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Impact of non-enzymatic antioxidants on the biochemical profile in buffaloes having uterine torsion

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Abstract

The present research aimed to assess the effects of combined vitamin E with selenium (Se) and vitamin C injections on the blood biochemical profiles of buffaloes affected by uterine torsion. A total of 18 buffaloes with uterine torsion were randomly divided into three groups (n = 6 animals/group). Group 1st received only routine supportive therapy and served as the control. Group 2nd was administered an intramuscular injection of vitamin E-selenium (α -tocopherol acetate 50mg and sodium selenite 0.3mg), while Group 3rd was injected with vitamin C (250mg sodium ascorbate/ml) intravenously, in addition to supportive therapy, immediately prior to detorsion. Heparinized and non-heparinized tubes were used to collect blood samples at the time of reporting, one hour after detorsion, and 24 hours after the correction of uterine torsion. Serum was separated by using centrifugation and was stored at -20 °C until biochemical analysis. The results revealed that the parenteral administration of both antioxidants significantly ($p \leq 0.05$) influenced the serum biochemical parameters, including total protein, albumin, A/G ratio, creatinine, BUN, ALT, AST, calcium, and phosphorus. The findings demonstrate the positive impact of vitamin E with Se and vitamin C on the serum biochemical parameters with uterine torsion affected buffaloes.

Keywords: Antioxidants, blood, dystocia, oxidative stress, uterine torsion

Introduction

Buffaloes are considered the backbone of animal husbandry in India, particularly calves, which are seen as the future of livestock wealth. Buffaloes are highly versatile animals with great potential to bring about social and economic transformation in agrarian communities. As of the latest census (DHAD 2024)^[9], the total buffalo population in India stands at 109.85 million, reflecting an increase of approximately 1.1% compared to the previous census.

Reproductive disorders can negatively impact the productivity and reproductive health of dairy animals (Fesseha & Ayele 2020)^[11]. Dystocia, also known as calving difficulty, occurs when the first or second stage of labour is prolonged and assistance is needed for delivery (Weldeyohanes and Fesseha 2020)^[32]. In buffaloes, uterine torsion is the leading cause of dystocia, accounting for 41.3% of cases (Juneja *et al.*, 2023)^[16]. Torsion of the uterus in buffaloes typically occurs in the pregnant uterine horn and is defined as the twisting of the uterus along its longitudinal axis.

Parturition is a stressful event in the life of an animal as evidenced by changes in certain haematological and biochemical parameters (Joshi *et al.*, 2018). Uterine torsion places additional stress on the bovine body, causing significant biochemical changes that vary depending on the severity and duration of the condition (Tripathi & Mehta 2016)^[30]. Oxidative stress occurs due to an imbalance between pro-oxidants and antioxidants, leading to cell damage. This disruption results in the production of reactive oxygen species (ROS) (Gutteridge & Halliwell 2018)^[13].

Non-enzymatic antioxidants, such as vitamin E along with selenium and vitamin C, helps in reduction of oxidative stress and improve the blood biochemical profile of buffaloes suffering from uterine torsion. Vitamin E, a fat-soluble vitamin, is a strong antioxidant agent act synergistically with the selenium (Xiao *et al.*, 2021)^[34]. Vitamin C is a potent reducing agent and scavenger of free radicals in biological systems (Pehlivan *et al.*, 2017)^[23]. This study aimed to improve the biochemical profile by utilizing non-enzymatic antioxidants,

which help reduce oxidative stress in buffaloes with uterine torsion.

Material and Methods

The present study was carried out, in uterine torsion affected buffaloes, presented to the department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Science, Bikaner and nearby areas of Bikaner city. Before attempting to relieve the torsion, a complete history of the case regarding age, parity, stage of gestation and findings of rectal palpation were recorded for confirmative diagnosis. Only those uterine torsion cases that were successfully relieved by the modified Schaffer's method were included in the present study.

A total of eighteen uterine torsion affected buffaloes were randomly divided in three groups (n = 6 animals/group). The animals of group 1st were received only routine supportive therapy and considered as control (C group). Animals of group 2nd were administered 10 ml intramuscular injection of vitamin E-selenium (VES group; Selexidant, containing tocopherol acetate 50mg and sodium selenite 0.3mg; *Intracin Pharmaceutical Private Limited, Nadiad, Gujrat, India*) whereas animals of group 3rd were injected 30 ml vitamin C (VC group; Ascorvet 250mg/ml; *Phoenix Life Science Private Limited, Rohtak, Haryana, India*) intravenously along with supportive therapy just prior to detorsion (Table-1).

