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Cardiovascular Haemodynamics and Some Biochemical Profiles of Endotoxemic Buffalo Calves on Infusion of HSS, Flunixin Meglumine & Blood

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ABSTRACT

Six apparently healthy male buffalo calves aged between 6 months to one year with body weight range of 70 - 140 Kg were used in the present investigation. One animal was used for pilot trial for this group. Five calves were randomly taken into this group. The *Eschrechia coli* endotoxin infused i/v @5 µg/kg BW/hr for 3 hours to all the animals caused symptoms of restlessness, respiratory distress, snoring, diarrhea, profuse salivation along with the significant fall in systolic, diastolic, pulse and mean arterial venous pressure, hypoproteinemia at 1, 2 & 3 hour and hypoglycemia at 3rd hour *i.e.*, end of endotoxin infusion. Respiration was increased significantly during endotoxin infusion at 2nd & 3rd hour and even afterwards till the end of the observation period *i.e.* 7 hrs. The treatment with hypertonic saline solution, flunixin meglumine and blood raised ($p < 0.05$) the systolic, diastolic, pulse, mean arterial, central venous pressure, plasma total protein, Albumin, Glucose and Creatinine to levels either close to or even higher than the normal pre-infusion levels while hematocrit and hemoglobin increased significantly at 7th hour and at 5th, 6th & 7th hour respectively *i.e.*, till the end of the observation period. There was no significant effect on Central Venous Pressure (CVP) and body temperature as measured from rectum.

1. INTRODUCTION

Endotoxemia is a state of high levels of endotoxin in blood circulation. It is a hypovolumic shock which is accompanied by decreased cardiac output and increased systemic vascular resistance. Endotoxins affect the host's peripheral circulation, thermal control mechanisms, prostaglandin metabolism, immune system and inflammatory process along with insufficient oxygen and substrate delivery to tissues. During shock there is substantial morbidity and mortality in cattle especially neonates [1]. Endotoxemia/endo-

toxic shock is a potentially devastating complication of several diseases of cattle and buffalo like enteric diseases, neonatal septicemia, metritis, coliform mastitis, colibacillosis and pneumonia [2].

Barton (1995) [3] was of view that despite plethora of new information, little progress has been made in the development of specific therapies to combat endotoxemia in clinical settings. During clinical conditions, the pathophysiological changes occur during shock as a complex cascade of overlapping events which necessitates the development of a comprehensive protocol for the treatment.

Septic or endotoxic shock results from rapid liberation of endotoxin into circulation that results into cardiovascular collapse accompanied by severe peripheral vasodilatation, pallor of mucosa, cool skin and extremities, diarrhoea, decreased systemic blood pressure and muscle weakness [4].

As the hemodynamics is seriously affected during endotoxemia, an effort was made through the present investigation to evaluate the efficacy of the combination of hypertonic saline solution, Flunixin meglumine and whole blood through the monitoring of some cardiovascular hemodynamic and biochemical parameters.

2. MATERIALS AND METHODS

While one calf was used for standardization of techniques and pilot trial, 5 apparently healthy male buffalo calves of age group from 6 months to one year with body weight range of 70 - 140 Kg procured from local market were used in the present investigation. These calves were kept under the same managemental conditions as are practiced at the dairy farm, COVS, GADVASU, Ludhiana. All the animals were dewormed and vaccinated for Haemorrhaegic Septicemia vaccine before the start of experiment.

The Endotoxin[1] was reconstituted by dissolving it in normal saline solution (0.9% NaCl Acq.) to make a stock solution of 1 mg/ml. Endotoxin concentration of 5 µg/ml was prepared by dissolving 1 ml of stock solution in 199 ml of normal saline to make total volume 200 ml. The endotoxin was infused intravenous @ 5 µg/kg BW/hr for 3 hrs followed immediately by infusion of HSS @ 4 ml/Kg body weight, Flunixin Meglumine @ 1.1 mg/Kg Body weight & blood @ 20 ml/Kg bw as one time infusion and these animals were observed further for 4 hours to study the changes in the cardiovascular, haemodynamic and biochemical profiles. The dose of endotoxin infused was as per Singh *et al*., 2005 [5]. The animals were casted in right lateral recumbancy on operation table. Before endotoxin infusion, an area over jugular furrow was shaved and disinfected with savlon.

