
INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN BIOLOGY AND MEDICINE

ISSN: 2455-944X

www.darshanpublishers.com

DOI:10.22192/ijcrbm

Volume 2, Issue 6 - 2017

Original Research ArticleDOI: <http://dx.doi.org/10.22192/ijcrbm.2017.02.06.003>

Hemodynamic and blood biochemical profile during endotoxemia and after therapeutic intervention in buffalo calves.

JP Singh¹ and DV Singh^{2*}¹Post-graduate student²Professor, Department of Vety. Physiology & Biochemistry, COVS, GADVASU, Ludhiana-141004*Corresponding author: digvijay231@rediffmail.com

Abstract

Five apparently healthy male buffalo calves aged between 6-12 months were infused with *Escherichia coli* endotoxin @5µg/kg BW/hr for 3 hours to induce the symptoms of endotoxemia like corneal opacity, increased respiration rate, hypoproteinemia, hypoalbuminemia, hypoglycemia, decrease in mean systolic, diastolic, pulse and mean arterial and central venous pressure along with inconsistent changes in heart rate in the present investigation. The endotoxemic animals were then treated with hypertonic saline solution (HSS) @ 4 ml/Kg BW, Dextran-40 @ 10 ml/Kg BW, Flunixin Meglumine 1.1 mg/Kg BW and blood @ 20 ml/Kg BW as a single infusion which raised the systolic, diastolic, pulse, mean arterial and central venous pressure along with hematocrit and hemoglobin to the levels either close or even higher than the normal pre-infusion levels. Respiration rate and body temperature showed non-significant alterations during endotoxin infusion and after therapeutic intervention. A non-significant increase in total protein was observed after treatment which reached close to pre-infusion level. Mean plasma fibrinogen showed non-significant rise throughout the period of observation while plasma albumin, globulin and creatinine varied non-significantly. A significant increase was seen in plasma glucose level at 5th, 6th and 7th hour as compared to pre-infusion normal values. Overall, treatment given to the animals was found to be effective.

Keywords: Buffalo calves, Endotoxin, Flunixin meglumine, HSS, Dextran-40, Blood.

Introduction

Endotoxemia is a life threatening inflammatory condition which can lead to shock, multiple organ failure, suppression of immune system and wound-healing processes (Ng *et al.*, 2008). Septic or endotoxic shock results from rapid liberation of endotoxins into circulation which leads to cardiovascular collapse accompanied by severe peripheral vasodilatation, pallor of mucosa, cool skin and extremities, diarrhea, decreased systemic blood pressure and muscle weakness (Radostits *et al.*, 2000).

Therefore, the consequences of endotoxemia are either a considerable morbidity or mortality of animals leading to severe economic losses to the dairy farmers.

In view of this, the present investigation was undertaken to elucidate the haemodynamic and biochemical changes observed during endotoxemia and after therapeutic intervention.

Materials and Methods

Five apparently healthy male buffalo calves aged between 6-12 months with body weight range of 70-140Kg were used in the present investigation. Jugular vein was exteriorized and catheterized under aseptic conditions using local anesthetic lignocaine for the infusion of endotoxin and therapeutic combination. Carotid artery was catheterized and attached to the mercury manometer for the record of blood pressure. Animals remained conscious throughout the experiment. Endotoxin was infused @ 5µg/kg BW/hr for consecutive three hrs for the development of endotoxemia and subsequently therapeutically intervenes for next 4 hrs. Animals were given HSS (7.2% NaClAcq.) @ 4 ml/Kg BW, Dextran-40 @ 10 ml/Kg BW, Flunixin Meglumine 1.1mg/Kg BW and blood @ 20 ml/Kg BW as a single infusion. The blood samples were collected before the start of experiment from healthy buffalo calves and immediately after 1,2,3,4,5,6 and 7 hrs of start of experiment. The cardiopulmonary and haemodynamic changes in buffalo calves were monitored through observation of general symptoms, recording of systolic, diastolic, pulse pressure, mean arterial pressure (MAP) from

carotid artery, central venous pressure (CVP) from jugular vein, heart rate, respiration rate, body temperature and estimation of haematocrit and hemoglobin till 7 hrs. Biochemical profile in buffalo calves were monitored through estimation of total plasma proteins, albumin, globulin, fibrinogen, plasma glucose and plasma creatinine levels. The data generated in the present investigation were analysed with CRD Anova (Snedecor and Cochran, 1976) and compared with the normal preinfusion values within the group.

