

## EVALUATION OF HYPERTONIC SALINE SOLUTION IN COMBINATION WITH CEFTIOFUR HCL AND FLUNIXIN MEGLUMINE IN THE TREATMENT OF HAEMORRHAGIC SEPTICAEMIA IN BUFFALOES

M. A. Zafar, G. Muhammad, Z. Iqbal and M. Riaz\*

Department of Clinical Medicine and Surgery, Department of Parasitology, \*Department of Livestock Management, University of Agriculture, 38040, Faisalabad-Pakistan  
Corresponding Author: drmarifzafar@hotmail.com

### ABSTRACT

This study was undertaken for the evaluation of efficacy of hypertonic saline solution (HSS) in combination with ceftiofur HCl and flunixin meglumine (FM) in haemorrhagic septicaemia in buffaloes. For this purpose, 50 buffaloes suffering from haemorrhagic septicaemia were selected from the field and randomly divided into two equal groups (A and B). Group A served as control and treated with ceftiofur HCl, IM and flunixin meglumine, IV @ 6 and 2 mg/kg BW, respectively. While animals of group B were additionally administered with rapid intravenous infusion of hypertonic saline solution (7.5% NaCl) @ 4 ml/kg BW followed by normal saline solution @ 10 ml/kg BW. Treatments were started after application of cold water on head for 15 minutes. Ceftiofur HCl and flunixin meglumine were repeated after 12, 24 and 48 hours in both the groups. Animals were then monitored for 24 hours after initiation of treatment. Haematological parameters, serum electrolytes and serum biochemical profiles were measured at baseline, 1, 3, 6, 12 and 24 hours after treatment and survival index was recorded at interval of one week. The survival percent was 50 and 80 amongst animals treated respectively with protocol A and B, respectively. In terms of reduction of severity of disease, group B showed significant difference ( $P < 0.05$ ) over group A. The treatment protocol B exploiting the use of hypertonic saline solution in combination with cephalosporin (ceftiofur HCl) plus a non-steroidal anti-inflammatory drug (flunixin meglumine) was more effective than the conventional treatment i.e. ceftiofur HCl and flunixin meglumine alone for the treatment of haemorrhagic septicaemia. It was concluded from the study that administration of hypertonic saline solution in combination with ceftiofur HCl and flunixin meglumine has beneficial effects in the treatment of haemorrhagic septicaemia in buffaloes.

**Key words:** Ceftiofur, flunixin meglumine, Haemorrhagic Septicaemia

### INTRODUCTION

Haemorrhagic septicaemia (HS) is one of the most alarming and devastating diseases of buffalo and cattle in Pakistan (Ajmal *et al.*, 1988; Raza *et al.*, 2000). This disease is associated with a gram-negative bacterium named *Pasteurella multocida* serotype B: 2. This organism produces endotoxin. All manifestations of the disease are due to these endotoxins (Horadagoda *et al.*, 2001). As endotoxins are associated with the effects on central circulation which lead to hypovolaemia, so, in addition to antibiotics, administration of fluids are recommended for the early compensation of the circulation to increase the survival rate (Gow *et al.*, 1998; Rivers *et al.*, 2001). Many studies have shown the importance of early volume resuscitation in septicaemia. Adequate volume therapy appears to be a cornerstone of managing the septic patient.

Recently, several studies have supported the concept of adequate fluid administration to counteract

vasodilatation by filling the intravascular space for the management of septic shock (Parillo *et al.*, 1990; Gow *et al.*, 1998; Somell *et al.*, 2007). Therefore, restoration of intravascular volume is of great importance to maintain an adequate cardiac output and blood pressure. Small volume resuscitation with hypertonic saline solution (HSS; 7-7.5% NaCl) promote immediate plasma volume expansion due to mobilization of fluids from the intracellular compartment, thus restore cardiac output and regional blood flows, improve microcirculation and modulate immune responses, thereby decreasing inflammatory responses triggered by endotoxins (Kramer, 2003; Rocha e Silva and Poli de Figueiredo, 2005).