The local anesthetic Lignocaine (2%) @ 90 - 120 ml was injected subcutaneously and intra-muscularly before catheterization of the jugular vein and carotid artery to alleviate pain. The skin was incised to expose and catheterize carotid artery and jugular vein. Siliconised polyethylene catheter was inserted into the carotid artery and was connected to mercury manometer through a 3-way cannula with a stopcock for the record of arterial pressure.The jugular vein was catheterized and attached to saline manometer[2] for the record of the central venous pressure (CVP)[3] and for administration of endotoxin and various treatment combinations. The cardiovascular haemodynamics of the buffalo calves were monitored through observation of general symptoms, recording of systolic, diastolic, mean arterial pressure (MAP), Central Venous Pressure (CVP), Pulse Pressure, Heart rate, body temperature and estimation of Haematocrit, Hemoglobin till 7 hrs.

All the animals were given rapid intravenous infusion of Hypertonic Saline Solution (HSS) @4 ml/kg body weight and HSS was administered within 6 minutes after endotoxin infusion was complete. Blood was collected in ACD collection bags containing anticoagulant and stored for 12 hours and was infused i.v. in endotoxemic buffalo calves after bringing it to room temperature. The blood samples from buffalo calves were collected immediately before and after 1, 2, 3, 4, 5, 6, 7 hrs of start of endotoxin infusion from carotid artery for estimation of some biochemical parameters like Total Protein, Albumin, Creatinine and Glucose in blood plasma. The blood biochemical constituents were estimated by blood chemistry analyzer

[1]*Eschrechia coli* endotoxin Lyophilized (Phenol extracted) 0111:B4 lipopolysacharide, SIGMA Chemicals USA.

[2]CVP saline manometer set-Ramsons Scientific and Surgical India Pvt Ltd Agra-India.

[3]Blood Chemistry analyzer-AMES technicon-RA50-Bayer diagnostics-Miles India ltd.-Baroda, Gujarat-India 33.

RA-50 1 using AUTOPAK analytical kits.

Total plasma protein was estimated by Biuret method based on the principle that the peptide bonds of protein form a blue violet colored complex with cupric ions in an alkaline medium. The intensity of color is proportional to the number of peptide bonds and the color is read at 540 nm, which is stable for eight hrs. Plasma Albumin was estimated by the Bromocresol green method using the principle that Albumin in a buffered solution reacts with the anionic Bromocresol green (BCG) a dye binding reaction to give a proportionate color that is measured at 628 nm. The final color is stable for 10 minutes while blood Glucose was estimated by glucose oxidase/peroxidase (GOD/POD) method based on the principle that Glucose is oxidized by glucose oxidase (GOD) into gluconic acid and hydrogen peroxide. Hydrogen peroxide in presence of peroxidase oxidizes the chromogen-4-aminoantipyrine phenolic compound to a red colored compound. The intensity of the red colored compound is proportional to the glucose concentration and measured at 505 nm. The final color is stable for 2 hours. Plasma Creatinine was determined by picrate method following the principle that Creatinine in alkaline solution reacts with picrate to form a red orange colored compound. Under specific conditions of the assay, the rate of development of color is proportional to the concentration of the Creatinine in the specified sample. It is measured at 510 nm. The data thus obtained was analyzed with Completely Randomised Design (CRD) Anova comparing the normal values with the values obtained from samples collected during and after endotoxin infusion and the treatment.

3. RESULTS & DISCUSSION

The results of present investigation are presented in **Table 1** and **Table 2**. All the animals exhibited restlessness, respiratory distress, forceful abdominal respiration, diarrhea and profuse salivation during the administration of endotoxin and its treatment. The respiration was forced, deep, rapid and abdominal with snoring in 3 animals. One animal developed diarrhea during endotoxin infusion.

Suzuki *et al*., (1998) [6] had also reported clinical signs of moist cough, dyspnea, increased salivation and diarrhea because of endotoxic shock. The animals struggled intermittently during endotoxin infusion and remained calm during and after treatment.After administration of hypertonic saline solution, the animals became quiet.

