tests.

From each animal 15 ml of blood was collected from jugular vein ensuring all aseptic measures. Out of which 5 ml of blood aimed was stored in EDTA containing vacutainers and were kept for estimation of hematological parameters. Approximately, 10 ml was stored sterile serum separation vials for estimation of serum parameter. Vials for serum separation were centrifuged at 3000 rpm for 15 minutes for separation of serum from coagulated blood after which it was stored in aliquots at -20°C till further processing.

The analytes like glucose, total bilirubin, serum albumin, serum globulin, total protein, blood urea, serum creatinine, serum ALT, serum AST, G6PD and serum alkaline phosphatase were estimated in serum by using autoanalyzer. Mineral estimation *viz* calcium, phosphorus, iron, molybdenum and copper were analyzed in serum by using atomic absorption spectrophotometer (Varian Spectr AA-5). Potassium levels were determined with flame photometer (Jenway PFP-7).

Hamatological parameters like PCV, ESR, RBC, neutrophil and Hb counts were analysed in the blood of both groups using proper methods.

The whole data was subjected to paired T-test to find out the level of significance in different treatments using SPSS software.

RESULTS AND DISCUSSION

Biochemical studies

Biochemical studies revealed high mean concentration of blood glucose, serum creatinine, blood urea, total bilirubin and enzymes like serum alkaline phosphatase, serum ALT, serum AST in the diseases animals (Group II) as compared to normal (Group I). While significantly low concentration of G6PDH, serum albumin, total protein were found (Figure 1). Increase in blood glucose level may be attributed to glycogenolysis and stress induced release of glucocorticoids in diseased animals (Benjamin, 1978; Singh *et al*., 1989; Latimer *et al*., 2003). Increase in blood urea level could be attributed to the endogenous release of corticosteroids, starvation and tubular epithelial necrosis occurring in Group II animals as compared to group I animals (Akhtar *et al*., 2007).

A significant increase in serum creatinine concentration in hypophosphataemic buffaloes was observed in present study as compared to healthy buffaloes. This is probably the first report worked out so far as per the literature available on the pregnant murrah buffaloes. Due to reduced GFR in hypophosphataemic animals there is decrease in creatinine clearance from the renal cells. This eventually leads to increase in concentrations of creatinine levels in serum of diseased animals (Latimer *et al*., 2003).

A significant increase in the serum total bilirubin in hypophosphataemic buffaloes as compared to healthy buffaloes was observed in the present study. These finding were in agreement with the results reported by Digraskar *et al*., 1996; Akhtar *et al*., 2007. In diseased animals, there occurs severe haemolysis which leads to hypoxia or decreases supply of oxygen concentration to the cells. Hypoxia induces stress in the hepatocellular tissue affecting structure and functions of liver. Loss of hepatic functions results in decreased capacity for bilirubin uptake by liver affecting bilirubin conjugation and ultimately leading to increase in level of serum bilirubin (Latimer *et al*., 2003). On the otherhand, the continuous destruction of RBCs in parturient paresis leads to increased formation of bilirubin, hence increasing serum bilirubin levels (Benjamin, 1978; Kurundkar *et al*., 1981; Pirzada and Ali, 1990; Akhtar *et al*., 2007).

A significant decrease in serum albumin concentration was first time observed in pregnant hypophosphataemic animals, which is indicative of poor liver function due to hypoxia. The total protein level showed a significant decrease which is due to decreased serum albumin level. The present study showed nonsignificant difference between the values of serum globulin in hypophosphaetmic buffaloes and healthy buffaloes. The level of serum ALT and AST showed significant rise in hypophosphaetmic animals which is indicative of liver damage in diseased animals in accordance with the findings of Singh *et al*., 1992.

The decrease in erythrocyte G6PD activity as observed in the current study leads to reduced glutathione levels thereby causing oxidative stress to erythrocytes. Among the two major pathways of glucose metabolism in the red blood cells, the pentose phosphate pathway (PPP) is of utmost significance for normal red cell survival. The first reaction in PPP is the catalytic action of the enzyme G6PD in oxidizing glucose 6 phosphate. Reducing potential in the cell in the form of NADPH is generated in this reaction by which glutathione is maintained in reduced state thus protecting the red cells from oxidative stress. Due to deficiency of this enzyme, oxidative stress increases in RBCs leading to hemolytic anemia as observed in parturient haemoglobinuria (Latimer *et al*., 2003).