Information on the use of hypertonic saline solution in the therapy of HS is totally nonexistent. So, this study was planned to evaluate the efficacy of hypertonic saline solution (7.5% NaCl) in haemorrhagic septicaemia in buffaloes with antibiotic (Ceftiofur HCl) and non-steroidal anti-inflammatory drug (Flunixin meglumine).

## MATERIALS AND METHODS

**Experimental Animals:** The experimental protocol for this study was approved by the Animal Ethics Committee, University of Agriculture, Faisalabad-Pakistan. A total of 50 buffaloes of either sex suffering from haemorrhagic septicaemia were selected from the field cases in different areas of Punjab. Disease was diagnosed on the basis of clinical features i.e. sudden onset of the disease, high rise in body temperature, anorexia, depression, oedematous swelling on throat, brisket and upper dewlap region, dyspnoea, nasal discharge, salivation and reluctance to move (Carter and De Alwis, 1989; Radostits *et al.*, 2007). The age spectrum of all the buffaloes ranged from six months to 2 years.

**Experimental Design:** These buffaloes were randomly divided into two groups, 25 buffaloes per group. Group A served as control and was treated with treatment already in vogue i.e. ceftiofur HCl (Excenel RTU<sup>®</sup>, Pfizer Animal Health Division, Pakistan) and flunixin meglumine (Lixin<sup>®</sup>, Selmore Pharmaceuticals, Pakistan) @ 6 and 2 mg/kg BW, IM and IV, respectively. While buffaloes of group B were treated with hypertonic saline solution (7.5% NaCl; 2400 mmol NaCl/L) in combination with ceftiofur HCl and flunixin meglumine. Hypertonic saline solution was rapidly infused through intravenous route @ 4 ml/kg BW, followed by normal saline @ 10 ml/kg BW, while ceftiofur HCl and flunixin meglumine were administered @ 6 and 2 mg/kg BW, IM and IV, respectively. Ceftiofur HCl and flunixin meglumine were repeated after 12, 24 and 48 hours in both the groups.

**Instrumentation:** For infusion of solutions and collection of blood samples, catheters of 14-gauges were placed in jugular veins, respectively. Jugular catheter was flushed with 1 ml saline (0.9% NaCl) solution at the time of infusion solutions and sampling, containing 5 U of sodium heparin/ml.

**Measurements and Analysis of Samples:** Venous blood samples were collected at baseline, 1, 3, 6, 12 and 24 hours after treatment. The venous blood samples with anticoagulant were used to determine hemoglobin concentration (Hb concentration) and hematocrit (Hct) values while the samples without anticoagulant were used to harvest serum after centrifugation and stored at -20 °C until assayed. The hemoglobin concentration was determined by cyanmethemoglobin method and Hct values were determined with microhematocrit method as described by Benjamin (1978). Serum sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>) and chloride (Cl<sup>-</sup>) were determined with the help of electrolyte's analyzer (Medica Corporation, Bedford). Total protein and albumin were measured through Biuret Method described by Oser (1976). All the

above parameters were recorded at baseline, 1, 3, 6, 12 and 24 hours after treatment and survival index was checked at interval of one week.

**Statistical analysis:** Statistical analyses were performed using student t-test. All analysis was performed using the Statistical Software Package (SPSS Version 11.5). Statistical significance was assigned at  $P < 0.05$ .

## RESULTS

Baseline values for the systemic parameters of the both groups are shown in Tables-1 and 2 and are not significantly different. After initiation of the respective treatments, the results were as under;

### Clinical Parameters

**Body temperature:** There was increased body temperature during the course of disease in all the animals of both the groups. After instituting the respective treatments, fall in the temperature was noted in both the groups but group B showed rapidity in decreasing the body temperature. In animals of group B, body temperature was near to normal within 3 hours and was normal within 6 hours (Table 1). But in animals of group A, it did attain its normal value within 12 hours.