Results and Discussion

All the animals exhibited symptoms of restlessness, respiratory distress, diarrhea and profuse salivation. The animals closed their eyes and struggled intermittently with progression of endotoxin infusion. Corneal opacity was observed in three animals immediately after end of experiment, from which two were able to recover subsequently in four days. Disseminantive Intravascular Coagulation (DIC) was a major problem encountered throughout the observation period in all the animals. (Pictures. 1 and 2)



Picture-1 showing a clot removed from carotid artery due to DIC



Picture-2 A Closer view of the clot removed from carotid artery due to DIC

But on treatment, all the animals remained calm, opened their eyes and profuse urination was observed one hour after infusion

Cardiovascular Haemodynamic

The mean systolic pressures 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 (145.20 ± 33.24) in mean systolic pressure was observed at 3rd hour (Table 1), which increased (174.80 ± 20.12) after treatment with Flunixin meglumine, HSS, Dextran-40 and Blood. Singh and Bansal (2008) observed a highly significant fall in mean systolic, diastolic, pulse pressure, MAP, CVP, hemoglobin till the end of the endotoxin infusion while respiratory rate was significantly increased along with a non-significant alteration in rectal temperature and hematocrit during infusion of the endotoxin. Non-significant changes were seen in mean diastolic pressure in experimental animals (Table 1). At the end of experiment, the mean diastolic pressure was higher (142.40 ± 15.19) than normal values indicating that the treatment given to animals effectively elevated the mean diastolic pressure. Singh

et.al., 2011 observed a highly significant fall in mean systolic, diastolic, pulse pressure, MAP, CVP and haemoglobin till the end of endotoxin infusion.

The changes in mean arterial pressure (MAP) were found to be non-significant throughout endotoxin infusion and attained near normal values in all the animals at 7th hour of the start of the endotoxin infusion. (Table 1). Singh *et. al.*, (2005) reported sharp fall in MAP after endotoxin infusion but upon treatment with hypertonic saline solution, MAP again tried to return to near normal values. The fall in MAP during endotoxin infusion (non-significant during the present study) may be due to the release of prostaglandins (Margolis *et al.*, 1987). The rise in MAP might be due to the fact that flunixin meglumine, one of the important components of the treatment, is a cyclo oxygenase inhibitor and prevents the formation of prostaglandin and hence improves tissue perfusion (Singh *et al.*, 2011). Another reason could be that HSS infusion increases the plasma osmolality and osmotically draws intracellular and interstitial water into vascular system. The plasma volume expansion is three ml for every one ml of hypertonic saline solution (Jean *et al.*, 1993).

There was non-significant fall in central venous pressure (CVP) during endotoxin infusion from 1st to 3rd hour in all the animals. However, after administering treatment combination, animals showed significant increase in CVP from 4th hour onwards (Table 1) which remained significantly high (20.10 ± 2.94) till the end of the observation period. The fall in CVP in present study might be attributed to the peripheral pooling of blood (Singh *et al.*, 2005). According to Singh (1979), failure of capacitance changes due to lack of venous constriction may be one of the important contributing factors in reducing CVP in calves.

A significant ($p < 0.05$) fall in pulse pressure was observed at 2nd and 3rd hour in the buffalo calves during endotoxin infusion. The administration of combination of Flunixin meglumine, HSS, Dextran-40 and Blood increased the mean pulse pressure non-significantly yet it was still lower than the normal pre-infusion values in all the animals. There was no significant change in heart rate during endotoxic shock and after therapeutic intervention (Table 1). However, there was a tendency of heart rate to be higher than the base values at the end of endotoxin infusion. In comparison to this, Reece and Whalstrom (1973) observed a decrease in heart rate through first 50 minutes after infusion of endotoxin with maximum decrease at 25 minutes after which increase in heart rate was observed which continued till the end of observation period of 24 hours. Waurick *et al.*, (1997) also reported elevated heart rate, cardiac output and oxygen delivery at 24th hour of endotoxin infusion in unanesthetised sheep.

Hematology

Non-significant alterations in hemoglobin were observed in experimental animals which could be due to haemodilution caused by Dextran-40 that constituted an essential part of treatment given to animals. No significant changes in hematocrit were observed in animals in spite of blood transfusion which may be due to haemodilution caused by intravenous infusion of Dextran-40 to buffalo calves indicating that treatment given to animals was effective in restoring near normal PCV (38.76 ± 4.38). Semrad (1993) reported decrease in hematocrit in endotoxemic neonatal calves at 48 to 96 hours of endotoxin infusion. Similar results have also been seen by Singh *et al.*, (2003) in endotoxemic calves who were treated with hypertonic saline solution and plasmex D-40.