Mineral profile

Mineral profile revealed high mean concentration of iron, molybdenum and potassium. On the otherhand significantly low concentration of phosphorous and copper were found in the current study (Figure 2). A significant decrease in the concentration of serum inorganic phosphorus in hypophosphataemic buffaloes might be due to supply of phosphorus deficient fodder, poor supplementation of phosphorus and heavy drainage of phosphorus through milk (Bhikane *et al*., 1995). Hypophosphataemic cases were frequently seen during third trimester of pregnancy which infers higher requirements of phosphorus during this stage (Karapınar *et al*., 2006). The present study showed nonsignificant difference between the values of serum calcium in hypophosphataemic buffaloes and healthy buffaloes. This was in accordance with the study reported by Kurundkar *et al.* (1981); Digraskar *et al.* (1996); Akhtar *et al.* (2007). Low levels of phosphorous disrupts the phospholipid layer of RBCs cell memebrane

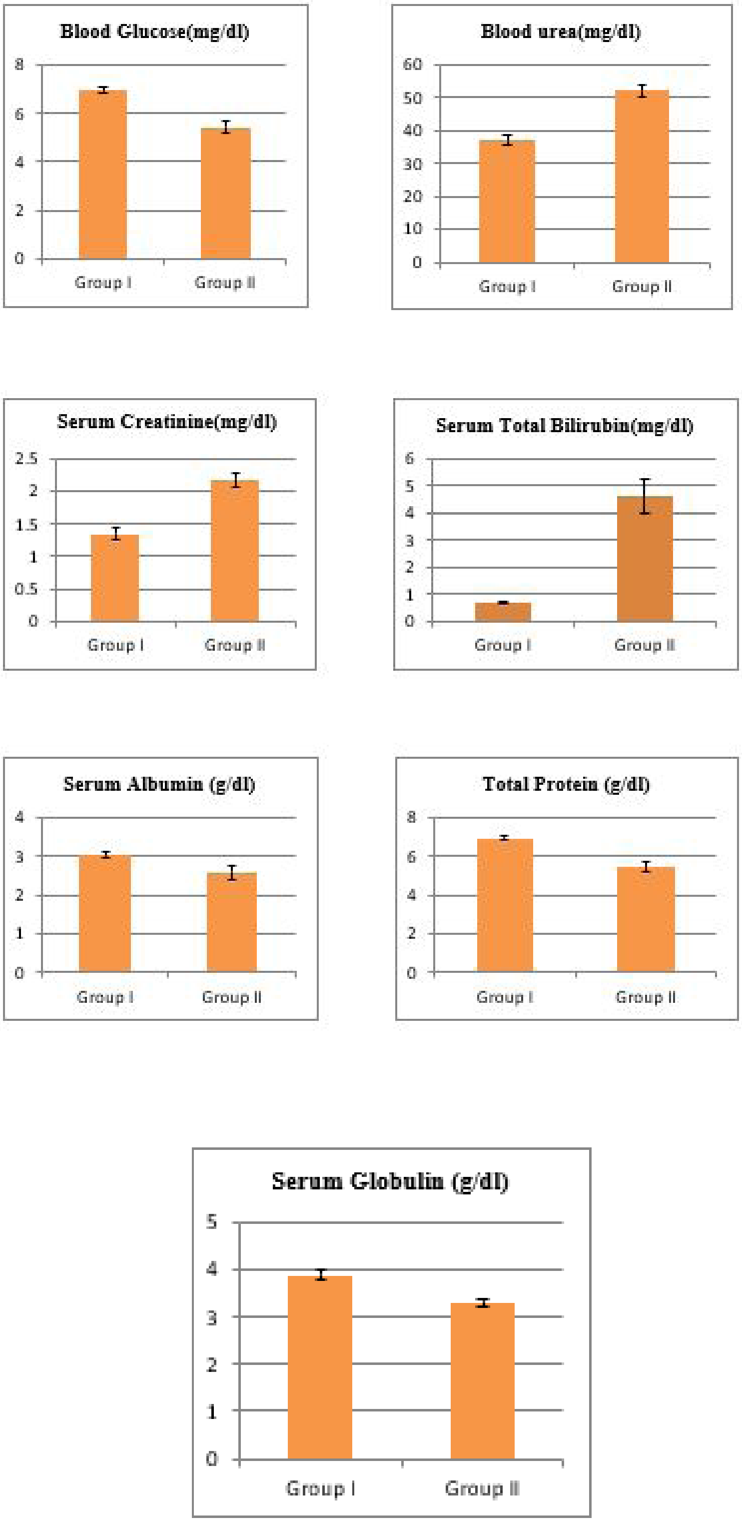

Figure 1. Comparison of Biochemical parameters (Mean±SE) in healthy and hypophosphaetmicbuffaloes.

