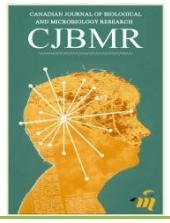
CANADIAN JOURNAL OF BIOLOGICAL AND MICROBIOLOGY RESEARCH

Journal homepage: www.mcmed.us/journal/cjbmr



ALDOSTERONE BASED OXIDATIVE STRESS APPRAISAL IN PREGNANT DOG

Sudesh Agarwal[1], B.S.Saini[2], Mamta Saini[2], Satyendra[2], Ashish Joshi[2] and Nalini Kataria*[2]

[1]Department of Obstetrics and Gynaecology, Sardar Patel Medical College, Bikaner, Rajasthan, India.
[2]Department of Veterinary Physiology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India.

Article Info

Received 07/04/2016
Revised 10/04/2016
Accepted 15/04/2016

Key words Aldosterone, electrolytes, German Shepherd, oxidative stress, pregnant.

ABSTRACT

The present endeavor was undertaken to appraise aldosterone based oxidative stress in pregnant dog. Sera were obtained to determine aldosterone, gamma glutamyl transferase (GGT) enzyme and electrolytes *viz.* Sodium, potassium, chloride, calcium, phosphorus and magnesium in healthy pregnant and non pregnant adult female German Shepherd dogs. Serum aldosterone levels were determined by radioimmunoassay. Results revealed significant ($p \leq 0.05$) increase in the values of serum aldosterone, GGT, sodium and chloride during pregnancy as compared to non pregnant state. Mean values of serum potassium, calcium, phosphorus and magnesium showed reverse trend and the values were significantly ($p \leq 0.05$) lower during pregnancy as compared to non pregnant state. Since serum GGT is an agreeably instituted indicator of oxidative stress, its raised levels denoted towards the expansion of oxidative stress in pregnant dogs. Greatest percent change was discerned in the mean value of serum GGT which was trailed by variation in serum aldosterone level. The present endeavor has tried to apprise the sorority of aldosterone with the oxidative stress as well as with electrolytes. It can be deduced that pregnancy produced the oxidative stress in the dogs. Interpretation of these variations will help to provide perceptive approach of various hidden mechanisms in the development of oxidative stress. Results clearly testified that apart from diseases, other stressors can also contribute in the development in oxidative stress and pregnancy is one of them. Canines must be supplemented with antioxidants regularly to protect them from the threats of oxidative stress particularly during pregnancy.

INTRODUCTION

In the organism, free radicals are produced via cellular metabolism. In physiological conditions, antioxidant enzymes regulate this phenomenon. However, when a large amount of free radicals is produced, these enzymes are overloaded, and free radicals induce major cellular damages. This is oxidative stress. Pregnancy is associated with increased oxidative stress, and exaggeration of oxidative damage is considered important in pregnancy complications. There have been many attempts to produce animal models that mimic the hypertensive disorders of pregnancy, especially preeclampsia, but most are incomplete when compared to the full spectrum of the human disease. Defining these animal models should help to understand the cause, as well as to test preventative and therapeutic strategies in the management of these hypertensive disorders of pregnancy. A number of researchers have investigated the impact of chronic reductions in uteroplacental perfusion pressure during pregnancy in a variety of animal species. In the pregnant rabbit, aortic clamping appeared to increase blood pressure within 24 hours and foetal compromise,

Corresponding Author

Nalini Kataria
Email:- nalinikataria@rediffmail.com

proteinuria, glomerular endotheliosis and intracapillary fibrinoid deposition are observed [1]. In the conscious pregnant sheep, stepwise reductions in uterine blood flow, down to approximately 20% of normal, could develop foetal hypoxia [2]. Researchers have observed pregnant ewes fed with a high salt diet result in increase in blood pressure. Hypertensive pregnant ewes can develop convulsions with reduced urine volume and respiratory distress due to pulmonary oedema [3]. Overall, it does appear that aortic constriction during pregnancy leads to hypertension in a variety of animal species. Whether this is entirely or partly caused by reductions in uteroplacental perfusion pressure is less clear because the more selective method of reducing uterine blood flow is much less reproducible as a model of hypertension in pregnancy.

Aldosterone is a steroid hormone which is responsible for the reabsorption of sodium and water across tubular epithelial cells in the distal nephron, the distal colon and the salivary glands. It maintains blood pressure by increasing plasma volume through sodium reabsorption. Potassium is excreted at the same time. Aldosterone is synthesized in the outermost zone of the adrenal cortex, the zona glomerulosa.

