



RESEARCH ARTICLE

Comparative Therapeutic Effect of Toldimfos Sodium and Sodium Acid Phosphate for Treatment of Post-Parturient Hemoglobinuria (PPH) In Buffalo: A Randomized Clinical Trial

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ARTICLE INFO	ABSTRACT
Received: Aug 16, 2024	Post-parturient hemoglobinuria (PPH) is a hemolytic metabolic disease of cattle and buffalo characterized clinically by hemoglobinuria, anorexia and anoxia. In this study, a randomized clinical trial was performed to assess the comparative efficacy of sodium acid phosphate and toldimfos sodium on the clinical outcome, hematological indices and biochemical parameters in buffalo with PPH. For this purpose, sixty-eight buffalo with PPH were studied. Animals were allocated randomly into two groups (n=34). Group I: buffalo injected with sodium acid phosphate, intravenously at 60 g in sterile water (20% concentration) and a similar dose was applied subcutaneously with repetition after 12 h. Group II: buffalo were injected with toldimfos sodium (I/M at 25 cc/animal repeated after 12 hrs. Clinical findings, urine characteristics, hematological indices and biochemical parameters were evaluated before and after treatment. Regarding the effect of treatment on serum phosphorus and calcium levels, there was improvement in both phosphorus and calcium levels, toldimfos sodium provided a significant improvement (p<0.05) in serum phosphorus level than sodium acid phosphate. Both AST and ALT activities decreased significantly with treatments, but toldimfos also provided better results than sodium acid phosphate. Glutathione peroxidase (GPx) and glucose 6-phosphate enzymes (G6PT) were improved after treatment with both drugs (p<0.05); However, more significant improvement was achieved by use of toldimfos sodium. In the same context, total erythrocytic count and hematological indices were improved. The findings of this study indicate that toldimfos is an effective alternative treatment of PPH in buffalo.
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INTRODUCTION

Post-parturient hemoglobinuria (PPH), the metabolic disease, which affects dairy buffalo and cattle (Purohit et al., 2018). PPH is prevalent in dairy cows with high milk yield (Rahmati et al., 2021a). PPH is characterized clinically by hemoglobinuria, and anemic anoxia (Sharma et al., 2020). In dairy animals, loss of phosphorus via milk is the main cause of PPH, especially when phosphorus intake is

lowered (Yadav et al., 2023). PPH is commonly affecting buffalo more than cattle (Bhikane and Syed, 2014). It commonly occurs at third to sixth month of gestation (Constable et al., 2016).

The exact cause and pathophysiology of the disease remains unknown, although the condition is associated with low serum phosphorus level (Stockdale et al., 2005). Likewise, copper deficiency and possible hemolyzing substances contained in certain feeds have been reported as potential etiology or predisposing factors for hypophosphatemia (Constable et al., 2016).

Many factors are able to alter the level of phosphorus in the blood. Serum calcium and dietary cation-anion ratio are the major factors (Heaney and Nordin, 2002). There are an association between the of PPH and oxidative stress in diseased large ruminants (Fayed et al., 2018). When there is hypophosphatemia, erythrocytes will unable to use glucose for making ATP with consequent decrease of glutathione and eventually the cells will be more liable to effects of oxidative stress (Sarma et al., 2014).

Several treatment regimens have been tried for management of dairy animals with PPH (Constable et al., 2016; Rahmati et al., 2021b). Sodium acid phosphate is the most common drug used for this metabolic disorder (Gahlawat et al., 2007; Shalini et al., 2015; Soren et al., 2014).

Toldimfos sodium is the sodium salt of 4-dimethylamino-2-methyl-phenyl-phosphinous acid. It is considered non-pharmacopeial drug, which is derived from phosphoric acid (Nazlawy et al., 2023). It is recommended when confronted with diseases related to parturition and transition period. It is also recommended for treatment of nutritional deficiency diseases (Soni et al., 2015). Toldimfos has been studied for many purposes in cattle (Dhamsaniya et al., 2016b; Karhe, 2012; Rede et al., 2016) and dogs (Stewart et al., 2017). Few studies reported on the clinical efficacy of toldimfos sodium on PPH in buffalo (Durrani et al., 2010). To the best of the authors' knowledge, little is known about comparative clinical treatment trials of PPH in buffalo. So, a randomized clinical trial was performed to assess the comparative effect of sodium acid phosphate and toldimfos sodium for treatment of PPH in buffalo.

MATERIALS AND METHODS

Buffalo

Sixty-eight buffalo suspected to suffer from PPH were studied in Middle and northern regions of Egypt. The diagnosis was based on clinical findings, serum biochemistry and urinalysis. The present study has been approved by the university of King Faisal (KFU 1452).