Some animals developed ruminal stasis which was evident by bloat and it persisted throughout the observation period. Bloat was removed by stabbing rumen at upper left flank region with a hypodermic needle leading to escape of gases. Eades (1993) [7] observed forestomach stasis, anorexia, depression and reduced reticuloruminal motility in experimentally induced endotoxemia.

Disseminated intravascular coagulation (DIC) remained a major problem throughout the observation period in all the animals. Deldar *et al*., (1984) [8] observed that disseminated intravascular coagulopathy (DIC) because of endotoxemia is characterized by two phases with an initial decline in blood platelets count and plasma fibrinogen, which was indicative of consumptive coagulopathy and followed by an increase in plasma fibrinogen concentration and blood platelets count during reparative phase of disseminated intravascular coagulopathy (DIC) in endotoxemic calves.

The mean systolic pressure decreased immediately in all the animals following endotoxin infusion and decreased steadily thereafter till the end of endotoxin infusion. A significant ($p < 0.05$) decline in mean systolic pressure was observed in 1st, 2nd and 3rd hour during endotoxin infusion. In all the animals, there was increase in mean systolic pressure after treatment 4th hour onwards (**Table 1**).

The mean diastolic pressure fell significantly ($p < 0.05$) at 1st, 2nd and 3rd hour *i.e*., throughout the endotoxin infusion (**Table 1**). After i/v infusion of the present combination, mean diastolic pressure in all the animals increased after 4th hour which continued throughout the observation period bringing it to higher levels as compared to normal pre-infusion levels indicating that the treatment combination effectively elevated the mean diastolic pressure.

The pulse pressure fell non-significantly during endotoxin infusion (**Table 1**) while a significant fall was observed in all animals at 6th hour of the observation period. Although the administration of the present treatment combination increased the mean pulse pressure non-significantly yet it was still lower than the normal pre-infusion values.

Table 1. Hemodynamic profile during endotoxic shock and after treatment with HSS, Flunixin meglumine & Blood in buffalo calves.

Parameter	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Systolic Pressure (mmHg)	182 ± 24.04	**140.80 ± 8.31***	**125.20 ± 19.26***	**121.40 ± 8.93***	187.60 ± 22.28	189.20 ± 18.47	181.20 ± 21.79	174.40 ± 32.78
Diastolic Pressure (mmHg)	148.40 ± 23.25	**111.20 ± 8.78***	**104.80 ± 24.80***	**103.00 ± 12.16***	168.80 ± 18.14	170.00 ± 16.67	167.80 ± 23.35	157.20 ± 26.81
Pulse Pressure (mmHg)	33.60 ± 10.52	29.60 ± 15.12	20.00 ± 8.12	18.40 ± 10.52	18.80 ± 7.15	19.00 ± 5.83	**14.40 ± 4.56***	17.20 ± 6.26
MAP (mmHg)	159.46 ± 22.91	**121.06 ± 4.86***	**111.46 ± 22.90***	**109.13 ± 10.03***	175.06 ± 19.33	178.66 ± 14.10	173.59 ± 22.10	164.93 ± 28.37
CVP (Cm Saline)	9.10 ± 3.17	5.80 ± 8.20	4.60 ± 6.38	5.50 ± 6.30	8.30 ± 3.70	10.90 ± 3.17	13.40 ± 3.63	14.20 ± 5.72
Respiration Rate/Min.	9.40 ± 1.81	16.40 ± 1.14	**19.80 ± 0.83***	**22.60 ± 1.51***	15.20 ± 9.39	13.80 ± 9.65	16.00 ± 14.71	17.80 ± 13.70
Hematocrit (Hct.) (%)	28.4 ± 2.19	27.60 ± 1.51	27.00 ± 1.73	27.36 ± 3.07	29.40 ± 3.71	30.20 ± 2.77	32.40 ± 1.51	**33.40 ± 2.19***
Hemoglobin (Hb) (Gm/dl.)	12.01 ± 0.31	11.88 ± 0.42	11.53 ± 0.38	11.50 ± 0.41	11.87 ± 0.29	**12.81 ± 0.68***	**14.22 ± 01.39***	**15.02 ± 2.79***
Heart Rate (Beats/Min.)	40.25 ± 14.66	40.03 ± 5.96	46.07 ± 9.79	46.40 ± 5.46	45.97 ± 6.07	54.90 ± 15.43	61.82 ± 27.12	59.56 ± 23.06
Body Temperature (˚F)	100.04 ± 0.49	99.64 ± 0.38	99.84 ± 0.79	100.40 ± 1.10	100.40 ± 0.76	100.68 ± 0.71	100.88 ± 0.99	101.20 ± 1.72

Each figure is a mean of 5 observation. *Significant at 5% level when compared with normal values.