Non-significant increase in Respiration rate was seen in all the animals during endotoxemia and even after treatment (Table 1). Griel *et al.*, 1975 reported that within 5 minute of endotoxin infusion, the respiration rate and amplitude of respiration was elevated by 3-4 times than normal. Dupe *et al.*, 1993 also reported significant increase in respiration rate after endotoxin infusion in cow calves. Increase in respiration rate might be due to fact that endotoxins causes pulmonary vasoconstriction resulting into increase in pulmonary ventilation (Germino *et al.*, 2001). The mean body temperature in all the animals showed non-significant alterations during endotoxin infusion and after treatment with Flunixin meglumine, HSS, Dextran-40 and Blood (Table 1). Similarly, Singh *et al.*, (2011) observed non-significant decrease in body temperature during endotoxin infusion and after treatment with NSAIDS.

Biochemical Profile

Plasma total proteins, albumin, globulin, fibrinogen, plasma glucose and plasma creatinine levels were assessed to check the biochemical status of the body in the experimental animals. Total plasma proteins decreased non-significantly after endotoxemia but increased to 8.58 ± 0.61 i.e., slight below pre-infusion levels after treatment with Flunixin meglumine, HSS, Dextran-40 and Blood transfusion (Table 2). Earlier Nagaraja *et al.*, (1979) observed hypoproteinemia on *E. coli* endotoxin infusion in cow calves. Singh *et al.*, (1997) 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 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).

Non-significant alterations were found in plasma albumin and globulin levels during endotoxin infusion and after treatment, however, slight decrease in plasma albumin level (3.18 ± 0.92) and increase in globulin level (4.74 ± 0.93) was found at the end of experiment as compared to preinfusion levels (Table 2). Singh (2000) and Kaneko *et al.*, (1997) also found the similar results in endotoxemic calves. Non-significant increase in plasma fibrinogen concentration was observed in the present investigation during endotoxemia well as after treatment (Table 2). This could be due to fact that endotoxin accelerates fibrinogen synthesis rate (Wycoff 1970).

Table 1: Hemodynamic profile during endotoxic shock and after treatment with HSS, Flunixin Meglumine, Dextran-40 and blood in buffalo calves.

Parameter	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Systolic Pressure (m.m. Hg.)	180.80 ±11.71	166.40 ±16.08	152.80 ±22.29	145.20±33.24*	176.40 ±15.58	173.20 ±18.47	178.00 ±18.05	174.80 ±20.12
Diastolic Pressure (m.m. Hg.)	136.40 ±9.94	134.00 ±13.56	132.00 ±21.02	122.40 ±38.22	136.80 ±18.03	137.60 ±17.28	141.20 ±11.36	142.40 ±15.19
Pulse Pressure (m.m. Hg.)	44.40 ±15.90	29.20 ±11.88	20.80 ±6.87*	20.80±4.6*	39.60 ±13.95	35.60±5.89	36.80 ±10.73	32.40 ±10.43
MAP (m.m. Hg.)	151.19 ±7.44	147.13 ±15.94	138.93 ±21.21	131.33 ±38.03	150.00 ±15.95	149.46 ±17.47	153.46 ±13.00	153.20 ±16.35
CVP (Cm Saline)	9.60 ±0.89	7.4 ±1.81	8.30 ±3.15	7.70 ±3.34	19.10 ±1.55*	21.30 ±2.70*	20.00 ±2.44*	20.10 ±2.94*
HeartRate (Beats/min.)	41.92 ±8.07	42.96 ±6.59	41.46 ±7.31	43.37 ±9.38	43.02 ±3.85	44.93 ±3.85	54.16 ±8.61	56.44 ±7.15
Hb (Gm/dl)	13.31 ±0.85	13.00 ±0.74	12.77 ±0.67	12.40 ±0.63	12.09 ±0.57	12.39 ±0.35	13.35 ±0.20	13.53 ±0.97
PCV (%)	38.40 ±4.77	37.20 ±5.26	35.20 ±2.38	34.80 ±3.11	34.60 ±2.30	36.20 ±5.49	38.84 ±4.73	38.76 ±4.38
Respiration Rate (/ Min.)	9.20 ±3.96	10.40 ±4.72	10.00 ±3.00	9.80 ±4.32	9.80 ±5.76	10.40 ±6.18	11.40 ±4.39	11.80 ±4.65
Body Temperature(°F)	99.12 ±0.60	99.32 ±0.75	99.28 ±0.83	99.64 ±0.95	99.40 ±0.80	99.40 ±0.80	99.84 ±0.51	99.84 ±0.76

*Significant at 5% level

Each figure is a mean of 5 observations

Table 2: Blood biochemical profile during endotoxic shock and after treatment with HSS, Flunixin Meglumine, Dextran-40 and blood in buffalo calves.

