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RESEARCH ARTICLE

The Synergistic Effects of Hypertonic Saline Solution and Ketoprofen in the Amelioration of Cytokine Level and Cardiac Performance in Induced Endotoxic Shock in Dogs

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ABSTRACT

This study was conducted to determine the effectiveness of rapid IV administration of hypertonic saline solution (HSS), ketoprofen and their combination thereof in amelioration of endotoxic shock through reduction in cytokine level and improved cardiac performance. For this purpose, 18 adult, healthy, mongrel dogs of either sex were used. Endotoxic shock was induced in all dogs through administration of *Escherichia coli* endotoxin @ 1 mg/kg BW, IV. Sixty minutes after infusion, all dogs were divided randomly into three groups equally viz. groups A, B and C. Animals of group A were treated with IV administration of ketoprofen alone @ 2 mg/kg BW. Group B received HSS @ 4 ml/kg BW, IV, and dogs of group C were treated with a combination of HSS and ketoprofen @ 4 ml/kg and 2 mg/kg BW, IV, respectively. Dogs were monitored for next 36 hours after institution of treatment. Efficacy was evaluated through interleukin (IL)-1α, IL-6, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), mean arterial pressure (MAP), hematological parameters, blood gases and serum electrolytes. Endotoxin had profound effects on inflammatory mediators and pulmonary function causing hypoxemia accompanied by a significant decrease in MAP, PaO_2 and relative plasma volume (rPV). Treatment protocol of group C induced a significant decrease in IL-1α, IL-6 and $PGF_{2\alpha}$ and rapid increase in MAP, rPV and PaO_2 when compared with isolated ketoprofen or HSS infusion. It was concluded from the results that ketoprofen as an adjunct to hypertonic resuscitation improved survival of dogs from endotoxic shock through amelioration of cytokine concentrations, improved cardiac performance and systemic oxygenation.

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INTRODUCTION

Endotoxemia is a life-threatening condition requiring emergency attention and is considered among one of the most common conditions that causes death in humans and animals. Endotoxin released from gram-negative bacteria are responsible for development of complex pathophysiological process through initiating a cascade of inflammatory mediators like cytokines i.e. IL-1, IL-2, IL-6 etc. (Minneci *et al*., 2003; Theobaldo *et al*., 2013). Endotoxin affect profoundly on circulatory system that causes pronounced vasodilatation and alterations in endothelial barrier culminates in decreased cardiac output and hypovolemia eventually results in impaired regional blood flow and disturbs end-organ function (Oliveira *et al*., 2002; Rasslan *et al*., 2014). Increased cytokine concentration and reduced cardiac output lead to multiple organ failure and death of patient (Kim *et al*., 2012; Santiago *et al*., 2013).

Recent data supported concept of adequate fluid administration to encounter vasodilatation through filling intravascular spaces for successful management of endotoxic shock (Batmaz *et al*., 2003; Hogue *et al*., 2012). Therefore, it is of great importance to restore intravascular volume for maintenance of adequate cardiac output. Hypertonic saline solution (HSS) is a small-volume resuscitation which mobilizes intracellular compartment fluids after its administration which results in increased MAP (Van Haren *et al*., 2012) thereby helps in maintaining adequate cardiac output and plasma volume expansion (Junger *et al*., 2013; Rocha e Silva, 2014).

Many researchers have investigated potential use of HSS in endotoxic shock in different species (Santiago *et al*.,

2013; Dong *et al*., 2014), however, up to author's knowledge; there is no controlled study that investigated effects of combination of HSS and non-steroidal anti-inflammatory drugs (NSAIDs). Arachidonic acid metabolites are well known to play an important role in the pathophysiology of multiple organ dysfunctions during endotoxic shock (Gouvy *et al*., 2005; Kim *et al*., 2013). Many studies proved that NSAIDs are inhibitors of arachidonic acid metabolites, thus, having beneficial effects to encounter endotoxic shock (Minneci *et al*., 2003). Ketoprofen is a NSAID which potently inhibit both pathways of arachidonic acid metabolites i.e. cycloxygenase and lipoxygenase (Sigurdsson *et al*., 1993). Inhibition of these pathways stops production of prostaglandins and prostacycline which aggravate the condition developed by cytokine concentration (Rasslan *et al*., 2014).