**Respiration Rate:** Decreased and difficult breathing was observed in diseased animals. After institution of treatment, mild change was observed towards normal in animals of group A, while the animals treated with HSS in combination with antibiotic and non-steroidal anti-inflammatory drug (group B) showed rapid improvement towards normal and respiration rate became normal within 12 hours (Table 1). After 24 hours, respiration rate was also near to normal in group A.

**Pulse Rate:** A sharp decrease in pulse rate was noted in diseased animals. After treatment, it increased more rapidly in group B as compared to group A. In group A, first its trend was decreasing after treatment but then it increased but could not achieved the normal limits even after within 24 hours. On the other hand, group B showed good recovery and values became normal within 6 hours (Table 1).

### Haematological Parameters

**Haemoglobin concentration:** Decreased level of haemoglobin concentration was observed in all the animals suffering from HS. After initiation of respective treatments, group B showed significant difference compared to group A ( $P < 0.05$ ) within 3 hours in reversing the Hb values toward normal (Table-1) and were normal within 24 hours but animals of group A could not achieve normal values within observing time and Hb concentration was still lower than the normal.

**Haematocrit:** Values of haematocrit (Hct) also decreased than its normal values during the course of disease. In group B, the Hct values started to become normal after infusion of HSS along with ceftiofur HCl and flunixin meglumine and it showed significant difference ( $P <$

0.05) over group A within first 3 hours. Group A showed recovery toward normal but did not return to normal within 24 hours. On the other hand, animals of group B showed rapid recovery and Hct value was in normal range within observing time (Table 1).

**Table 1. Results of survival index, clinical and haematological parameters for samples obtained from buffaloes suffering from haemorrhagic septicaemia before and after treatment in both the groups**

Variables	Groups	Time after treatment					
		Baseline	1 hr	3 hrs	6 hrs	12 hrs	24 hrs
Survival Index (No. of buffaloes)	Group A	25	23	19	15	12	12
	Group B	25	25	24*	23*	20*	20*
Body Temp. (°C)	Group A	41.3 ± 0.8	40.2 ± 1.1	39.6 ± 1.2	38.8 ± 0.9	38.3 ± 0.7	38.5 ± 0.6
	Group B	41.2 ± 1.4	40.0 ± 0.6	38.8 ± 0.8*	38.1 ± 0.6*	38.3 ± 0.4	38.1 ± 0.2
Resp. Rate (breaths/min)	Group A	12 ± 2	12 ± 3	13 ± 3	12 ± 2	14 ± 2	16 ± 1
	Group B	13 ± 2	14 ± 2	16 ± 1*	16 ± 3*	18 ± 2*	18 ± 2
Pulse Rate (beats/min)	Group A	44 ± 6	40 ± 3	40 ± 4	44 ± 2	49 ± 6	54 ± 4
	Group B	40 ± 4	44 ± 5	52 ± 6*	58 ± 3*	58 ± 5*	60 ± 2
Hb conc. (g/dl)	Group A	10.4 ± 0.8	10.2 ± 1.4	10.8 ± 1.2	11.2 ± 0.9	11.4 ± 1.3	12.8 ± 1.6
	Group B	9.8 ± 1.2	10.6 ± 0.8	12.2 ± 0.4*	12.8 ± 1.1*	14.2 ± 1.6*	14.4 ± 1.2*
Haematocrit (%)	Group A	26 ± 1.8	27 ± 2.0	27 ± 1.8	28 ± 1.1	29 ± 2.1	30 ± 2.8
	Group B	25 ± 1.4	28 ± 1.6	30 ± 2.2*	32 ± 1.8*	35 ± 2.6*	35 ± 1.8*

\*Significantly different from other group ( $P < 0.05$ )

Group A= Treated with Ceftiofur HCl plus flunixin meglumine; Group B= Hypertonic saline solution in combination with Ceftiofur HCl and flunixin meglumine.