Huge variations can result in problems in vital organs. Stress and strenuous exercise can increase aldosterone levels. Physiological modulations in the aldosterone levels are important to help the body to overcome immediate threats. It is challenging for a clinician to differentiate between the transient rise and chronic elevations, so that corrective measures can be taken timely. Pregnancy is associated with striking increase in rennin and aldosterone secretion. This is attributed to increase in glomerular filtration rate and progesterone secretion. Aldosterone secretion is compromised in dogs with hypoadrenocorticism with and without electrolyte abnormalities [4], however, increase in aldosterone level does not mean adrenal pathology every time. Earlier research [5] has shown elevation of plasma aldosterone levels in dogs after injection of ACTH which indicated its association with stress mechanisms. Focus of earlier research concerning aldosterone in dogs has been coupled with hypoadrenocorticism, however, there is paucity of research to bracket together aldosterone and pregnancy associated oxidative stress in dogs. It is imperative to correlate aldosterone with other physiological mechanisms linked with stress. Scientists have shown that in many conditions like hypoxia, pregnancy etc. dissociation between renin activity and aldosterone may occur and various controllers may interact to regulate aldosterone secretion [6]. Higher aldosterone levels are associated with systemic evidence of oxidative stress in human medicine [7]. Due to dearth of research on this aspect in pet animals, application of scientific force is required to hook up the function of aldosterone with the development of oxidative stress. Therefore a study to correlate aldosterone concentration with oxidative stress during pregnancy becomes important for scientific management of animals. Determination of serum electrolyte levels in animals is important to assess the fluid and electrolyte levels of the body particularly during pregnancy. This can also reflect about the proper renal functioning and hormonal influences.

During the last decade it has become more widely accepted that pet ownership and animal assistance in therapy and education may have a multitude of positive effects on humans [8]. Dogs of German shepherd breed are trustworthy and good protectors. As a result they are kept as companions by large number of families. Hence it becomes crucial to keep the pets stress free and well-timed uncovering of stress can strengthen the measures which help in preventing the incidence of diseases. Rareness of literature on this viewpoint in the dogs was adequate to embark on the initiation of a project with comprehensible directions to assess oxidative stress in pregnant dogs. Further looking towards the important role played by the aldosterone in homeostatic mechanisms related with the salt and water balance and significance of oxidative stress during gestation period, the present investigation was carried out to appraise the role of aldosterone as an oxidative stress marker in German shepherd dogs.

MATERIAL AND METHODS

The present endeavor was undertaken in sixteen healthy adult (between 3 and 5 years) German shepherd female pregnant (8) and non pregnant (8) dogs kept by the private owners with almost similar type of management conditions. These animals were free from endo- and ecto-parasites as assessed by routine faecal and skin examination, respectively. Blood sample were collected in sterile tubes to harvest sera during morning hours. Pregnant animals were in mid gestation phase. Serum aldosterone along with GGT as marker of oxidative stress and electrolytes *viz.* Sodium, potassium, chloride, calcium, phosphorus and magnesium were determined.

Serum aldosterone was determined by immunoradiometric assay using Radio immuno assay kit (DiaSorin) in the RIA laboratory of Department of Veterinary Physiology, CVAS, Bikaner, Rajasthan, India as per the manufacturer's protocol.The assay is based on the competition between the labeled aldosterone and aldosterone contained in the calibrators and serum samples assayed for a fixed and limited number of antibody binding sites. After the incubation the amount of labeled aldosterone bound to the antibody on the tube walls is inversely related to the concentration of unlabelled aldosterone present in calibrators or samples. In the method antibody coated tubes were used. All reagents were brought to room temperature before assaying. Tubes were arranged as per the protocol provided by the manufacturer. Coated tubes were labeled for zero, 50, 100, 250, 500 and 1000 pg/ml calibrators starting from A to F, respectively. For serum samples coated tubes were labelled accordingly. Samples and reagents were dispensed in the bottom of the tubes by appropriate micropipettes as per protocol. The

contents of the tubes were vortexed except T tube. All the tubes (except T tubes) were incubated at 26-28 ^{0}C for 18-22 hrs. The incubation mixture was aspirated carefully leaving no trace of dye except T tube. Then the radioactivity of each tube was measured including T tube using Gamma Counter (^{125}I Gamma counter, ECIL, India).