Therefore, it seems plausible to assume synergism of ketoprofen and HSS in resuscitation of patients from endotoxic shock. Thus, our aim was to test the hypothesis whether HSS and ketoprofen combination provides beneficial effects in significant reduction of cytokine level, improve cardiac performance through expanding rPV and increased MAP, blood oxygenation and survival.

MATERIALS AND METHODS

Animals: Experimental protocol of study was approved by Institutional Ethical Committee. Eighteen adult, healthy mongrel dogs of either sex were acclimatized for a period of one week prior to experiment. All animals fed on same feed and fresh water a*d libitum* and their health status was ascertained through complete blood count, fecal and urine examination.

Instrumentation: Day prior to the induction of endotoxic shock, dogs were sedated with medetomidine (@ 0.025 mg/kg, IV) for aseptic placement of intravenous catheters. For HSS infusion and blood sample collection, catheters of 18-gauges placed in jugular and cephalic veins, respectively. Femoral artery was cannulated with 22-gauge catheter for measurement of MAP. Jugular and cephalic catheters used to flush with 1 ml and 0.5 ml normal saline solution (0.9% NaCl) containing 5 U of heparin/ml at time of HSS administration and sampling, respectively.

Induction of endotoxic shock: Endotoxic shock was induced with slow IV administration (over 15 min) of *Escherichia coli* (O111:B4) endotoxin (Sigma Chemical Co, St. Louis, Mosby, USA) @ 1 mg/kg BW, dissolved in normal saline solution (Batmaz *et al*., 2003) in conscious dogs. The criterion for development of endotoxic shock was MAP <60 mm Hg (Cohen *et al*., 1996).

Experimental Design: On meeting aforementioned criteria, treatment phase of the study was initiated, dogs were randomly divided into three groups, 6 dogs/group. Group A treated with IV administration of ketoprofen (Profenid®, Sanofi-Aventis Limited Karachi-Pakistan) @ 2 mg/kg BW. Dogs of group B were infused IV with HSS (7.5% NaCl) @ 4 ml/kg BW, respectively. Group C was treated with combination of HSS and ketoprofen @ 4 ml/kg and 2 mg/kg BW, IV, respectively.

Measurements and analysis of samples: Blood samples were collected with anticoagulant to measure hemoglobin concentration (Hb conc) and hematocrit (HCT), while samples collected without anticoagulant were used to harvest serum after centrifugation and stored at -20ºC until further analyses. Cyannmethemoglobin and microhematocrit methods were used to determine Hb conc and HCT, respectively as described by Benjamin (1978). Serum sodium (Na^+), potassium (K^+) and chloride (Cl^-) were determined with electrolyte's analyzer (Electrolyte Analyzer, Medica Corporation, Bedford). Changes in relative plasma volume (rPV) were calculated from Hb conc. and HCT, using accepted formula (Constable, 1999).

Blood samples for cytokine analyses were collected, centrifuged and the resultant plasma were stored at -80 °C and used within 48 hours. The levels of IL-1α, IL-6 and $PGF_{2\alpha}$ were determined using an ELISA technique according to manufacturer's instructions (Amersham Biosciences Inc; Piscataway New Jersy, USA).

Arterial and venous blood samples were obtained anaerobically in 1-ml heparinized syringe for determination of blood gases. Syringe was then placed on ice and processed within an hour of collection. Blood gases i.e. partial pressures of arterial oxygen (PaO_2), arterial carbon dioxide ($PaCO_2$), venous oxygen (PvO_2), arterial and venous pH and HCO_3 were measured with automatic gas analyzer (Blood Gas Analyzer, Medica Corporation, Bedford) at 37°C.

All parameters were measured at baseline (before shock), during shock and 1, 3, 6, 12, 24 and 36 hours after treatment except cytokine levels. The cytokine concentrations i.e. IL-1α, IL-6 and $PGF_{2\alpha}$ were measured at baseline (before shock), during shock and 3, 6, 12 and 24 hours post-treatment.