**Table 2. Results of serum electrolytes and serum biochemical profiles for samples obtained from buffaloes suffering from haemorrhagic septicaemia before and after treatment in both the groups**

Variables	Groups	Time after treatment					
		Baseline	1 hr	3 hrs	6 hrs	12 hrs	24 hrs
Sodium (mEq/L)	Group A	145 ± 6	145 ± 3*	142 ± 2*	140 ± 2*	136 ± 4*	138 ± 2
	Group B	142 ± 4	154 ± 4	162 ± 3	154 ± 2	146 ± 3	136 ± 3
Chloride (mEq/L)	Group A	110 ± 1.8	110 ± 2.0	107 ± 1.2*	105 ± 2.3*	106 ± 3.4*	104 ± 3.0
	Group B	112 ± 2.0	116 ± 1.4	120 ± 1.8	118 ± 2.8	114 ± 1.1	106 ± 2.4
Potassium (mEq/L)	Group A	3.2 ± 0.86	3.2 ± 0.88	3.3 ± 0.90	3.5 ± 1.0	3.7 ± 0.64	3.8 ± 0.66
	Group B	3.0 ± 0.98	2.9 ± 0.68	2.8 ± 1.10	3.2 ± 1.2	3.6 ± 0.88	3.8 ± 0.86
Total Protein (g/dl)	Group A	4.8 ± 0.4	4.9 ± 0.5	5.1 ± 0.3	5.8 ± 0.4	6.6 ± 0.1	6.4 ± 0.3
	Group B	5.3 ± 0.2	5.8 ± 0.4*	6.1 ± 0.2*	6.9 ± 0.3*	7.1 ± 0.2	7.3 ± 0.4*
Albumin (g/dl)	Group A	2.8 ± 0.4	2.8 ± 0.2	2.9 ± 0.1	3.2 ± 0.2	3.1 ± 0.3	3.2 ± 0.1
	Group B	3.0 ± 0.3	3.5 ± 0.3	3.8 ± 0.2*	3.6 ± 0.1	3.8 ± 0.2*	3.8 ± 0.1*

\*Significantly different from other group ( $P < 0.05$ )

Group A= Treated with Ceftiofur HCl plus flunixin meglumine; Group B= Hypertonic saline solution in combination with Ceftiofur HCl and flunixin meglumine.

### Serum Electrolytes

**Sodium:** An increased level of sodium ions ( $\text{Na}^+$ ) concentration was observed during course of disease in all the animals of both groups. After instituting the treatments, group A showed good recovery towards normal and showed significant differences ( $P < 0.05$ ) throughout the study period over group B (Table 2). While in group B, there was an increasing trend in the  $\text{Na}^+$  ions concentration for first 3 hours and values reached up to 162 mEq/l but after that there falling trend

was noted and values were below the baseline within 24 hours

**Chloride:** Same observations were observed in the chloride ions ( $\text{Cl}^-$ ) concentration as for sodium ( $\text{Na}^+$ ) ions. After instituting respective treatments, group A showed good recovery towards normal and significant differences ( $P < 0.05$ ) were observed over group B from 60 minutes to 6 hours. After that, group B showed rapid recovery and it was below the baseline within observing time (Table 2).

**Potassium:** Decreasing trend was noted in the potassium ions ( $K^+$ ) concentration during disease conditions. After administration of allotted treatments to the animals of both the groups, no significant differences were observed at each observational time in recovering the values towards normal (Table 2), and it never became normal within 24 hours in both the groups. So, there was no significant difference between groups A and B.

**Serum Biochemical Profile:** Time dependant changes in the total protein and albumin values of both the groups are presented in the table 2. There was a decreasing trend in the diseased animals. After allotted treatments to group A, the values of total protein and albumin were not significantly increased as in group B. Administration of HSS along with ceftiofur and flunixin meglumine to the animals of group B made a significant increase in the values of total protein and albumin and showed significant difference ( $P < 0.05$ ) over group A.