Table 1: Experimental regime of research work

Group I (C)	Group II (VES)	Group-III (VC)
Only supportive therapy	Vitamin E-Se + Supportive therapy	Vitamin C + Supportive therapy

Blood sampling schedule

A total of three blood samples (10 ml/animal) were taken from each animal at the time of reporting, one hour after detorsion and 24 hours of correction of uterine torsion to access the biochemical profiles. Blood samples collected were than centrifuged at 3000 rpm for 15 minutes to separate serum and were stored at -20 °C until the analysis of biochemical parameters.

Estimation of biochemical parameters-

Serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), blood urea nitrogen (BUN), albumin (ALB), globulin (GLB), albumin-to-globulin ratio, creatinine (CR), calcium (Ca) and phosphorus (P) were analysed by a semi-auto analyser (Spectra lab Genie Biochemistry Analyzer) as per the

protocols given in the respective kits manufactured by Spectrum Medical Industries Private Limited, India.

Statistical analysis

Data obtained were subjected to analysis by one-way analysis of variance (ANOVA) technique (Snedecor & Cochran 2004) [27] using the SPSS software version 21. To know statistical significance the means of different experimental groups were tested (P<0.05) by Duncan's Multiple Range Test (Duncan, 1955) [10].

Results and Discussion

The results of the current experiment are presented to define the effects of parenteral administration of vitamin E with Se and vitamin C injections on serum biochemical profiles in uterine torsion affected buffaloes (Table-2).

Table 2: The impact of vit E + Se and vit C administration on serum biochemical in uterine torsion affected buffaloes

Group of animals	C			VES			VC		
	AR	AC	24 hrs	AR	AC	24 hrs	AR	AC	24 hrs
TP (g/dl)	5.88 ^{Aa} ±0.11	6.06 ^{Aa} ±0.10	6.31 ^{Ab} ±0.19	6.01 ^{Aa} ±0.09	6.05 ^{Aa} ±0.08	7.55 ^{Bb} ±0.25	6.04 ^{Aa} ±0.08	6.16 ^{Aa} ±0.13	7.28 ^{Bb} ±0.17
ALB (g/dl)	1.55 ^{Aa} ±0.01	1.53 ^{Aa} ±0.01	2.21 ^{Ab} ±0.02	1.49 ^{Aa} ±0.02	1.52 ^{Aa} ±0.01	3.70 ^{Cb} ±0.07	1.52 ^{Aa} ±0.02	2.31 ^{Bb} ±0.12	2.95 ^{Bc} ±0.14
GLB (g/dl)	4.33±0.12	4.25±0.10	4.02±0.09	4.52±0.08	4.36±0.18	4.16±0.24	4.53±0.07	4.12±0.12	4.33±0.13
A: G	0.36 ^{Aa} ±0.01	0.36 ^{Aa} ±0.01	0.55 ^{Ab} ±0.01	0.33 ^{Aa} ±0.01	0.35 ^{Aa} ±0.02	0.91 ^{Cb} ±0.08	0.34 ^{Aa} ±0.004	0.56 ^{Bb} ±0.05	0.68 ^{Bc} ±0.04
BUN (mg/dl)	27.83 ^{Aa} ±0.70	28.28 ^{Ba} ±0.70	26.34 ^{Ba} ±0.49	29.01 ^{Ab} ±0.89	26.85 ^{ABb} ±0.81	18.78 ^{Aa} ±0.43	28.39 ^{Ac} ±0.83	25.93 ^{Ab} ±0.75	19.01 ^{Aa} ±0.70
CR (mg/dl)	2.15 ^{Aa} ±0.09	2.32 ^{Ba} ±0.12	2.10 ^{Ba} ±0.13	2.22 ^{Ab} ±0.13	2.02 ^{ABb} ±0.15	1.48 ^{Aa} ±0.11	2.28 ^{Ab} ±0.12	1.77 ^{Aa} ±0.13	1.52 ^{Aa} ±0.11
ALT (U/L)	41.04 ^{Aa} ±0.54	42.05 ^{Aa} ±0.42	39.98 ^{Ca} ±0.81	38.94 ^{Ab} ±0.62	41.58 ^{Ab} ±0.59	26.64 ^{Aa} ±0.63	40.40 ^{Ab} ±0.62	39.96 ^{Ab} ±0.64	32.13 ^{Ba} ±0.97
AST (U/L)	111.82 ^{Aa} ±1.36	111.78 ^{Aa} ±1.67	110.32 ^{Ba} ±1.16	113.35 ^{Ab} ±2.54	111.22 ^{Ab} ±2.48	93.19 ^{Aa} ±1.49	114.00 ^{Ab} ±2.03	112.00 ^{Ab} ±1.98	96.88 ^{Aa} ±2.02
Ca (mg/dl)	7.08 ^{Aa} ±0.15	7.11 ^{Aa} ±0.15	7.13 ^{Aa} ±0.14	6.96 ^{Aa} ±0.19	7.15 ^{Aa} ±0.21	10.15 ^{Cb} ±0.29	7.04 ^{Aa} ±0.17	7.08 ^{Aa} ±0.16	8.72 ^{Bb} ±0.23
P (mg/dl)	3.39 ^{Aa} ±0.16	3.41 ^{Aa} ±0.17	3.49 ^{Aa} ±0.18	3.44 ^{Aa} ±0.26	3.45 ^{Aa} ±0.26	5.94 ^{Cb} ±0.21	3.39 ^{Aa} ±0.18	3.44 ^{Aa} ±0.18	4.65 ^{Bb} ±0.36