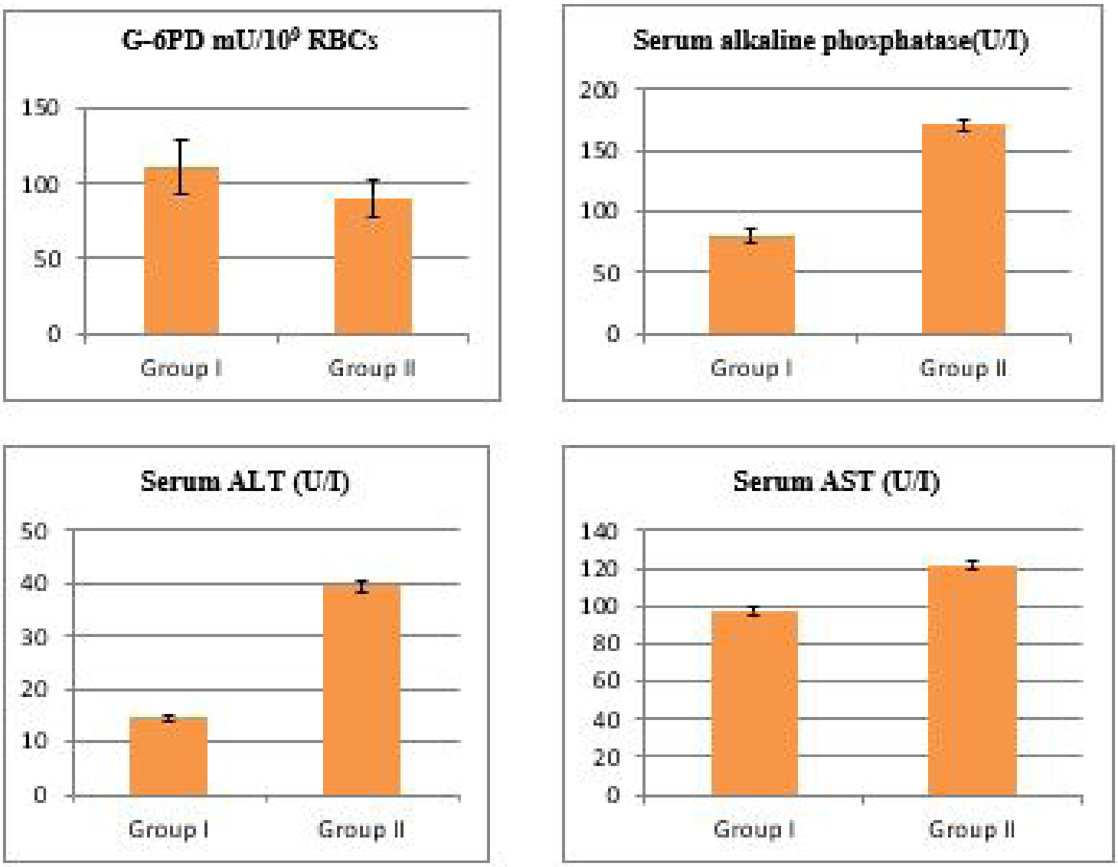

Figure 2. Comparison of Biochemical parameters-enzymes (Mean±SE) in healthy and hypophosphaetmic buffaloes.