Serum gamma glutamyl transferase (GGT) was determined by spectro-photometric method [9]. Sodium and potassium in serum were determined by the standard method using a flame photometer [10]. Serum chloride was determined by the titrimetric method of Schales and Schales. Serum calcium was determined by the titrimetric method of Clark and Collip, modified by Kramer & Tisdall. Serum phosphorus was determined by the spectrophotometric method of Fiske & Subarrow [10]. Serum magnesium was determined by the titan yellow method [11]. Statistical significance for individual parameter between pregnant and nonpregnant was analysed [12]. Mean value of healthy non pregnant dogs for each parameter was considered as control.

RESULTS

The mean±SEM values of serum aldosterone, GGT and electrolytes along with per cent variations in the values in pregnant and non pregnant dogs are presented in table 1. All the comparisons for each parameter during pregnancy have been made from respective non pregnant value, keeping it as control. Results indicated significant ($p\leq0.05$) increase in the levels of serum aldosterone, GGT, sodium and chloride during pregnancy as compared to non pregnant state. Mean values of serum potassium, calcium, phosphorus and magnesium showed reverse trend and the values were significantly ($p\leq0.05$) lower during pregnancy as compared to non pregnant state. Maximum percent change was observed in the mean value of serum aldosterone which was followed by change in serum GGT level.

Table 1. Serum levels of aldosterone, gamma glutamyl transferase and electrolytes in dogs (n=8, mean ± SEM values)

Serum Parameters	Physiological status		Per cent change
	Non-pregnant	Pregnant	
Aldosterone (nmol L^{-1})	0.20±0.001	0.37±0.001 ^{d}	85.00 %
Gamma glutamyl transferase (UL^{-1})	30.00±0.21	278.00±0.18 ^{d}	82.66%
Sodium (mmol L^{-1})	126.00±0.03	150.00±0.17^d	19.04%
Potassium (mmol L^{-1})	5.22±0.001	4.59±0.001 ^{d}	- 12.06%
Chloride (mmol L^{-1})	110.00±0.09	128.00±0.17 ^{d}	16.36%
Calcium (mmol L^{-1})	2.90±0.001	1.94±0.001 ^{d}	- 33.10%
Phosphorus (mmol L^{-1})	1.96±0.001	1.48±0.001 ^{d}	- 24.48%
Magnesium (mmol L^{-1})	1.41±0.001	1.10±0.001 ^{d}	- 21.98%

Superscript 'd' indicates that a given parameter differs significantly ($p\leq0.05$) from respective moderate mean value.

DISCUSSION

There is paucity of literature regarding information on serum aldosterone levels in the dogs of German Shepherd breed during pregnancy. Highest percent change was observed in the mean value of serum aldosterone indicating the development of oxidative stress [13]. It was followed by the percent change in GGT levels reflecting its association with oxidative stress.

Aldosterone

Researchers have shown the relationship of aldosterone and oxidative stress [14-17]. Observations in animal models suggest that elevated levels of aldosterone promote oxidative stress [7]. Chronic stress over an expanded period can disturb the internal regulatory mechanisms which control aldosterone. Aldosterone increases sodium absorption by the kidney and helps control the homeostasis of blood sodium levels. Increase in aldosterone perhaps is essential to support absorption of more salt and water from gastrointestinal tract. Pregnancy is known to alter many physiological mechanisms for improved water retention process in body, which is helpful in maintaining fluid volume to meet the confronts progressed due to water deficit. The results of present investigation regarding aldosterone status are also pointing towards its role as one of the contributing factors in the development of oxidative stress. Direct effect of aldosterone on oxidative stress has been shown through its ability to increase the levels of an important subunit of NADPH oxidase, essential for superoxide anion generation [18]. Reduced nitric oxide bioavailability can be involved in the development of oxidative stress by aldosterone [19]. In women, the plasma aldosterone to renin ratios rise as gestation progresses, and the highest values are observed with overt preeclampsia [20]. The focus on adrenal mineralocorticoid production and a healthy robust volume expansion is indeed of interest, but a bit surprising. In pregnancy, a vast part of the increased mineralocorticoid activity relates to the greatly increased production of deoxycorticosterone whose circulating levels, like those of aldosterone, are markedly high in pregnancy [21]. Above discussion has given a direction to appreciate the additional role of aldosterone as a stress hormone with its involvement in the development of oxidative stress.