Parameter	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
Plasma Total Proteins (gm/dl)	8.70 ±0.71	8.14 ±0.75	7.86 ±0.67	7.78 ±0.66	8.10 ±0.52	8.00 ±0.77	8.46 ±0.70	8.58 ±0.61
Plasma Albumin (gm/dl)	3.58 ±0.79	3.38 ±0.69	3.12 ±0.68	2.94 ±0.80	2.76 ±0.55	2.82 ±0.92	2.94 ±0.68	3.18 ±0.92
Plasma Globulin (gm/dl)	4.66 ±0.71	4.22 ±0.73	4.18 ±0.81	4.2 ±1.00	4.7 ±1.08	4.50 ±1.20	4.86 ±0.93	4.74 ±0.93
A/G ratio	0.76	0.80	0.74	0.70	0.58	0.62	0.60	0.67
Plasma Fibrinogen (gm/dl)	0.46 ±0.08	0.54 ±0.08	0.56 ±0.11	0.64 ±0.13	0.58 ±0.13	0.68 ±0.13	0.66 ±0.19	0.66 ±0.14
Plasma Glucose (mg/dl)	90.80 ±7.79	84.60 ±6.76	85.60 ±5.41	82.40±10.80	91.20 ±5.97	104±7.96*	102.60±4.03*	103.60±11.16*
Plasma Creatinine (mg/dl)	1.00 ±0.11	0.88 ±0.10	0.84 ±0.11	0.79 ±0.09	0.80 ±0.12	0.89 ±0.14	0.89 ±0.14	0.86 ±0.13

*Significant at 5% level

Each figure is a mean of 5 observations

Non-significant hypoglycemia was evident from the start of experiment till 4th hour followed by a significant ($p < 0.05$) increase in plasma glucose level at 5th, 6th and 7th hour of observation (Table 2). Singh et. al., (2004) observed significant hypoglycemia in buffalo calves after subjecting them to endotoxic shock with i/v infusion of E coli endotoxin. Endotoxin induced alterations in glucose concentration are postulated to be caused by changes in cellular calcium utilization. Insulin like activity of endotoxin has also been implicated in causing the hypoglycemia during endotoxemia (Rose and Semrad 1993). Plasma glucose level significantly ($p < 0.05$) increased after the treatment probably due to beneficial effect of Dextran-40 which gets converted into glucose with the passage of time through metabolism in liver.

The plasma creatinine level in endotoxemic buffalo calves showed non-significant alterations during the study period, however, remained slightly below (0.86 ± 0.13) the normal pre-infusion values (Table 2). The haemodilution caused by i/v infusion of HSS and whole blood has probably significantly reduced the circulating plasma levels of creatinine.

It can be concluded from the results of present investigation, that the treatment with Flunixin meglumine, HSS, Dextran-40 and Blood transfusion was found to be effective against endotoxemia as it improved the general physiological conditions of animals at the end of observation period.