The normal MAP ranged between 159.46 ± 22.91 to 178.66 ± 14.10 mm Hg (Table 1) which is close to 153.88 ± 2.0 mm Hg as reported earlier by Singh et al (1997) [9]. The fall in MAP throughout endotoxin infusion was significant ($p < 0.05$) at 1st 2nd and 3rd hour while MAP attained near normal or non-significantly higher than normal values in all the animals from 4th hour onwards till 7th hour *i.e.* end of observation period of experiment.

Table 2. **Some biochemical profile during endotoxic shock and after treatment with HSS, Flunixin meglumine & Blood in buffalo calves.**

Parameter	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Total Protein (Gm/dl)	6.80 ± 0.59	**6.24 ± 0.61***	**5.48 ± 1.1***	**4.80 ± 0.40***	**5.38 ± 1.04***	6.34 ± 0.62	**6.10 ± 0.78***	6.74 ± 0.61
Plasma Albumin (Gm/dl)	2.96 ± 0.52	2.78 ± 0.39	2.32 ± 0.37	2.24 ± 0.47	2.42 ± 0.49	2.68 ± 0.49	2.50 ± 0.60	2.98 ± 0.11
Plasma Glucose (mg/dl)	92.80 ± 5.54	84.80 ± 1.92	77.80 ± 4.71	**72.00 ± 3.39***	88.00 ± 21.52	78.60 ± 16.86	80.20 ± 10.35	91.40 ± 6.06
Plasma Creatinine (mg/dl)	0.94 ± 0.10	0.86 ± 0.08	**0.77 ± 0.07***	**0.73 ± 0.08***	**0.66 ± 0.08***	**0.65 ± 0.09***	**0.66 ± 0.04***	**0.70 ± 0.12***

Each figure is a mean of 5 observation. *Significant at 5% level when compared with normal values.

Templeton *et al.*, (1988) [10] reported a significant decline in mean arterial pressure as early as 25 minutes after start of endotoxin infusion that remained significantly below normal till 250 minutes after endotoxin administration. Singh *et al.*, (2005) [5] reported sharp fall in MAP after endotoxin infusion but upon treatment with hypertonic saline solution, MAP again tried to return to normal or near normal values.

The rise in MAP after infusion of the present combination may be due to the fact that HSS infusion increases the plasma osmolality and osmotically draws intracellular and interstitial water into vascular space. The plasma volume expansion is three ml for every one ml of hypertonic saline solution [11].

There was non-significant changes in CVP during endotoxin infusion and after the i.v. infusion of H.S.S., Flunixin meglumine and whole blood throughout the period of observation (**Table 1**).

The respiration rate increased significantly ($p < 0.05$) at the 2nd and 3rdhour of endotoxin infusion (**Table 1**). Respiration rate showed a general increase even after administration of the treatment along with a non-significant increase in respiration indicating no beneficial effect of the present treatment combination on respiration of endotoxemic buffalo calves.

According to Templeton *et al.*, (1988) [10], cattle in comparison to other species have more abundant smooth muscles in the pulmonary vascular tree and this may partially or completely explain why the response to endotoxin is so extreme in lungs of cattle leading to pulmonary edema, atelactasis and respiratory acidosis. This is true not only for cattle but also for mature cattle and onset of clinical respiratory signs can be detected minutes after *E. coli* endotoxin administration as was also observed in present investigation. Dupe *et al.*, (1993) [12] also reported significant increase in respiration rate after endotoxin infusion in cow calves. The period of respiratory distress lasted throughout the experiment in the present investigation.