Hematology

Blood samples were obtained from each buffalo under investigation (two samples). One sample for hematological examination was collected into a tube with anticoagulant, while the second sample was collected to obtain serum. Hematological investigation was carried out by use of cell counter (MS 9, Franc).

Biochemical Analyses

Serum electrolytes (Potasium and sodium) and macro-element (calcium, phosphorus, magnesium) were measured using spectrophotometry (test kits: Tecodiagnosics, USA) according to the standard technique. Glutathione peroxidase (GSH-PX) activity was measured in erythrocytes (Allen et al., 1975). Glucose 6-phosphate was measured with specific kits (Span diagnostics, India). Moreover, AST and ALT were also measured (SEppim, France).

Urinalysis

After following standard method for urine sample collection, urine samples were examined physically, and microscopically according to standard technique (Schinkel et al., 2002)

Treatments

Diseased animals were allocated randomly to two equal groups (34 each):

Group I was treated with sodium acid phosphate, intravenously at 60 g in 300 mL of sterile water and a similar dose was applied subcutaneously and was repeated after 12 h.

Group II was treated with toldimfos sodium (Fosfan, Payer, Germany) I/M at 25 cc/animal repeated after 12 hrs. Blood samples were collected before treatment and on day 3 post-treatment. Clinical, hematological, and biochemical examinations were conducted before treatment and post-treatment.

Statistical analysis

Sigma Plot, a statistical software was used for all statistical analyses (SigmaPlot v15, Canada). Firstly, groups were tested for homogeneity using Mann-Whitney u test. The groups were found homogenous; therefore, the results were presented as mean and SD. Furthermore, unpaired t-test was applied before and after treatment for each drug and between treated groups at each time point of treatment. At $P < 0.05$ in each test, the outcome was considered significant.

RESULTS

Clinically, the standard findings of diseased buffalo were discolored urine (mainly coffee color), depression, anorexia, and/or dehydration. There was no alteration in the rectal temperature. Polypnea with rapid pulse were the significant findings ($p < 0.05$). While the contractility of the rumen decreased and became less frequent. The feces were normal to hard or sometimes even loose in consistency. There was a significant increase in pulse rate (72.9 ± 9.90 vs 54.75 ± 3.78), respiratory rate (23.58 ± 4.27 vs 14.65 ± 0.93), but significant decrease of ruminal contraction (2.32 ± 0.99 vs 3.60 ± 0.5).

The color of urine in hemoglobinuric buffalo ranged from red (28%), dark red (20%) to coffee colored (52%) depending on the duration and severity of the disease. The urine pH was significantly higher ($p < 0.05$) in diseased animals than in healthy (8.39 ± 0.26 vs 7.91 ± 0.25). The urine of the affected buffalo was positive for hemoglobin (100%) and albumin (100%). Microscopic examination of urine of buffalo discovered no intact erythrocytes but few epithelial cells and crystals were found. The comparative effect of sodium acid phosphate and toldimfos sodium on urine characters was presented in Table 1.

Regarding the effect of treatment on serum phosphorus and calcium levels, there was improvement in both phosphorus and calcium levels, toldimfos sodium provided a significant improvement in serum phosphorus level than sodium acid phosphate (Figure 1). Both AST and ALT activities decreased significantly with treatments, but toldimfos also provided better results than sodium acid phosphate (Figure 2). Glutathione peroxidase and glucose 6 phosphate enzymes were improved after treatment with both drugs; However, more significant improvement was achieved by use of toldimfos sodium (Figure 3). In the same context, total erythrocytic count and hematological indices were improved (Figure 4). The remaining parameters did not show significant variation between the two treatments.

Table 1: Change in urine color pre- and post-treatment in buffalo with post-parturient hemoglobinuria (PPH).

Treatment	Total buffalo (n=68)	Day1	Day2	Day3
Sodium acid phosphate	n=34	red	red	clear
Toldimfos sodium	n=34	clear	clear	clear

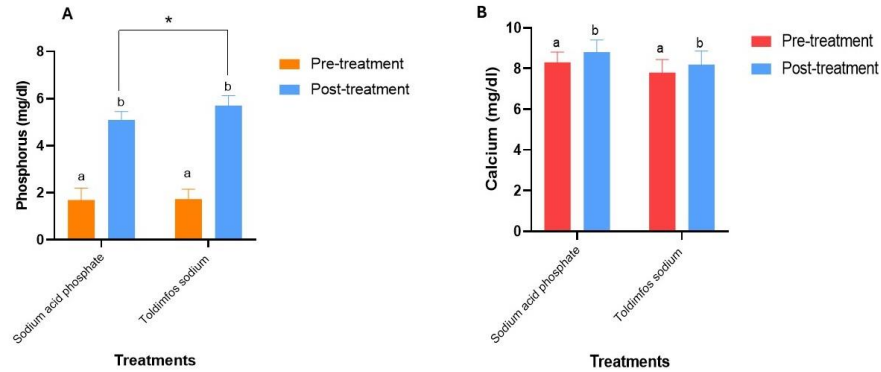


Figure 1: Comparative effects of Sodium acid phosphate and toldimfos sodium on serum phosphorus (A) and calcium (B) level in buffalo with PPH. a,b: Significance between pre-treatment and post-treatment in the same group at $p < 0.05$. *: Significant differences in post-treatment values among treatment groups at $p < 0.05$.