Statistical analyses: Data obtained were analyzed statistically using 2-factor Complete Randomized Design. Variables involving repeated measures were analyzed with multivariate repeat measures ANOVA. When a significant ($P<0.05$) group or time interaction was observed, additional testing performed using Duncan's Multiple Range Test to determine differences between groups.

RESULTS

Endotoxin administration had a profound effect on pulmonary function with severe hypoxemia. Endotoxin decreased PaO_2 significantly accompanied by a significant reduction in PvO_2 and increased $PaCO_2$ from baseline (Table 1). After treatment, values of PaO_2 and PvO_2 did not return to baseline in group A, while these were recovered in groups B and C. Group B showed significant difference over group A, while group C differed significantly ($P<0.05$) from both groups A and B at different observational time (Table 1). Values of $PaCO_2$ also decreased in a better way in group C toward baseline than group B, while it differed significantly over group A (Table 1).

Mixed venous pH and HCO_3 values decreased significantly during endotoxic shock, however, arterial blood pH values did not differ significantly between

Table 1: Results of blood gases for samples obtained from endotoxemic dogs before and after treatment

Variables	Groups	Time after treatment (hours)							
		Baseline	Shock	1	3	6	12	24	36
Number of Animals	Group A	6	6	6	6	5	5	4	3
	Group B	6	6	6	6	6	6	6	6[a]
	Group C	6	6	6	6	6	6	6	6[a]
PaO_2	Group A	88±5	39±3	44±2	47±3	52±5	57±3	62±2	69±1
(mm of Hg)	Group B	88±4	40±9	50±2[a]	54±5[a]	64±4[a]	70±3[a]	74±5[a]	80±2[a]
	Group C	90±9	42±7	52±2[a]	60±3[c]	74±5[c]	77±4[c]	84±3[c]	92±2[c]
PvO_2	Group A	34±2	14±2	19±3	23±2	27±3	29±5	26±2	28±4
(mm of Hg)	Group B	35±2	17±1	23±3	29±1[a]	35±2[a]	32±3	33±4[a]	35±1[a]
	Group C	37±4	16±2	24±2[a]	29±3[a]	37±1[a]	40±1[c]	40±2[c]	38±2[a]
$PaCO_2$	Group A	36±3	46±1	47±2	49±4	47±3	45±2	39±1	42± 4
(mm of Hg)	Group B	38±2	45±3	44±2	41±3[a]	43±1	42±1	40±1	39±2
	Group C	36±1	47±2	43±1	40±2[a]	40±2[a]	38±2[a]	36±1	35±3[a]
Arterial pH	Group A	7.4±.03	7.3±.02	7.3±.02	7.3±.04	7.3±.03	7.3±.01	7.3±.02	7.4±.01
	Group B	7.4±.02	7.3±.05	7.3±.01	7.2±.03	7.2±.06	7.3±.07	7.3±.01	7.4±.05
	Group C	7.4±.04	7.3±.02	7.3±.02	7.3±.05	7.3±.05	7.3±.03[b]	7.4±.03[c]	7.4±.01
Mixed	Group A	7.3±.01	7.1±.04	7.1±.04	7.1±.04	7.1±.03	7.1±.05	7.2±.03	7.2±.02
Venous pH	Group B	7.4±.03	7.1±.05	7.1±.01	7.1±.07	7.1±.04	7.2±.02	7.3±.05[a]	7.3±.01[a]
	Group C	7.4±.02	7.1±.03	7.1±.05	7.1±.04	7.2±.03[c]	7.3±.04[c]	7.4±.02[c]	7.4±.01[a]
HCO_3	Group A	23±1.6	12±1.6	12±1.4	12±1.7	16±2.0	15±1.9	18±2.1	20±1.7
(mEq/L)	Group B	22±1.7	14±1.5	12±1.1	11±0.9	14±1 .3	17±1.6	17±1.3	20±1.6
	Group C	24±2.0	15±1.7	15±1.6	17±1.1[c]	18±1.7[b]	20±2.3[a]	23±1.7[c]	25±1.3[c]

[a]Significant difference (P<0.05) from group A; [b]Significant difference (P<0.05) from group B; [c]Significant difference (P<0.05) from other groups; Values are reported as mean±SD; Dogs (*n*=6/group) were treated as follow: Group A = Ketoprofen, IV; Group B = Hypertonic Saline Solution, IV; Group C = Hypertonic Saline Solution and Ketoprofen, IV.