PCV decreased non-significantly up throughout the endotoxin infusion period while a significant increase was observed at 7th hour of start of endotoxin infusion *i.e.*, the end of the experiment while the mean hemoglobin showed a general declining trend with a significant ($p < 0.05$) increase above normal value at 5th, 6th and 7th hour of observation (**Table 1**). Semrad (1993) [2] reported decrease in PCV in endotoxemic neonatal calves at 48 to 96 hours of endotoxin infusion. After treatment, both hematocrit and

hemoglobin showed a significant ($p < 0.05$) increase which can be attributed to the hemoconcentration caused by infusion of whole blood which was part of treatment. Singh *et al.*, (2003) [13] found that packed cell volume *i.e.*, hematocrit and hemoglobin showed a sharp decline in endotoxemic calves who were treated with hypertonic saline solution and plasmex D-40.

There was no significant change in heart rate in all the animals during endotoxic shock and treatment (**Table 1**). However, there was a tendency of heart rate to be higher than the base values at the end of endotoxin infusion which continued till the end of experiment. The mean body temperature in all the animals showed non-significant change during endotoxin infusion and after treatment at the end of the observation period (**Table 1**).

A significant hypoproteinemia was observed at 1st, 2nd and 3rd hour *i.e.*, throughout the endotoxin infusion in all the animals (**Table 2**) and even later after the treatment at 4th and 6th hour. However the total plasma protein level was very close to normal pre-infusion level at the end of the observation period *i.e.*, 7 hours which could be an effect of the whole blood transfusion. Earlier Nagaraja *et al.*, (1979) [14] observed hypoproteinemia on *E. coli* endotoxin infusion in cow calves. Singh *et al.*, (1997) [9] reported a slight decrease in plasma proteins in endotoxemic buffalo calves. The hypoproteinemia as observed in present investigation was perhaps due to the increased protein breakdown and increased ability of the carbon skeleton of amino acids to enter kreb cycle. Additionally the decreased ability of anoxic liver to metabolize amino acids may also partially contribute to hypoproteinemia (Singh *et al.*, 2004) [15]. A generalized non-significant hypoalbuminemia was observed during endotoxin infusion and after treatment.

A significant ($p < 0.05$) hypoglycemia was observed at 3rd hour of start of endotoxin infusion followed by a non-significant increase in plasma glucose level at 4th, 5th, 6th and 7th hour of observation bringing these close to pre-infusion level (**Table 2**) which could be due to the whole blood transfusion. Singh *et al.*, (2004) [15] observed significant hypoglycemia in buffalo calves after subjecting them to endotoxic shock with I/v infusion of E coli endotoxin. When glycogen stores are depleted and hepatic production of glucose decrease, hypoglycemia results. Increased utilization of glucose by muscle tissue, inhibition of glucose synthesis from non carbohydrate sources, decreased hepatic glucose synthesis, depletion of glycogen storage pools and macrophage, insulin like activity of endotoxin, limited amount of available carbon for glucose synthesis and macrophage derived glucocorticoids antagonizing factor reportedly contribute to hypoglycemia during endotoxemia. Endotoxin induced alterations in glucose concentration are postulated to be caused by changes in cellular calcium utilization. Further Insulin like activity of endotoxin has also been implicated in causing the hypoglycemia during endotoxemia [16].

Endotoxemia induced hypoglycemia was evident by 3 hours of endotoxin infusion and persisted throughout the period of study in endotoxemic calves, mean plasma lactate concentration was also increased in all endotoxin exposed calves [2]. A similar endotoxin induced hypoglycemia till 3 hours of endotoxin infusion was observed in the present investigation as well.

Plasma creatinine showed significant decline from 2nd hour onwards till 7th hour *i.e.*, throughout the period of observation. The level of plasma creatinine remained slightly below the normal pre-infusion values after i/v administration of Hypertonic saline, Flunixin meglumine and whole blood (**Table 2**) The overall changes in plasma creatinine which were probably due to hemodilution caused by whole blood transfusion were within normal range of 1 - 2 Mg/dl.

4. CONCLUSION

On the basis of the present investigation, it can be concluded that a significant decrease in systolic, diastolic, pulse, mean arterial and Central Venous Pressure (CVP) was effectively restored back to normal or non-significantly higher levels than normal on infusion of hypertonic saline, Flunixin meglumine and blood but hematocrit, hemoglobin and respiratory rate of endotoxemic buffalo calves could not be restored to normal and the CVP & body temperature as measured from rectum was not affected either with endotoxin infusion or with the i/v infusion of the above mentioned treatment combination.

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