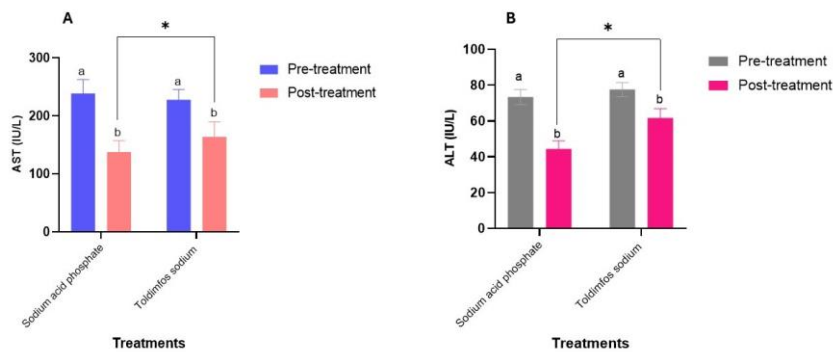


Figure 2: Comparative effects of Sodium acid phosphate and toldimfos sodium on serum AST (A) and ALT (B) activities in buffalo with PPH. a,b: Significance between pre-treatment and post-treatment in the same group at $p < 0.05$. *: Significant differences in post-treatment values among treatment groups at $p < 0.05$.

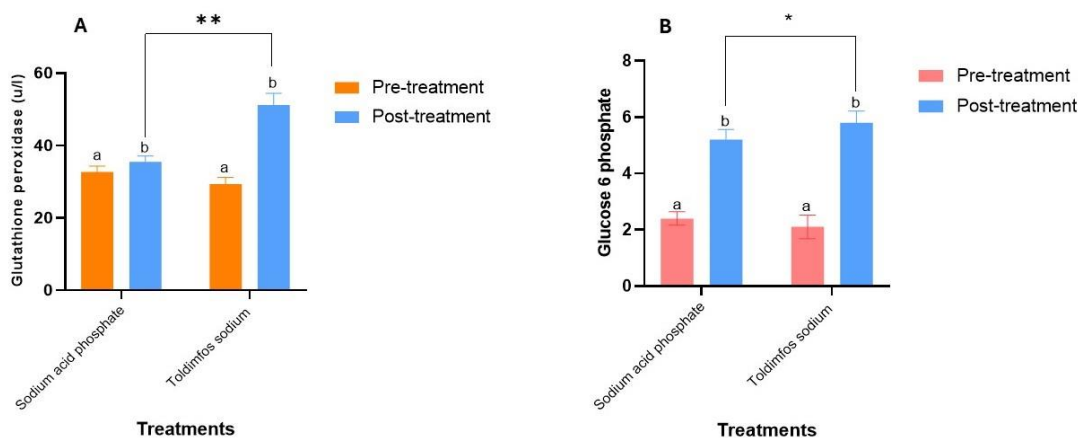


Figure 3: Comparative effects of Sodium acid phosphate and toldimfos sodium on serum GPx (A) and level of G6PT (B) in buffalo with PPH. a,b: Significance between pre-treatment and post-treatment in the same group at $p < 0.05$. *: Significant differences in post-treatment values among treatment groups at $p < 0.05$.

