



ISSN (E): 2277- 7695  
ISSN (P): 2349-8242  
NAAS Rating 2017: 5.03  
TPI 2017; 6(11): 125-128  
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www.thepharmajournal.com  
Received: 21-09-2017  
Accepted: 22-10-2017

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## A study on comparison of haemato-biochemical changes in haemoglobinuria buffaloes

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### Abstract

The present study was carried out to investigate the haemato-biochemical urological changes in post parturient haemoglobinuric buffaloes. Six buffaloes presented to the TVCC Dept. of Veterinary College, Bidar. Blood samples were collected from the study group animals for the estimation of various haemato-biochemical parameters and compared with healthy control group. Haemogram reduced significantly in haemoglobinuria buffaloes when compared to healthy buffaloes with corresponding decrease in MCH and MCHC values indicative of severe anaemia. Mean MCV values increased significantly in haemoglobinuria buffaloes compared to healthy animals will substantiate macrocytic hypochromic anaemia. TLC values were within normal physiological limits in both study and control groups differ insignificantly. Marked decrease in the serum phosphorus in haemoglobinuria buffaloes differ significantly when compared to healthy buffaloes similar decrease in the mean values of calcium indicative of hypophosphataemia with concurrent hypocalcaemia induced haemolytic crisis due to reduced glycolytic activity and membrane instability of RBCs. Haemoglobinuria buffaloes were successfully treated with sodium acid phosphate (Inj Novizac®) along with other supportive treatment. Haemato-biochemical parameters were estimated on 5<sup>th</sup> day post treatment and were within normal physiological limits.

**Keywords:** buffaloes, haemoglobinuria, serum phosphorus, hypophosphataemia, macrocytic hypochromic anaemia

### Introduction

India has diverse population of livestock and ranks first in the milk production. Total population of buffaloes in India is 108.7 million (Livestock census, 2012) constitutes up to 21.23 per cent of total livestock. Under production stress several diseases are noticed in buffaloes and succumb to infection. Day by day numbers of cases of production disease in buffaloes are in increasing trend. Among common production disease, post parturient haemoglobinuria pose a potent threat to the milking buffaloes in Indian subcontinent affecting a considerable number of buffaloes every year during advance pregnancy and early lactation and has great potential for causing severe economic loss to the farmers (Dalir-Naghadeh *et al.*, 2005; Gahlawat *et al.*, 2007; Akhtar *et al.*, 2008; Ghanem and El-Deeb 2010) [7, 12, 2, 13]. It is a non infectious haemolytic syndrome of buffaloes and cattle which is characterized by intravascular haemolysis, anaemia and haemoglobinuria (Akhtar *et al.*, 2007a) [1]. In Indian subcontinent it is associated with diversified etiological factors as underlying cause that is responsible for occurrence of hemoglobinuria in buffaloes. Feeding of phosphorus deficient diets for longer period of time with cruciferous plants, berseem (saponin), maize, sorghum, sugarcane, lucerne, sarson *etc.*, are speculated to be a common cause (Akhtar *et al.*, 2007) and even hypermolybdenosis and hypocuprosis also associated with impairment in absorption of phosphorus leading to hypophosphataemia in buffaloes (Radostits *et al.*, 2007) [19]. Hypophosphataemia reduces erythrocyte glycolytic activity leading to diminished ATP production which is essential for maintenance of normal integrity of RBCs resulting in to haemolytic crisis. Therefore, underlying cause for haemolytic crisis in buffaloes need to be elucidated before taking up rational treatment. Hence, the present paper describes the haemato-biochemical and urological changes in haemoglobinuria buffaloes and its treatment.

### Materials and Methods

A total of six haemoglobinuria buffaloes were included in the study group which were presented at Veterinary Clinical Complex Hospital, Veterinary College Bidar, Karnataka.

Cases presented were from different localities from in and around Bidar with a history of recent calving and advanced pregnancy. The clinical diagnoses of the affected animals were made based on the characteristic clinical signs exhibited by animals viz., haemoglobinuria, anaemia, pallor of mucous membranes, tachycardia, dyspnoea and difficulty in passing faeces which was further differentiated with other diseases causing haemoglobinuria (haemoprotozoans and leptospirosis) through suitable diagnostic aid (Coles *et al.*, 1973) [5] like dark field microscopy examination and blood smear examination. Haemato-biochemical and urological changes in haemoglobinuria buffaloes were compared with healthy control group.

### Haematological Examinations

Blood samples (2 ml) were collected by jugular venipuncture of the selected animals in to the sterile vials containing disodium salts of EDTA as anticoagulant under aseptic conditions, for the estimation of haematological parameters (Haemogram, Leucogram and Erythrocytic Indices) on the 0<sup>th</sup> day (before treatment) and 5<sup>th</sup> day (post treatment). Parameters were estimated on fully automated haematology cell counter- Automatic Blood Cell Counter, Model PCE 210, Manufactured by ERMA Inc., Tokyo, Japan. Blood smear examination was performed to rule out haemoprotozoan infection.