Gamma glutamyl transferase

In clinical physiology, serum GGT levels can be used effectively as markers of oxidative stress. Enhanced serum GGT activity suggested the development of oxidative stress. Research in animals have correlated higher GGT activity to stress [22]. The primary role of cellular gamma glutamyltransferase is to metabolize extracellular reduced glutathione , allowing for precursor amino acids to be assimilated and reutilized for intracellular glutathione synthesis. Paradoxically, recent experimental studies indicate that cellular GGT may also be involved in the generation of reactive oxygen species in the presence of iron or other transition metals. Although the relationship between cellular GGT and serum GGT is not known and serum GGT activity has been commonly used as a marker for liver diseases, earlier research suggest that serum GGT within its normal range might be an early and sensitive enzyme related to oxidative stress. More importantly, serum GGT level within its normal range predicts oxidative damage product. Researchers have shown strong associations of serum GGT with many cardiovascular risk factors and related to oxidative stress. Serum GGT might be useful in studying oxidative stress-related issues [23]. Scientists have projected serum GGT activity as a biochemical marker in preeclamptic pregnant women. Elevated levels of serum GGT indicates the tissue damage related to endothelial vascular damage and are the main cause of the occurrence of preeclampsia [24]. Scientists in human medicine have used the levels of GGT as a potent marker of oxidative stress in pregnant women [25]. There is paucity of such studies in pregnant dog. Increased quantity of serum GGT ought to be looked in stipulations of oxidative stress along with liver problems [26].

Sodium

Higher sodium concentration in pregnant dogs indicated water retention due to increased aldosterone [14]. Modulation in sodium value could be due to oxidative stress mechanisms. Pregnancy affects essentially all aspects of kidney physiology. The orchestration of changes that occur is a physiologic feat. Kidney and systemic hemodynamics are marked by significant volume expansion and vasodilatation. A rise in serum aldosterone results in a net gain of sodium in women [27]. Progesterone in dog is not natriuretic.

Potassium

The lower value of serum potassium during pregnancy indicated the effect of increased aldosterone level [14]. Aldosterone favours sodium retention and potassium excretion. Enzyme systems, metabolic functions and performance measures depend on the homeostasis. The kidneys face remarkable demands during pregnancy, and it is critical to understand the normal kidney adaptations to pregnancy. A parallel rise in progesterone protects from hypokalemia [27].

Chloride

Mineral metabolism is modulated during pregnancy. Chlorides in the body follows sodium ions in maintenance of acid base balance [28]. The higher value during pregnancy indicated the retention of water in the body. In present study, serum sodium level was significantly ($p \leq 0.05$) higher in pregnant animals.

Calcium

Decrease in serum calcium level is known to be associated with potentiating the oxidative stress. Oxidative stress causes calcium influx into the cytoplasm from the extracellular environment, then its rising concentration in cytoplasm causes its influx into mitochondria, disrupting normal metabolism [29]. Probably movement of the calcium from plasma into the cells lowered the calcium levels in pregnant dog. Different studies concluded that calcium homoeostasis is an important aspect of maternal and foetal physiology during gestation. A certain calcium level is required for production of endothelial derived releasing factor which maintains vasodilatation in normal pregnancy. Alteration of calcium metabolism has been implicated in pathogenesis of hypertension during pregnancy. Studies in human medicine have determined calcium and creatinine to find out the presence of oxidative stress associated with pregnancy induced hypertension. Further scientists recommend the use of antioxidants additionally in the treatment of pregnancy induced hypertension [30]. Since oxidative stress is associated with many diseases and the aging process, understanding how oxidants alter calcium signaling can help to understand process of disease, and may lead to new strategies for their prevention.

Inorganic phosphorus

It has a close connection with calcium .In present study; serum calcium level was lower in pregnant dog as compared to non pregnant. Perhaps, this could be the reason of low phosphorus. Formation of free radicals alters oxidative metabolism which leads to disruption of ionic environment of the cell. This causes low phosphorus levels. Researchers have correlated calcium phosphorus levels to oxidative stress in human medicine [31]. Decreased phosphorus levels could be correlated with oxidative stress.