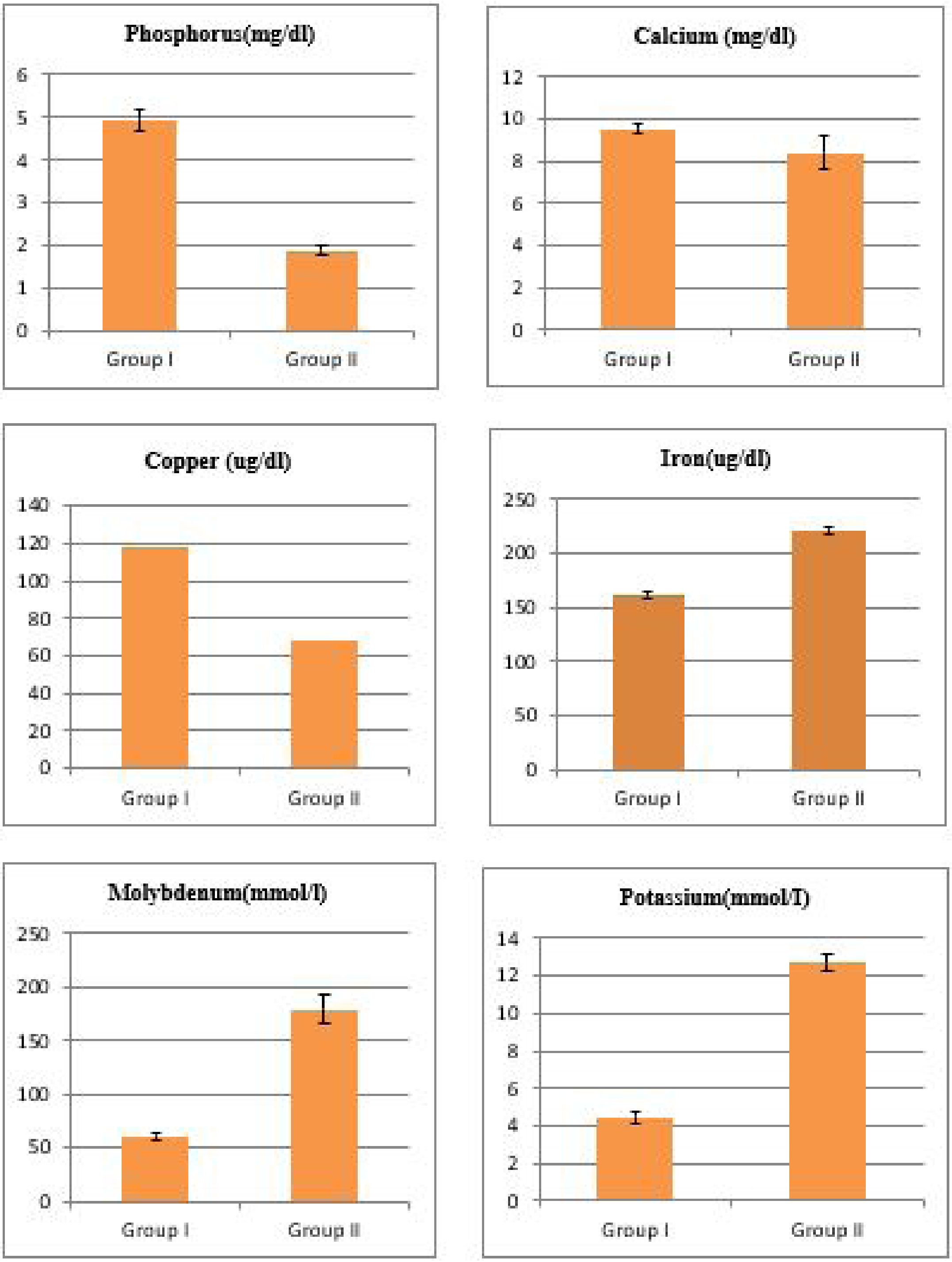

Figure 3. Serum mineral profile (Mean ± SE) in healthy and hypophosphaetmic buffaloes.

resulting in hemoglobinuria, loss of appetite, reduction in milk production and ultimately death. The concentration of copper in hypophosphataemic buffaloes was significantly lower and the concentration of serum molybdenum in affected buffaloes was significantly higher than in healthy buffaloes. This finding was in accordance with the results of Digraskar *et al.* (1996); Akhtar (2007). Decreased levels of copper in present study could be due to a three way interaction between copper, molybdenum and sulfur (McDonald, 2002). As reported by Suttle (1991), this interaction can occur with concentrations of molybdenum and sulfur that are naturally present in feed stuffs and is involved in the formation of thiomolybdates in the rumen (Spears, 2003). Sulfides are produced by the rumen micro organisms via reduction of sulphate and also degradation of sulfur amino acids. These sulfides react with molybdate to form thiomolybdates which bind with copper and forms highly insoluble complexes that do not release copper even under acidic conditions and render it unavailable to the animal for utilization resulting in copper deficiency (Sarwar and Hasan, 2001). The higher concentration of serum iron could be due to acute intravascular haemoloysis (Latimer *et al*., 2003) as intravascular hemolysis commonly occurs in hypophosphataemic cases (Radostits, 2000). The serum potassium concentration in hypophosphataemic buffaloes was significantly higher than the concentration in healthy buffaloes. These observations were in accordance with the values reported by Pandey and Misra (1987) in hypophosphataemic buffaloes. The increase in potassium could be attributed to cell membrane damage. (Latimer *et al*., 2003).

Hematological studies

A decrease in RBC, Hb and PCV indicated severe anaemia in hypophosphataemic buffaloes that could be attributed to intravascular haemolysis (Benjamin, 1978; Pandey and Misra, 1987; Smith, 1990; Digraskar, 1991). In the present study ESR showed significant increase in hypophosphataemic buffaloes as compared to ESR in healthy buffaloes The present findings were in agreement with the results recorded by Dhillon *(*1972); Arif (1997); Akhtar*et al.* (2007). In the present study, total leucocyte count (TLC), monocytes and eosinophil counts showed non significant differences, however neutrophil and lymphocyte counts showed significantly increasing and decreasing trend in hypophosphataemic buffaloes respectively as compared to healthy ones. Increasing and decreasing trend in neutrophil and lymphocyte counts in the present study could be attributed to the endogenous release of corticosteroids. Great oxidative stress to erythrocytes in hypophosphatemia is the source of the release of corticosteroids (Bhardwaj *et al*., 1988) that results in increased neutrophils and depressed lymphocyte counts.

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