Mean values having different superscripts in a column (Capital letter) differ significantly (p<0.05)

Mean values having different superscripts in a row (Small letter) differ significantly (p<0.05)

AR-At reporting of case; AC-At one hr after correction of UT; 24 hrs-At 24hrs of UT correction

Effect of administration of antioxidants on serum total proteins, albumin, and globulin-

In the present study, parenteral administration of antioxidants significantly (p<0.05) affected serum total proteins, albumin, and the A/G ratio in buffaloes 24 hours after the correction of uterine torsion. However, no significant effect was observed on serum globulin levels. After 24 hours of correction, intramuscular administration of

vitamin E and selenium resulted in the highest serum albumin levels and A/G ratios, followed by vitamin C.

These findings align with those of (Helal *et al.*, 2009) [14], who reported a significant increase in serum total protein levels following oral supplementation of vitamin E and selenium in buffaloes. The lack of significant effect on serum globulin levels in buffalo calves, as observed by (Shinde *et al.*, 2009) [26], also supports the current findings.

Similarly, (Sushma *et al.*, 2015) ^[29] did not observe any significant changes in serum globulin levels in ram lambs supplemented with selenium. According to (Mogal *et al.*, 2019) ^[21], who found significantly higher ($p \leq 0.01$) serum total protein and albumin levels in uterine torsion-affected buffaloes treated with intramuscular vitamin E + Se compared to untreated animals. These findings are also in agreement with (Sultana *et al.*, 2022) ^[28], who reported significantly higher serum total protein, albumin levels, and A/G ratios in heat-stressed Holstein Friesian calves supplemented with vitamin E and selenium. Similarly, (Gade *et al.*, 2010) ^[12] observed a significant increase in A/G ratio in heat-stressed buffaloes supplemented with ascorbic acid compared to the control group. Furthermore, the present study is consistent with previous reports, such as (Youssef *et al.*, 2013) ^[37], who recorded significantly higher albumin levels in buffalo bulls treated with vitamin C. In line with the current findings, (Barwary *et al.*, 2011) ^[5] reported no significant effect of dietary ascorbic acid supplementation on serum globulin levels in male Meriz kids. Additionally, (Abd-Allah & Zanonny 2014) ^[1] observed a significant increase in serum total protein levels in heat-stressed ram lambs supplemented with vitamin C during the summer season.

The exact mechanism behind the increased serum total protein in antioxidant-treated animals remains unclear, but it may be attributed to improved protein anabolism and reduced protein catabolism. Vitamin E can boost immunoglobulin production, which may explain the rise in albumin levels. It plays a crucial role in protein synthesis and cellular function. Selenium, in conjunction with vitamin E, helps prevent and repair cellular damage caused by reactive oxygen species (ROS), thereby minimizing oxidative stress. Similarly, vitamin C plays a vital role in amino acid metabolism and protein synthesis, potentially contributing to the increased blood protein concentrations observed in this study. This increase may also be attributed to the ameliorative effects of ascorbic acid in reducing the catabolic activity of stress hormones (Borges *et al.*, 2007) ^[8].

Effect of administration of antioxidants on serum enzymatic variables

In this study, the parenteral administration of antioxidants had a significant ($p \leq 0.05$) effect on serum ALT and AST levels 24 hours after the correction of uterine torsion in buffaloes. Both antioxidant treatments resulted in significantly lower serum ALT and AST levels compared to the control group. Previous research supports these findings. For instance, (Mahmoud *et al.*, 2014) ^[20] demonstrated that intramuscular administration of antioxidants, and (Alhidary *et al.*, 2015) ^[3] showed that oral supplementation with vitamin E and selenium led to a significant reduction in serum ALT and AST levels in sheep. These results are consistent with those of (Yenilmaz *et al.*, 2022) ^[36], who observed a substantial decrease in ALT levels ($p \leq 0.05$) after intramuscular vitamin E administration in cattle, which helped maintain ALT levels and mitigate liver damage caused by oxidative stress during calving. Similarly, (Bhardwaj *et al.*, 2022) reported a significant reduction ($p \leq 0.05$) in serum ALT and AST levels following oral supplementation of vitamin E and selenium in buffaloes exposed to heavy metals.