### Biochemical Examinations

For biochemical estimations 10 ml blood was collected in vials coated with clot activators and blood was allowed to coagulate. Serum was separated by centrifugation at 2500 rpm for 10 minutes and serum was collected in eppendorf tubes, labelled accordingly and maintained at -20°C until analysis. Biochemical Parameters like calcium and phosphorus were analysed by ARTOS<sup>®</sup> semi automatic biochemical analyser.

### Urological study

The urine sample from affected animals were collected in clean sterilized glass beaker and they were analysed by reagent urine strips (Uro Colour<sup>®</sup>10 Standard Diagnostics Inc.) predominantly used to differentiate haemoglobinuria with haematuria and other abnormal constituents of urine viz. bilirubin, urobilinogen, specific gravity, leucocytes, nitrates, proteins, ketone bodies, glucose and pH. Further urine samples were subjected to dark field microscopic study to rule out leptospirosis.

### Statistical Analysis

The haematological and biochemical values obtained in affected group and control group were subjected to statistical analysis by one way ANOVA using Statistical Package for Social Sciences (SPSS) version 20. Significance was set at 5 per cent ( $p < 0.05$ ).

### Results and Discussion

The haemato-biochemical and urine parameters were evaluated in haemoglobinuria buffaloes. Several metabolic adaptations are mediated by the endocrine system to adequately support the changes during early lactation and can be attributed to hypophosphataemia resulting in haemoglobinuria in buffaloes. The comparative analyses of haematological parameters are tabulated in Table (1). The haemogram (Haemoglobin, total erythrocyte count and packed cell volume) in affected animals were reduced significantly ( $p < 0.05$ ) when compared with healthy control

group indicative of severe anaemia. The results of the present study were in close confirmation with (Bhikane *et al.*, 2004; Akhtar, 2006; Dua, 2009) [4, 3, 8]. Hypophosphataemia results in the decreased RBC glycolysis and ATP synthesis which results in altered function and structure of the RBC lead to increased fragility and intra vascular haemolysis (Ogawa *et al.*, 1987) [16]. The mean values of leucogram in haemoglobinuric buffaloes showed insignificant change when compared to healthy control group and are within the normal physiological limits.

The mean values of erythrocyte indices are the arithmetic derivative of haemogram. The mean MCV ( $56.43 \pm 1.66$ ) concentrations in haemoglobinuria buffaloes differ significantly higher when compared healthy control group Table (1). However, the mean values of MCH ( $13.24 \pm 0.53$ ) and MCHC ( $23.53 \pm 1.02$ ) in haemoglobinuria animals decreased significantly ( $P < 0.05$ ) when compared to healthy control group. The erythrocyte indices are used for morphological classification of anaemia. Significantly increase in the MCV whereas significant decrease in MCH and MCHC in haemoglobinuria buffaloes indicates macrocytic hypochromic anaemia (Decie and Lewis 1991 and Mahmood *et al.*, 2013) [6, 14]. Increased mean values of MCV also indicate erythroid regenerative response resulting in release of immature cells of increased size (Feldman *et al.*, 2000). The findings with respect to erythrocytic indices are in agreement with Dalir- Naghadeh *et al.* (2005) [7]; Akhtar (2006) [3]; Radwan and Rateeb (2007) [20]; Durrani *et al.* (2010) [9] and Mahmood *et al.* (2013) [14]. Mean values of platelets of haemoglobinuria buffaloes were within normal physiological limits and no significant difference was noticed when compared to healthy control group.

The mean values of serum inorganic phosphorus ( $2.22 \pm 0.24$ ) significantly ( $P < 0.05$ ) decreased in haemoglobinuria buffaloes when compared to healthy buffaloes Table (1). The results were in accordance with Ellison *et al.* (1986) [10]; Bhikane *et al.* (2004) [4] and Mahmood *et al.* (2013) [14]. Hypophosphataemia can be attributed to feeding of cruciferous plants and copper deficiency (Radostists *et al.*, 2007) [19]. Utilization of Phosphorus for foetal bone development and its heavy drainage through the milk in high producing animals maintained on low phosphorus diet (Akhtar, 2006) [3]. It is postulated that excessive feeding of sugar cane tops (0.12 per cent DM basis) which are deficient in phosphorus, lead to hypophosphataemia so it is recommended to provide sugar cane tops with phosphorus supplementation (Akhtar *et al.* 2007 and Preston 1977) [18]. Saponin present in sugar beet leaves, alfa alfa hay and berseem have been incremented as haemolytic factor causing haemoglobinuria in buffaloes (Mohamed *et al.*, 1988) [15]. Impaired calcium phosphorus ratio and excess molybdenum interfere with the phosphorus absorption in the intestine can substantiate increase in incidence of hypophosphataemia (Dua, 2009) [8].