Magnesium

Scientists have associated the changes in the levels of magnesium with oxidative stress. Magnesium deficit and oxidative stress are common features. Deviations in magnesium levels may openly manage the cellular redox state and its deficiency is coupled with the increased creation of reactive oxygen species along with the initiation of immune and inflammatory mechanisms. Antioxidant enzyme activity is known to increase with magnesium supplementation in disease conditions. Scientists have demonstrated that alloxanic diabetes is

associated with decreased magnesium status and increased oxidative stress and that magnesium supplementation can in part restore the antioxidant parameters and decrease the oxidative stress in experimental diabetic rats [32]. Studies carried out in human medicine tried to focus on the relationship of magnesium and its association with oxidative stress and inflammation in preeclamptic women to identify the predictor variables of the disorder [33]. There is paucity of studies in pregnant dogs. Pattern of changes in serum magnesium level in the present study is pointing towards the development of oxidative stress and relationship of magnesium with antioxidant capacity of the animals [34].

CONCLUSION

Increase in serum aldosterone levels substantiated the presence of oxidative stress in the pregnant dogs. Rise in serum GGT level suggested its relationship with oxidative stress. Precedent of variation in serum electrolytes propped up the probable physiological changes under the impact of enhanced aldosterone concentrations. It can be deduced that pregnancy produced the oxidative stress in the dogs. Laboratory analysis of various electrolytes become important as current perception in this area reveals their fundamental function in skeleton remodeling and bone metabolism. Owing to pregnancy associated changes in electrolyte, logical insight of changes in acid-base balance can be deduced. It may help in enforcing several modifications in the management of pregnant dogs to shield them from the danger of ensuing oxidative stress.

ACKNOWLEDGEMENTS

The authors are thankful to the dog owners for allowing collecting blood to be used in this study.

CONFLICT OF INTEREST

No Interest.

REFERENCES

1. Abitbol MM, Gallo GR, Pirani CL, *et al*. (1976). Production of experimental toxemia in the pregnant rabbit. American Journal of Obstetrics & Gynecology,124, 1976,460–470.
2. Clark KE, Durnwald M, Austin JE. (1982). A model for studying chronic reduction in uterine blood flow in pregnant sheep. *American Journal of Physiology*, 242, H297–H301.
3. Leffler CW, Hessler JR, Green RS. (1986). Effects of sodium chloride on pregnant sheep with reduced uteroplacental perfusion pressure. *Hypertension*, 8, 62–65.
4. Baumstark ME, Sieber-Ruckstuhl NS, Müller C, Wenger M, Boretti FS, Reusch CE. (2014). Evaluation of aldosterone concentrations in dogs with hypoadrenocorticism. *Journal of Veterinary Internal Medicine*, 28(1),154-159.
5. Willard MD, Refsal K, Thacker E. (1987). Evaluation of plasma aldosterone concentrations before and after ACTH administration in clinically normal dogs and in dogs with various diseases. *Americal Journal of Veterinary Research*, 48(12), 1713-1718.
6. Merrill DC, Ebert TJ, Skelton MM,Cowley JR. (1989). Effect of plasma sodium on aldosterone secretion during angiotensin II stimulation in normal humans. Hypertension, 14, 164-169.
7. Kotlyar E, Vita JA, Winter MR, Awtry EH, Siwik DA, Keaney JF Jr, Sawyer DB. (2006). The relationship between aldosterone, oxidative stress, and inflammation in chronic, stable human heart failure. *Journal of cardiac failure*, 12(2), 122-127.
8. Beetz A, Uvnäs-Moberg K, Julius H, Kotrschal K. (2012). Psychosocial and psychophysiological effects of human-animal interactions: The possible role of oxytocin. *Frontiers in psychology*,3, 234- 240.
9. Wolf PL, Williams D. (1973). In: Practical clinical enzymology, Wiley-Inetrscience Publication, John Wiley & Sons, New York, 22-156.
10. Oser BL. (1976). In: Hawks physiological chemistry, Tata McGraw Hill Publishing Co Ltd New Delhi, 900-1125.
11. Varley H. (1988). Tests in liver and biliary tract disease In:Practical Clinical Biochemistry, CBS publishers New Delhi, 158-467.
12. Kaps M, Lamberson WR. (2004). Biostatistics for Animal Science, CABI Publishing Oxford shire, 36-270.
13. Lee DH, Blomhoff R, Jacobs D. (2004). Is serum gamma glutamyltransferase a marker of oxidative stress. *Free radical research*, 38(6), 535-539.
14. Kataria N, Kataria AK, Agarwal VK, Garg SL, Sahani MS, Singh R. (2000). Effect of water restriction on serum aldosterone and cortisol in dromedary camel during winter and summer. *Journal of camel practice and research*, 7(1), 1-7.
15. Kataria N, Kataria AK. (2006). Endocrine and metabolic responses of Marwari sheep in arid tract. *Slovenian veterinary research*, 43(3), 135-142.
16. Kataria N, Kataria AK. (2004). Use of blood analytes in assessment of stress due to drought in camel. *Journal of camel practice and research*, 11(2), 129-133.
17. Ojeda M, Barrera CJ, Alberu J, Perez VR, Morales LE, Bobadilla NA. (2013). Mineralocorticoid receptor blockade reduced oxidative stress in renal transplant recipients: A double-blind randomized pilot study. *American journal of nephrology*, 37(5), 481-490.