Table 2: Results of laboratory analyses for samples obtained from endotoxemic dogs before and after treatment

Variables	Groups	Time after treatment (hours)							
		Baseline	Shock	1	3	6	12	24	36
MAP	Group A	116±4.9	50±3.2	58±4.2	70±2.8	76±3.5	84±3.2	92±4.4	98±1.9
(mm of Hg)	Group B	118±7.5	60±3.6	70±3.8[a]	78±3.0[a]	88±3.7[a]	94±4.4[a]	102±6.5[a]	110±6.0[a]
	Group C	114±8.7	56±4.0	74±3.6[a]	88±6.1[c]	96±5.3[c]	104±3.7[c]	112±4.6[c]	120±4.2[c]
Sodium	Group A	136±1.9	148±1.6	148±2.2	147±0.8	144±1.6	140±1.8	142±0.9	139±1.2
(mEq/L)	Group B	138±2.6	144±2.6	143±2.9	145±1.7	142±0.9	138±1.6	138±2.0	136±3.3
	Group C	138±2.4	147±2.4	154±1.5	161±2.1	155±2.4	150±2.4	144±2.1	139±2.1
Chloride	Group A	106±1.9	118±2.2	118±2.0	115±1.8	110±2.4	107±0.8	106±1.2	108±0.8
(mEq/L)	Group B	108±2.8	115±2.5	115±1.6	113±2.7	114±1.3	112±2.1	108±2.1	108±3.1
	Group C	108±2.9	114±2.5	120±1.7	119±2.5	114±2.3	112±2.0	111±1.6	109±1.6
Potassium	Group A	4.6±0.6	2.9±0.3	2.8±0.3	3.0±0.4	3.3±0.1	4.3±0.2	4.2±0.2	4.7±0.3
(mEq/L)	Group B	4.3±0.5	2.7±0.1	2.7±0.2	2.6±0.2	3.0±0.2	3.1±0.2	3.6±0.3	3.9±0.5
	Group C	5.1±0.7	3.3±0.5	3.0±0.4	3.6±0.4[b]	4.0±0.4[b]	4.1±0.5[b]	4.8±0.5[b]	5.1±0.6[b]
Hematocrit	Group A	33±1.1	43±1.0	40±1.1	41±1.3	40±1.1	38±0.9	38±2.1	39±1.4
(%)	Group B	35±1.4	43±1.0	42±1.7	43±2.4	43±2.0	40±2.2	39±1.8	37±1.8
	Group C	34±1.4	42±1.2	40±1.7	38±2.4[c]	36±2.0[c]	34±2.2[c]	34±2.0	34±2.1[a]
Hb. conc.	Group A	14.2±1.1	17.0±0.6	17.0±0.9	16.7±0.7	16.9±0.6	16.4±0.8	16.0±0.9	15.2±0.4
(g/dL)	Group B	13.5±1.3	16.0±0.8	17.0±1.2	16.5±0.8	17.3±0.8	16.8±0.7	15.8±0.7	14.9±0.8
	Group C	13.6±1.3	16.0±0.9	16.3±0.5	15.6±0.5	15.3±1.2[b]	14.3±1.2	14.0±1.4	14.0±1.4
Body Temp. (°C)	Group A	38.8±.06	41.3±.03	40.8±.06	40.0±.04	39.0±.05	39.0±.01	38.6±.04[b]	38.7±.04
	Group B	38.7±.04	41.0±.05	40.7±.03	40.2±.06	39.7±.04	39.6±.08	39.5±.05	39.1±.05
	Group C	38.6±.06	41.1±.05	40.5±.07[a]	39.7±.08[b]	39.0±.06	38.7±.04[b]	38.7±.05[b]	38.7±.02

See Table 1 for footnote.

samples obtained before or after endotoxin challenge (Table 1). Infusion of HSS in dogs of group B induced metabolic acidosis by decreasing values of arterial and venous pH and HCO_3^- within 1h after infusion. Then these values returned near to normal at 12h post-infusion, while dogs of groups A and C did not show any metabolic acidosis after treatment and values decreased and returned near to baseline at 12h and 6h in groups A and C, respectively (Table 1).