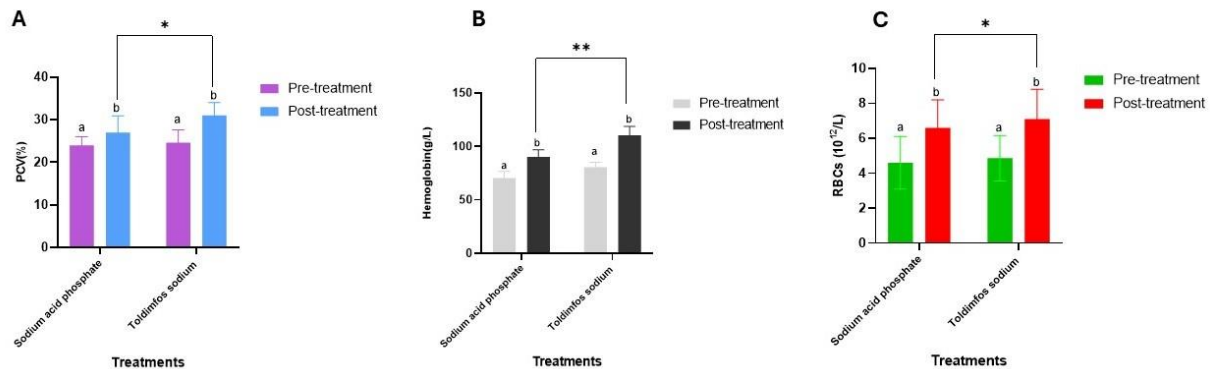


Figure 4: Comparative effects of Sodium acid phosphate and toldimfos sodium on PCV% (A), hemoglobin (B) and total erythrocytic count (C) in buffalo with PPH. a,b: Significance between pre-treatment and post-treatment in the same group at $p < 0.05$. *: Significant differences in post-treatment values among treatment groups at $p < 0.05$.

DISCUSSION

PPH, the metabolic disorder in ruminants, occurs usually during transition period (Bhikane and Syed, 2014). PPH in large ruminants exhibits several clinical signs. The evidence of abnormal coloration of urine, depression, decrease production, anemia, and increased pulse and respiratory rates (Madheswaran et al., 2017).

Clinically, the present cases showed the typical findings of the disease which come in accordance with that previously reported in Pakistan (Almubarak et al., 2023; Durrani et al., 2010). In the present study, treatments with toldimfos sodium and sodium acid phosphate provided improvement of all clinical variables. The color of urine became clear by the third day of treatment. Similar finding has been reported in cattle (Smith, 1973).

Regarding the effect of treatments on serum phosphorus and calcium levels, there was improvement in both phosphorus and calcium levels, toldimfos sodium provided a significant improvement in serum phosphorus level than sodium acid phosphate. Both AST and ALT activities decreased significantly with treatments, but toldimfos also provided better results than sodium acid phosphate. Glutathione peroxidase and glucose 6-phosphate enzymes were improved after treatment with both drugs; However, more significant improvement was achieved by use of toldimfos sodium. In the same context, total erythrocytic count and hematological indices were improved. Similar finding in buffalo with PPH has been reported in a previous study in Pakistan (Durrani et al., 2010) and for treatment of PPH in Murrah buffalo (Kumar et al., 2014). It has been also found that intravenous single dose of toldimfos could treatment of PPH in cattle (Jagadeeswaran and Jagadishkumar, 1998).

In this Study using toldimfos sodium provided good results on both clinical and biochemical levels. Complete recovery of diseased animals after treatment is attributed to restoring the normal phosphorus level with stop of intravascular hemolysis. This condition is associated with a decrease of ATP, which makes the RBCs more liable to fragility eventual short life span (Abdul Samad, 1997).

Toldimfos sodium is the sodium salt of 4-dimethylamino-2-methyl-phenyl-phosphinous acid. It is considered non-pharmacopeial drug, which is derived from phosphoric acid (Nazlawy et al., 2023). It is recommended when confronted with diseases related to parturition and transition period. It is also recommended for treatment of nutritional deficiency diseases (Soni et al., 2015). The commended beneficial dose of toldimfos is 10 mg/kg bw intravenously, intramuscularly or subcutaneously. Under field conditions, the injection is normally repeated up to ten injections.

Toldimfos is indicated for use in the following food producing species: horses, cattle, sheep, pigs and goats (Akhy, 2020)

The pharmacodynamic properties of toldimfos sodium in Surti buffalo have been described. Toldimfos did not significantly affect levels of all minerals except copper and cobalt (Dhamsaniya et al., 2016a).

In dogs with hypophosphatemia, toldimfos provided reliable results for treatment of this clinical condition (Stewart et al., 2017). However, toldimfos has been used for many purposes in cattle other than treatment of PPH. It was used to induce estrous in cattle (Dhamsaniya et al., 2016b; Karhe, 2012; Rede et al., 2016). Although toldimfos provided good results in several studies, it has been found that its excessive use may have harmful residues in production animals (Nazlawy et al., 2023).

CONCLUSION

The present results conclude that PPH could induce several metabolic alterations which have a detrimental influence on animal production and health. The findings of this study indicate that toldimfos is an effective alternative treatment of PPH in buffalo.

AUTHORS' CONTRIBUTIONS

Conceptualization: M. M., A. A.; Methodology: M.A., M. K.; Analysis: M. K.; Drafting: M.A., A.A.; Review: H.B.; Supervision: M.M

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

All data in manuscript.

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