18. Calò La, Zaghetto F, Pagnin E. (2004). Effect of aldosterone and glycyrrhetinic acid on the protein expression of PAI-1 and p22phox in human mononuclear leukocytes. *Journal of clinical endocrinology and metabolism*, 89, 1973–1976.
19. Queisser N, Schupp N. (2012). Aldosterone, oxidative stress and NF-kB activation in hypertension related cardiovascular and renal diseases. *Free Radical Biology and Medicine*, 53, 314-327.
20. Brown MA, Zammit VC, Mitar DA, Brown MA. (1992). Renin aldosterone relationship in pregnancy induced hypertension. *American Journal of Hypertension*, 5, 366-371.
21. Winkel CA, Milewich L, Parker CR. (1980). Conversion of plasma progesterone to deoxycorticosterone in men, nonpregnant and pregnant women and adrenalectomized subjects,Journal of clinical investigation, 66, 803-812.
22. Kataria N, Kataria AK. (2007). Compartmental water management of Marwari sheep during dehydration and rehydration. *Veterinarski Arhiv*, 77(6), 551-559.
23. Lee DH, Blomhoff R, Jacobs DR. (2004). Is serum gamma glutamyltransferase a marker of oxidative stress. *Free Radical Research*, 38, 535-539.
24. Sarkar PD, Sogani S. (2013). Evaluation of serum lactate dehydrogenase and gamma glutamyl transferase in preeclamptic pregnancy and its comparison with normal pregnancy in third trimester. *Int J Res Med Sci*, 1, 365-368.
25. Korkmaz V, Ozkaya E, Seven BY, Duzguner S, Karsli MF, Kucukozkan T. (2014). Comparison of oxidative stress in pregnancies with and without first trimester iron supplement: a randomized double-blind controlled trial. *J matern Fetal Neonatal Med*, 27, 1535-1538.
26. Kataria N, Kataria AK. (2012). Use of serum gamma glutamyl transferase as a biomarker of stress and metabolic dysfunctions in Rathi cattle of arid tract in India. *Journal of stress physiology and biochemistry*, 8(3), 23-29.
27. Cheung KL, Lafayette RA. (2013). Renal Physiology of Pregnancy. *Adv Chronic Kidney Dis*, 20, 209-214.
28. Leaf A. (1962). The clinical and physiologic significance of the serum sodium concentration. *The new England journal of medicine*, 267, 77-83.
29. Ermak G, Davies KJ. (2002). Calcium and oxidative stress: from cell signaling to cell death. *Molecular Immunology*, 38, 713-721.
30. Yadav B, Paneri S, Yadav S. (2014). Evaluation of oxidative stress and urinary calcium creatinine ratio in pregnancy induced hypertension. *Global Journal of Medical Research: E Gynecology and Obstetrics*, 14, 1.
31. Walwadkar SD, Suryakar AN, Katkam RV, Kumbar KM, Ankush RD. (2006). Oxidative stressand calcium-phosphorus levels in rheumatoid arthritis. *Indian journal of clinical biochemistry*, 21, 134-137.
32. Hans CP, Chaudhary DP, Bansal DD. (2003). Effect of magnesium supplementation on oxidative stress in alloxanic diabetic rats. *Magnesium Research*, 16, 13-19.
33. Rocha VS, Rosa FBD, Ruano R, Zugaib M, Colli C. (2015). Association between magnesium status, oxidative stress and inflammation in preeclampsia: A case–control study. *Clinical Nutrition*, 34, 1166-1171.
34. Kataria N, Kataria A K, Pandey N, Maan R, Joshi A, Sankhala LN, Chaturvedi M, Pachaury R, Pilania PK. (2012). Use of Serum Magnesium as a Biomarker of Oxidative Stress in Marwari Goat from Arid Tracts in India. *Veterinary Research*, 5(4), 91-96.