A Significant decrease ( $P < 0.05$ ) observed in the mean values of serum calcium ( $7.08 \pm 0.18$ ) concentration in haemoglobinuria buffaloes when compared to healthy buffaloes are tabulated in table (1). The results of present study were in accordance with Radwan and Rateeb (2007) [20]. Hypocalcaemia is most common conditions in early lactation can be attributed to increased demand for the foetal development, physiological functions and drain through the colostrum (Pal and Acharya 2013) [17] there by impairment of calcium and phosphorus ratio in the body resulting in hypophosphataemia.

Urine of haemoglobinuria buffaloes were analysed by Reagent urine strips (Standard diagnostics, INC.). The samples were positive for haemoglobin (Haemolysed) is indicated by colour change from yellow to light green (++) on urine strips would differentiate haemoglobinuria from haematuria. Urine samples were also positive for proteins (30mg/dl) indicated by colour change from light green to green. The haemoglobinuria and proteinuria indicate haemoglobinuria nephrosis. However, samples were within normal limits for glucose, ketone bodies, bilirubin and leucocytes. Urine examination of healthy buffaloes showed that all the parameters were within the normal physiological

limits with minor fluctuations.

The affected animals were treated with sodium dihydrogen phosphate dehydrate (130mg/ml) (Novizac<sup>®</sup>) at the total dose of 30ml per animal. Supportive treatment includes plasma volume expanders (Haemacel<sup>®</sup>) infused 500ml intravenously, tranexamic acid (500mg/5ml) (Inj Pause<sup>®</sup>) as antifibrinolytic agent at the dose rate of 10 mg/kg of body weight and iron supplements (Inj Ferritas<sup>®</sup>) 5ml intramuscular daily up to 5 days. The haemato-biochemical and urine parameters were observed on 5<sup>th</sup> day post treatment and were found to be within normal physiological limits. All the buffaloes recovered from haemoglobinuria condition without any untoward effect.

**Table 1:** Comparative analysis of haemato-biochemical changes in haemoglobinuria buffaloes in comparison with healthy buffaloes

Parameters	Groups					
	Normal buffaloes (n=6) (Control)		Haemoglonuric buffaloes (n=6) (Before treatment)		Haemoglonuric buffaloes (n=6) (After treatment)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
TEC ( $\times 10^6/\mu\text{L}$ )	6.82 ± 0.31 <sup>a</sup>	6.10 – 8.10	3.32 ± 0.28 <sup>b</sup>	2.70 – 4.20	5.95 ± 0.33	4.80 – 7.20
Hb (g/dL)	10.23 ± 0.57 <sup>a</sup>	8.70 – 12.10	4.38 ± 0.41 <sup>b</sup>	3.60 – 6.20	7.85 ± 0.44 <sup>c</sup>	6.50 – 9.50
PCV (%)	33.78 ± 0.87 <sup>a</sup>	32.10 – 37.80	18.60 ± 1.38 <sup>a</sup>	14.80 – 22.90	25.38 ± 1.77 <sup>c</sup>	20.60 – 31.40
Platelets ( $\times 10^3/\mu\text{L}$ )	238.33 ± 18.74	196.00 – 312.00	281.50 ± 20.13	215.00 – 338.00	223.00 ± 15.32	185.00 – 278.00
WBC ( $\times 10^3/\mu\text{L}$ )	8.50 ± 0.54	6.90 – 10.50	9.25 ± 0.66	7.40 – 10.50	8.32 ± 0.56	6.80 – 10.40
MCV (fL)	49.86 ± 1.66 <sup>a</sup>	46.52 – 56.56	56.43 ± 1.66 <sup>b</sup>	52.86 – 62.41	42.78 ± 2.24 <sup>c</sup>	34.92 – 50.65
MCH (pg)	15.02 ± 0.56 <sup>a</sup>	13.33 – 16.81	13.24 ± 0.53 <sup>b</sup>	11.61 – 14.76	13.32 ± 0.81	11.02 – 15.02
MCHC (g/dL)	30.27 ± 1.43 <sup>a</sup>	26.96 – 36.12	23.53 ± 1.02 <sup>b</sup>	21.43 – 27.07	31.20 ± 1.28 <sup>a</sup>	26.33 – 35.65
Ca (mg/dL)	10.05 ± 0.43 <sup>a</sup>	8.90 – 11.40	7.08 ± 0.18 <sup>b</sup>	6.50 – 7.80	7.82 ± 0.28 <sup>a</sup>	6.90 – 8.60
P (mg/dL)	4.38 ± 0.26 <sup>a</sup>	3.60 – 5.40	2.22 ± 0.24 <sup>b</sup>	1.40 – 2.90	3.88 ± 0.17 <sup>b</sup>	3.4 – 4.5

\*Means with dissimilar superscripts differ significantly

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