## DISCUSSION

Rational therapy of a bacterial disease requires consideration of its pathophysiology. In this context, the aspect of haemorrhagic septicemia, the number one killer disease of buffalo in Pakistan, warrant special attention that; (a) this disease is associated with a Gram-negative bacterium known as *Pasteurella multocida* serotype B:2 (Carter and De Alwis, 1989). As such the antibiotic administered must be specifically effective against Gram-negative organism (Raza *et al.*, 2000). (b) Moderate to severe pyrexia is a hallmark of this disease, so, measure(s) should be adapted to lower the elevated body temperature such as water hosing on the head for 10-15 minutes. (c) All manifestations of the disease are due to endotoxins released by Gram-negative bacteria (Horadagoda *et al.*, 2001). These endotoxins trigger arachidonic acid metabolites resulting in the production of prostaglandins and leukotrienes. These mediators would ensue vasodilation, hypotension and other circulatory perturbations (Slauson and Cooper, 1990). Selective inhibitors of arachidonic acid metabolism i.e. non-steroidal anti-inflammatory drugs (NSAIDs) e.g. flunixin meglumine and steroids have been shown beneficial influence on the pathophysiological consequences of endotoxaemia (Conlon, 1988; Slauson and Cooper, 1990; Raza *et al.*, 2000).

In addition to antibiotic therapy, fluid administration plays a pivotal role in the management of sepsis and septic shock (Parillo *et al.*, 1990; Zafar *et al.*, 2009). The goal of fluid administration is to restore intravascular volume to maintain an adequate blood pressure and cardiac output. Restoring a normal cardiac output requires intravascular replacement of fluid losses, compensation for venous pooling of blood and a sufficiently high left ventricular filling pressure to

compensate for decreased ventricular contractility during sepsis (Parker *et al.*, 1984). Despite limited data in humans and animals, a number of studies suggested that sepsis is accompanied by impaired tissue oxygen extraction and decreased cardiac output (Nelson *et al.*, 1987; Constable *et al.*, 1996; Gow *et al.*, 1998; Somell *et al.*, 2007).

In the present study, regarding to aforementioned pathophysiological features of HS, a treatment protocol was constructed that animals should be treated with the bolus administration of 7.5% saline solution in addition to a third generation cephalosporin (Ceftiofur HCl) through intramuscular route along with non-steroidal anti-inflammatory drug (flunixin meglumine) intravenously. The percent survival among animals treated with protocol A and B was 50 and 80, respectively. Difference in recovery rate between these protocols was highly significant. This higher (80%) recovery rate with treatment protocol B than protocol A (50%) was attributed to the addition of hypertonic saline solution in the former treatment protocol. Raza *et al.* (2000) reported survival rates of 85, 80 and 30% in cases of HS treated with treatment protocols A, B and C. They treated the animals suffering from HS with norfloxacin, diclofenec sodium alone (group B) and in combination with a circulatory stimulant Novacoc Forte (group A), while group C was treated with gentamicin sulphate and metamizole only.

Sodium ( $Na^+$ ) concentration is of great importance to resuscitate the patient from hypovolaemic or septic shock by elevating extracellular volume more than the transfused amount (Constable, 1999). Hypertonic saline solution infusion to the septic shocked buffaloes induced significant increase in serum sodium and chloride concentration, which were accompanied by a transient decrease in serum potassium concentration. However, a rapid increase in serum sodium concentration could cause salt poisoning (Ajito *et al.*, 1999). The increase in serum sodium may also cause hypernatraemia, but at risk. In our study, the administration of 4 ml/kg of HSS produced transient high sodium level and animals were hypernatraemic (160 mM/l) for a short interval but after 3 hours its value became below than this margin and no adverse effect was observed during the whole study. These results are inline with the results reported by other scientists (Velasco *et al.*, 1980; Tyler *et al.*, 1994; Constable *et al.*, 1996). The mild decrease in potassium concentration after HSS infusion has been observed previously (Constable *et al.*, 1991; Suzuki *et al.*, 1998), but was not considered clinically important.