Immunomodulatory effects of all treatment protocols in *E. coli* endotoxin induced shock are reflected in Fig 1, Fig 2 and Fig 3. Compared to groups A and B, a significant (P<0.05) reduction in concentration of IL-1α observed in dogs of group C (Fig. 1) throughout study period. Likewise, animals of group C exhibited significant difference (P<0.05) in decreasing concentration of IL-6 over groups A and B, while no significant difference was observed between these groups except 12h and 24h when group B showed a significant difference over group A in lowering level of IL-6 (Fig. 2). Furthermore, concentrations of $PGF_{2\alpha}$ decreased significantly in group C at each interval, while it also showed significant difference (P<0.05) over groups A and B (Fig. 3).

Administration of endotoxin induced significant decrease in MAP. Peak effects observed at end of endotoxin administration followed by rapid return toward normal in groups B and C after treatment (Table 2), while group A showed least significant improvement in MAP. Among groups B and C, latter group induced progressive and significant improvement in MAP at each observing time and showed a significant difference (P<0.05) over groups A and B. All dogs were hypovolemic after endotoxin infusion, as evidenced by decrease in rPV (Fig 4). Administration of ketoprofen alone in group A did not show any significant improvement in rPV, while administration of HSS (groups B and C) increased rPV more effectively. Among these, group C showed a remarkable increase in rPV toward baseline and recovered

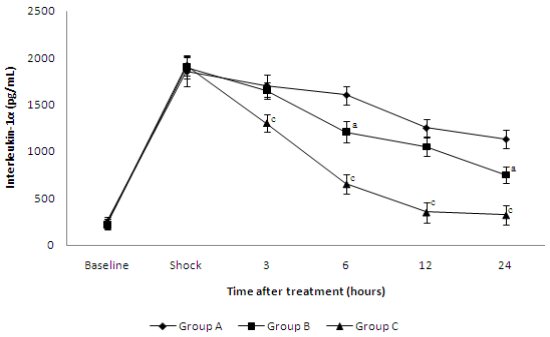

Fig. 1: Interleukin-1α (pg/ml) in dogs with induced endotoxic shock. [a]Significant difference (*P*<0.05) from group A; [b]Significant difference (*P*<0.05) from group B; [c]Significant difference (*P*<0.05) from other groups. Values are reported as mean±SD.

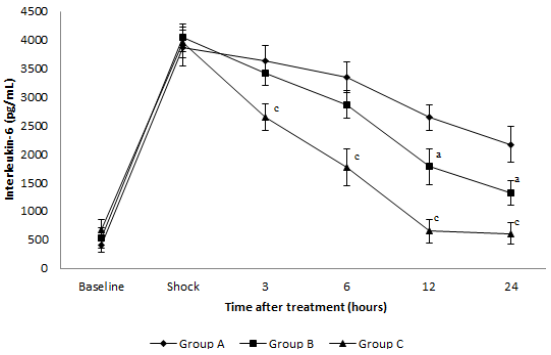

Fig. 2: Interleukin-6 (pg/ml) in dogs with induced endotoxic shock. [a]Significant difference (*P*<0.05) from group A; [b]Significant difference (*P*<0.05) from group B; [c]Significant difference (*P*<0.05) from other groups. Values are reported as mean±SD.

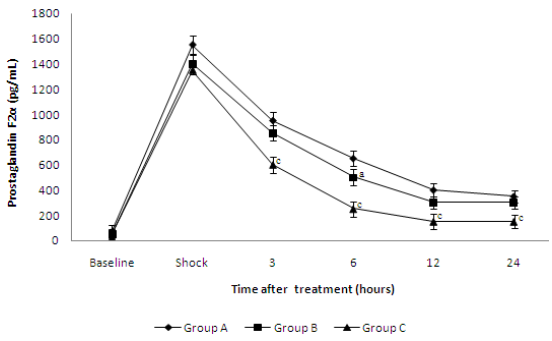

Fig. 3: Prostaglandin-$F_{2\alpha}$ (pg/ml) in dogs with induced endotoxic shock. [a]Significant difference (*P*<0.05) from group A; [b]Significant difference (*P*<0.05) from group B; [c]Significant difference (*P*<0.05) from other groups . Values are reported as mean±SD.