Moreover, the results are aligned with previous studies such as those by (Kassab & Mohammed, 2014) ^[18], who found a

significant decrease ($p \leq 0.01$) in serum ALT and AST levels after oral administration of ascorbic acid in sheep.

Hepatic cells play a crucial role in various metabolic processes and contain enzymes like ALT and AST, which are widely used as biomarkers for liver damage and the effectiveness of hepato-protective therapies (Rosser & Gores, 1995) ^[24]. The administration of antioxidants like vitamin E, vitamin C, and selenium helps preserve liver integrity by protecting it from oxidative stress and supporting its metabolic functions.

Effect of administration of antioxidants on serum metabolites-

In the current study, parenteral administration of vitamin E + Se and vitamin C significantly ($p \leq 0.05$) reduced serum creatinine and BUN levels 24 hours after the correction of uterine torsion.

These findings are in line with previous research, such as the study by (Kassab & Mohammed, 2014) ^[18], which reported a significant reduction in serum creatinine levels in transportation-stressed rams treated with ascorbic acid. Likewise, (Khan *et al.*, 2015) ^[19] observed the beneficial effects of vitamin E and mineral supplementation in reducing plasma BUN levels in pre-partum buffaloes compared to the non-supplemented control group. (Semsirboon, 2022) ^[25], also reported a significant reduction ($p \leq 0.05$) in plasma BUN and creatinine levels following high-dose ascorbic acid supplementation in stressed dairy goats under tropical conditions.

Elevated BUN and creatinine levels are commonly associated with increased cortisol concentrations, which can trigger the breakdown of proteins and nucleic acids in muscles during stress (Kannan *et al.*, 2002) ^[17]. Non-enzymatic antioxidants, such as vitamin E, vitamin C, and selenium, may serve as effective agents in protecting tissues from oxidative damage due to their potent free radical-scavenging and antioxidant properties (Yanardag & Ozsoy-Scan, 2007) ^[35].

Effect of administration of antioxidants on serum minerals profile-

In the present study, parenteral administration of antioxidants significantly ($p \leq 0.05$) affected serum calcium (Ca) and phosphorus (P) levels 24 hours after the correction of uterine torsion in buffaloes. After 24 hours, intramuscular administration of vitamin E and selenium resulted in significantly higher serum Ca and P levels, followed by vitamin C, in comparison to the untreated group.

These findings are in line with those of (Khan *et al.*, 2015) ^[19], who observed higher plasma Ca and P levels following oral supplementation of vitamin E and a commercial mineral mixture in pre-partum buffaloes. Similar results were reported by (Behera *et al.*, 2020) ^[6], who noted significantly increased serum calcium and phosphorus levels 46 days after vitamin C administration in heat-stressed ducks. The present study also aligns with the findings of (Achi *et al.*, 2022) ^[2] who found a significant increase in milk phosphorus levels in cows supplemented orally with vitamin E and selenium.

Vitamin E helps prevent calcium loss caused by increased oxygen consumption and the production of free radicals (White *et al.*, 2001) ^[33]. A deficiency in vitamin E has also been linked to hypocalcaemia, as it inhibits the conversion of vitamin D to its active metabolites, which may reduce calcium absorption from the intestine (Norazlina *et al.*,

2004)^[22]. Selenium, known for its protective effect against oxidative stress, also plays a vital role as a trace element essential for regulating thyroid hormones, maintaining the redox state of vitamin C, and supporting the function of other molecules (Arthur *et al.*, 1997)^[4]. Additionally, the bioconversion of vitamin D3 to its active form, 1,25-(OH)2D3, is enhanced by utilizing ascorbic acid as a cofactor, which in turn elevates plasma concentrations of 1,25-(OH)2D3. This increase boosts serum Ca levels by enhancing the activity of duodenal calcium-binding proteins (Volker & Weiser, 1993)^[31].

Conclusion

The parenteral administration of both non-enzymatic antioxidants can be considered an effective alternative for improving serum total protein, albumin, A/G ratio, Ca, and P levels, while significantly reducing creatinine, BUN, and the serum enzymatic activities of ALT and AST. Overall, the study demonstrated a positive impact of vitamin E with Se and ascorbic acid on serum biochemical parameters in uterine torsion-affected buffaloes.

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