Haemoglobin concentration and haematocrit (Hct) values are the indicators of total cells including erythrocytes, leukocytes and platelets. Any increase or decrease in values of both parameters showed the signs of dehydration and hypovolaemia, respectively (Reece, 1997). In the present study, decreased values of both Hb

and Hct clearly indicated reduction in the number of cells and ultimately plasma volume. Reduced plasma volume resulted in decreased cardiac output escorted weak and declined pulse rate. In group B, HSS generated plasma volume expansion by rapidly increase in Hct and Hb concentration values and played a pivotal role in restoring plasma volume and also increased osmosis and promoted movement of endogenous fluid from the extravascular to intravascular space (Constable *et al.*, 1991; Tyler *et al.*, 1994). As no fluid was administered in animals of group A, so, increase in plasma volume and pulse rate was slow. These observations strongly recommend that fluid administration is the basic tenet to resuscitate the patients from septic shock.

Flunixin meglumine (a NSAID) is responsible to diminish the effects of arachidonic acid metabolites which culminate in decreased level of prostaglandins and leukotrienes and ultimately lowers the body temperature (Plumb, 1999). In the present study, this drug helped in achieving the target of exerting the beneficial influence on the pathophysiologic consequences of HS endotoxaemia and lowering the elevated body temperature of the animals suffering from HS. These results are in line of the previous studies (Verhoef *et al.*, 1986; Anderson and Hunt, 1989).

The higher recovery rate in animals of group B was only the administration of hypertonic saline solution and may be repetition of antibiotic and anti-inflammatory therapy at 12 hour interval which is two order of the frequency recommended by manufacturer. Ceftiofur HCl and flunixin meglumine were repeated at 12 hours instead of 24 hours intervals because our own observations and those of others (Raza *et al.*, 2000) would indicate that the HS cases respond temporarily with partaking of feed and water but worsens again if the repetition is deferred until 24 hours. Animals treated with the conventional treatment did not show so better recovery rate because animals of that group were not administered with any fluid.

**Conclusion:** From the study, it is deduced that the treatment protocol B exploiting the use of hypertonic saline solution plus Ceftiofur HCl (an antibiotic) and flunixin meglumine (a non-steroidal anti-inflammatory drug) was more effective than the conventional line of treatment in view of preliminary nature of this work.

## REFERENCES

- Ajito, T., K. Suzuki, and S. Iwabuchi (1999). Effect of intravenous infusion of a 7.2% hypertonic saline solution on serum electrolytes and osmotic pressure in healthy beagles. *J. Vet. Med. Sci.*, 61: 637-641.
- Ajmal, M., M. Arshad, and M. Ahmad (1988). Major livestock diseases in Pakistan. An overview. In: Afzal, M., A. H. Cheema, and A. S. Akhtar (eds.): *Proc. Livestock Diseases in SAARC Countries*. PARC Islamabad. May 22-25, pp. 22-26.
- Anderson, K. L. and E. Hunt (1989). Anti-inflammatory therapy in acute endotoxin induced bovine mastitis. *Vet. Res. Comm.*, 13: 17-26.
- Conlon, P. D. (1988). Nonsteroidal drugs used in the treatment of inflammation. *Vet. Clin. N. Amer. Small Anim. Pract.*, 18: 1115-1131.
- Constable, P. D. (1999). Hypertonic saline. *Vet. Clin. North Amer. Food Anim. Pract.*, 15: 559-585.
- Constable, P. D., H. M. Gohar, D. E. Morin, J. C. Thurmon (1996). Use of hypertonic saline-dextran solution to resuscitate hypovolemic calves with diarrhea. *Amer. J. Vet. Res.*, 57: 97-104.
- Constable, P. D., L. M. Schmall and W. W. Muir III (1991). Respiratory, renal, hematologic and serum biochemical effects of hypertonic saline solution in endotoxemic calves. *Amer. J. Vet Res.*, 52: 990-998.
- Gow, K. W., P. T. Phang, S. M. Tebbutt-Speirs, J. C. English. and M. F. Allard (1998). Goddard CM, Walley KR: Effect of crystalloid administration on oxygen extraction in endotoxemic pigs. *J. Appl. Physiol.*, 85: 1667-1675.
- Horadagoda, N. U., J. C. Hodgson, G. M. Moon, T. G. Wijewardana and P. D. Eckersall (2001). Role of endotoxin in the pathogenesis of haemorrhagic septicaemia in the buffalo. *Microb. Pathogen.*, 30: 171-178.
- Kramer, G. C. (2003). Hypertonic resuscitation: Physiological mechanisms and recommendations for trauma care. *J. Trauma*, 54: 89-99.
- Nelson, D. P., C. Beyer, R. W. Samsel (1987). Pathological supply dependence of O<sub>2</sub> uptake during bacteremia in dogs. *J. Appl. Physiol.*, 63: 1487-1492.
- Oser, B. L. (1976). *Hawks Physiological Chemistry*. Tata Macgraw Hill Publisher and Company, New Delhi, India.
- Parker, M. M., J. H. Shelhamer, S. L. Bacharach, M. V. Green, C. Natanson, T. M. Fredrick, B. A. Damske and J. E. Parrillo (1984). Profound but reversible myocardial depression in patients with septic shock. *Ann. Intern. Med.*, 100: 483-490.
- Parrillo, J. E., M. M. Parker, C. Natanson, A. F. Suffredini and R. L. Danner (1990). Cunnion RE, Ognibene FP: Septic shock in humans: advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. *Ann. Intern. Med.*, 113: 227-242.