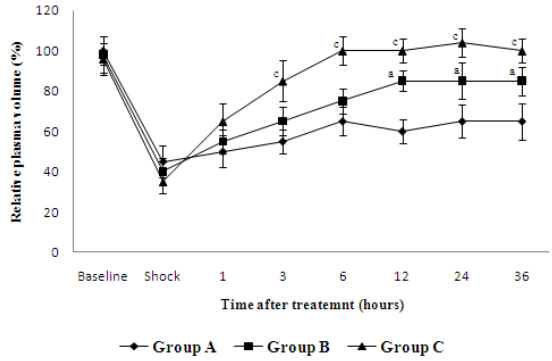

Fig. 4: Graph depicting changes in relative plasma volume (%) in dogs with induced endotoxic shock. [a]Significant difference (P<0.05) from group A; [b]Significant difference (P<0.05) from group B; [c]Significant difference (P<0.05) from other groups. Values are reported as mean±SD.

normal value within 6h and showed significant difference (P<0.05) over group B (Fig. 4).

Concentrations of serum Na^+ and Cl^- ions increased significantly (P<0.05) while marked decrease in K^+ concentration was observed after endotoxin administration. Infusion of HSS produced significant increase in Na^+ and Cl^- ions concentration in group C, compared with baseline and post-endotoxin values (Table 2) and then returned to normal values within study period. While K^+ ions concentration transiently decreased after HSS infusion in group C and then returned to normal. Groups A and B showed better recovery and K^+ concentration increased significantly (P<0.05) to baseline (Table 2). Body temperature increased significantly in endotoxic shock in all groups. Infusion of ketoprofen alone and in combination with HSS in groups A and C, respectively caused a significant and sustained reduction in body temperature to baseline (Table 2).

DISCUSSION

This is the first study conducted to evaluate beneficial effects of HSS and ketoprofen combination in the amelioration of cytokine concentration and other resuscitative effects during endotoxic shock in dogs. Our most conspicuous findings relate to the reduction of cytokine concentration, substantial improvement in cardiac performance and systemic oxygenation, and reversal of acidosis.

Endotoxin are known to elicit acute inflammatory mediators such as cytokines i.e. IL-1α, IL-2 and IL-6 and enzymes, such as cyclo-oxygenase which are responsible for all devastating effects of endotoxemia (Elgawaby *et al*., 2011; Kim *et al*., 2013). In our study, cytokine level (IL-1α and IL-6) reduced efficaciously after administration of HSS in group B as it is reported in recent studies (Mazandarani *et al*., 2012; Theobaldo *et al*., 2012) but amelioration intensity observed more rapid and synergistic in dogs treated with combination of HSS and ketoprofen along with improvement in level of $PGF_{2\alpha}$. Anti-inflammatory drugs are also reported beneficial in the treatment of endotoxemia (Minneci *et al*., 2003; Kim *et al*., 2012), therefore, these conspicuous findings established our hypothesis of synergistic effects of HSS and ketoprofen combination in the resuscitation of patients suffering with endotoxic shock.

Another hallmark of inflammatory process is metabolic acidosis due to accumulation of metabolic waste products and lack of buffer system during endotoxic shock and endotoxin also induced hypoxemia accompanied by increase in $PaCO_2$ (Van Haren *et al*., 2012). Hypoxemia can be caused by hypoventilation or by diffusion impairment such as pulmonary edema, shunting or ventilation-perfusion inequalities. Causes of these pulmonary responses may be linked to endotoxin-induced activation of host granulocytes and release of endogenous arachidonic acid metabolites, such as thromboxane and

$PGF_{2\alpha}$, which are important contributors to endotoxin-induced pulmonary injury (Wang and Ma, 2008; Kim *et al*., 2012). Other than these, decreased arterial and venous blood pH and decreased HCO_3 during endotoxic shock were indicators of metabolic acidosis in dogs. As ketoprofen is a potent inhibitor of arachidonic acid metabolites (Sigurdsson *et al*., 1993; Schaeffer *et al*., 2011) and HSS rectify acidemia (Van Haren *et al*., 2012; Dong *et al*., 2014), that is why group C improved acidosis more potently than other treatment regimens.