References

1. Dupe R, Bywater R J and Goddard M. 1993. A hypertonic saline infusion in treatment of experimental shock in calves and clinical shock in dogs and cats. *Veterinary Record* **133**: 585-90.
2. Gerbino A J, Altemier W A, Schimmel C, Glennly R W. 2001. Endotoxemia increases relative perfusion to dorsal-caudal regions. *Journal of Applied Physiology*. **90**: 1508-15.
3. Griel L C, Zarkower A and Eberhart R J. 1975. Clinicopathological effect of *E. coli* endotoxin in mature cattle. *Canadian Journal of Comparative Medicine* (**39**): 1-6.
4. Jean GS, Constable PD and Yorchock K. 1993. The clinical use of hypertonic saline solution in food animals with hemorrhagic and endotoxic shock. *Agricultural Practice*. **14** (7): 6-11.
5. Kaneko J J, Harvey J W, Bress M L. 1997. Clinical biochemistry of domestic animals, pp.126-134, 893, 5th edition, Academic press, New York.
6. Margolis J H, Buttom G D and Fessler J F. 1987. The efficacy of dexamethasone and flunixin meglumine in treating endotoxin induced changes in calves. *Veterinary Research Communications* **11**: 479-491.
7. Nagaraja T G, Bartley E E, Anthony H D, Leipold H W and Fina L R. 1979. Endotoxic shock in calves from intravenous injection of rumen bacterial endotoxin. *Journal of Animal Sciences* **49**: 567-82.
8. Ng S W, Zhang H, Hedge A and Bhatia M. 2008. Role of preprotachykinin -A gene products on multiple organ injury in LPS-induced endotoxemia. *Journal of Leukocyte Biology* **83**:288-295.
9. Radostits OM, Gay CG, Blood DC, Hinchcliff KW (2000) *Textbook of diseases of Cattle, sheep, pigs, goats and Horses*, pp. 41-47,410. Chapter II and IX, WB Saunders Company ltd.
10. Reece O J and Whalstrom J D. 1973. *E.coli* endotoxemia in conscious calves. *American Journal of Veterinary Research* **34**: 765-69.
11. Rose M L and Semrad S D. 1993. Clinical efficacy of tirilazad mesylate for treatment of endotoxemia in neonatal calves. *American Journal of Veterinary Research* **53**: 2305-10.
12. Semrad S D. 1993. Comparative efficacy of flunixin, ketoprofen, ketorolac for treating endotoxemic neonatal calves. *American Journal of Veterinary Research*. **54** (9): 1511-16
13. Singh D V. 2000. Physiological and pharmacological studies on bovine endotoxic shock and its treatment. Ph.D. thesis, Punjab Agricultural University, Ludhiana, India.
14. Singh D V, Singh R V and Sodhi S P S. 2003. Use of combination of hypertonic saline solution and plasmex D-40 to resuscitate endotoxemic buffalo calves. *Buffalo Journal*. **2**: 169-78.
15. Singh D V, Singh R V and Sodhi S P S. 2004. Blood biochemical parameters during bovine endotoxemia and after i/v infusion of hypertonic saline solution and plasmex -D-40. *Indian Journal of Animal Sciences* **11**: 1098-1101
16. Singh K, Krishnamurthy D and Peskin P K. 1997. Endotoxin shock with and without dexamethasone pretreatment in anaesthetized buffalo calves- II. *Indian Journal of Veterinary Surgery* **18**: 8-11.
17. Singh D V, Singh R V and Sodhi S P S. 2005. Effect of blood transfusion in combination with Dextran-40 and hypertonic saline solution on

- cardiopulmonary haemodynamics of endotoxic shock in buffalo calves: *Veterinary Research Communications* **29**: 421-30.
18. Singh D V, Bansal S K. 2008. Hemodynamics during endotoxin administration and after i/v infusion of Flunixin meglumine in buffalo calves. Abstract published and paper presented at XXIV conference of ISSAR at KVAFSU, Hebbal, Bangalore.
 19. Singh D.V., S.K. Bansal and G.S.Ghumman 2011 Effect of flunixin meglumine alone and in combination on haemodynamics during bovine endotoxic shock and after treatment. *J. Biomedical Science and Engineering*, 2011, 4, 29-33.
 20. Singh J (1979) An investigation into pathophysiology and treatment of shock in bovines, Ph.D. dissertation, Punjab Agricultural University, Ludhiana, India
 21. Snedcor GW and Cochran WG (1976) Statistical methods, Iowa state college press. Iowa, USA.
 22. Waurick R, Boe G H, Meyer J, Booke M, Meibner A, Prien T and Aken H V. 1997. Haemodynamic effects of dopamine and nitric oxide synthase inhibition in healthy and endotoxaemic sheep. *European Journal of Pharmacology*. **333**: 181-86.
 23. Wycoff H D. 1970. Production of fibrinogen following the endotoxin injection. *Proceedings of experimental biological medicine* **133**: 940-43.

Access this Article in Online	
	Website: www.darshanpublishers.com
	Subject: Veterinary Sciences
Quick Response Code	

How to cite this article:

JP Singh and DV Singh. (2017). Hemodynamic and blood biochemical profile during endotoxemia and after therapeutic intervention in buffalo calves. *Int. J. Curr. Res. Biol. Med.* 2(6): 13-19.

DOI: <http://dx.doi.org/10.22192/ijcrbm.2017.02.06.003>