- Raza, M. A., G. Muhammad, M. Athar and M. Z. Khan (2000). Comparative efficacy of three protocols for the treatment of haemorrhagic septicaemia in buffaloes and cattle. *Pakistan Vet. J.*, 20: 35-39.
- Reece, W. O. (1997). *Physiology of Domestic Animals*. 2<sup>nd</sup> Edition. In; *Blood and its Functions*. Lippincott Williams & Wilkins, Baltimore, USA.
- Rivers, E., B. Nguyen, S. Havstad, J. Ressler, A. Muzzin, B. Knoblich, E. Peterson and M. Tomlanovich (2001). Early goal-directed therapy in the treatment of severe sepsis and septic shock. *New Engl. J. Med.*, 345: 1368-1377.
- Rocha, E., M. Silva and L. F. Poli De Figueiredo (2005). Small volume hypertonic resuscitation of circulatory shock. *Clinics*, 60: 159-172.
- Slauson, D. O. and B. J. Cooper (1990). *Mechanisms of Disease-A Textbook of Comparative General Pathology*. 2<sup>nd</sup> Edition, Lippincott Williams & Wilkins, Baltimore, USA. Pp: 506-519.
- Somell, A., E. Weitzberg, A. Suneson, H. Hjelmqvist (2007). Effects of the dual endothelin receptor antagonist tezosentan and hypertonic saline/dextran on porcine endotoxin shock. *Acta Physiol.*, 190: 291-302.
- Suzuki, K., T. Ajito and S. Iwabuchi (1998). Effect of infusion of hypertonic saline solution on conscious heifers with hypoxemia caused by endotoxin infusion. *Am. J. Vet. Res.*, 59: 452-457.
- Tyler, J. W., E. G. Welles, R. J. Erskine, H. C. Lin, M. A. Williams, J. S. Spano, T. Gaslin, and K. A. McClure (1994). Clinical and clinicopathologic changes in cows with endotoxin-induced mastitis treated with small volumes of isotonic or hypertonic sodium chloride administered intravenously. *Amer. J. Vet. Res.*, 55: 278-287.
- Velasco, I. T., V. Pontieri, E. Rocha, M. Silva, and O. U. Lopes (1980). Hyperosmotic NaCl and severe hemorrhagic shock. *Amer. J. Physiol.*, 239: 664-673.
- Verhoef, J., A. C. Wierda, V. Vulpen and J. Dolrresteijin (1986). Flunixin meglumine in calves with natural bovine respiratory syncytial virus infection. *Vet. Rec.*, 118: 14-16.
- Zafar, M. A., G. Muhammad, M. H. Hussain, T. Ahmad, A. Yousaf and I. Sarfaraz (2009). Comparative efficacy of hypertonic saline and normal saline solutions in experimentally induced endotoxic shock in dogs. *Pakistan Vet. J.*, 29: 115-120.