Infusion of HSS to endotoxic dogs significantly increased concentrations of serum Na^+ and Cl^- accompanied by a transient decrease in serum K^+ concentration. The key feature for successful resuscitation of hypovolemia and/or endotoxemia in animals is administration of total amount of sodium (Hogue *et al*., 2012). In group C, Na^+ ions concentration increased beyond the limit of hypernatremia that is 160 mEq/L (Shih *et al*., 2012), but this increase was temporary and values became below this level at 3h. So, infusion of 7.5% NaCl is safer and it does cause hypernatremia but not for a prolonged period (Dong *et al*., 2014). Rapid expansion of plasma volume in animals of group C following HSS administration along with ketoprofen may lead to relatively rapid decrease in Na^+ and Cl^- concentration. Mild decrease in K^+ concentration after HSS infusion has also observed in previous studies but not considered clinically important (Zafar *et al*., 2010; Santiago *et al*., 2013).

Vasoactive substances released in bloodstream in response to endotoxin administration leads to arterial hypotension and circulatory failure (Elgawaby *et al*., 2011; Shih *et al*., 2012). In our study, hypotension was characterized by decreased MAP and decreased rPV during endotoxic shock. Cohen *et al*. (1996) reported that MAP $\leq$ 60 mm Hg accepted as typical hypotension after endotoxin administration. In addition to hypotension, decreased rPV may be interpreted as an indicator of hypovolemia (Constable, 1999), as well as pooling of venous blood in peripheral vasculatures. Increased values of HCT and Hb conc also indicated circulatory failure and hemoconcentration. After administration of HSS and ketoprofen in group C, rPV restored and ultimately MAP increased to normal values more effectively than groups A and B (Zafar *et al*., 2010).

Endotoxin induced significant decrease in PaO_2 and PvO_2. Increased PaO_2 following HSS administration indicated a better systemic oxygen delivery. Most probable reason could be the hypovolemia which resulted in the reduction of O_2 extraction from limited supply (Oliveira *et al*., 2002). It suggested mismatching of tissue blood flow with tissue oxygen requirements as impaired efficiency of oxygen extraction by some tissues through vascular endothelial damage have been demonstrated in endotoxemia (Rocha e Silva, 2014). Administration of HSS potentially increase inner capillary diameter, thus, reduces hydraulic resistance through shrinkage of endothelial cell, resulting in improved tissue perfusion (Oliveira *et al*., 2002; Wang *et al*., 2013). In this study, group B showed relative increase in oxygen delivery but effects appeared greater in group C.

Although we documented some beneficial effects of combination of HSS and ketoprofen in the treatment of endotoxic shock, however, some limitations related to this study are deserved to be mentioned. In our model, we could not measure production of nitric oxide during endotoxic shock and after treatment. It is reported that high production of nitric oxide is mainly responsible for development of acidosis during endotoxemia. Further investigations are recommended to evaluate relation of these events and effects of HSS and ketoprofen combination on production of nitric oxide.

Conclusion: It was concluded from the study that ketoprofen as an adjunct to hypertonic resuscitation improved survival of dogs from endotoxic shock through amelioration of cytokine concentrations, improved cardiac performance and increased systemic oxygenation. However, further evaluations are required to evaluate effects of this resuscitation strategy on multiple organ dysfunction in endotoxic shock.

Author's contribution: MAZ floated the idea, developed methodology, conducted the experiment and wrote discussion of manuscript. AR modified and finalized work plan in view of pharmacological aspects and analyze the data statistically. RZA and MUH gave the intellectual input in finalizing the final draft. SUR helped in data collection. AY finalized all work plan and critically looked over all matters of the study.

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