RESEARCH ARTICLE Indian Journal of Animal Research, Volume 55 Issue 1: 25-30 (January 2021)

Evaluation of Physico-Chemical and Antioxidant Properties of Dairy Cow, Goat and Buffalo Urine in Two Different Seasons in A Sub-Tropical Region of India

Khushboo[1], Mamta Kumari[1], Neha Kalotra[1], Arup Giri[1,2]

10.18805/ijar.B-3916

ABSTRACT

The present study was conducted to evaluate the physicochemical and anti-oxidative properties of cow, buffalo and goat urine samples in two different successive seasons (summer and winter). For the physicochemical study, pH, electrical conductivity (EC), total dissolved solids (TDS) was measured and for the antioxidant properties, FRAP, DPPH and ABTS level was evaluated by standard methods. The data set were executed through the independent 't' test by using IBM SPSS software version 22.0. The results showed that the EC level in all the (except cow) was significantly ($p<0.5$) lower in the winter season than in the summer season. TDS level in buffalo was significantly ($p<0.5$) lower in the winter season than the summer season but significantly higher in the winter season of the goat urine sample. In the case of antioxidant markers, FRAP and DPPH levels were significantly ($p<0.5$) higher in the summer season in all the animals, but there were no significant changes in the case of ABTS in all the animal's urine samples. From these results, it is concluded that in the study area, the stress in the summer season as heat stress prevails in dairy cows, buffalo and goats. From the overall findings, it may be concluded that urine could be a good biomarker to evaluate the status of livestock health.

Key words: ABTS, Buffalo, Dairy cow, DPPH, FRAP, Goat, Urine.

INTRODUCTION

Urine is the excretory metabolic product through which most of the nitrogenous excreted materials are expelled out from the body through the urethra after the proper urination mechanism (Hall, 2016). The excretion of urine is essential for different bodily functions (Chauhan, 2001). Livestock requires proper management because of the higher stocking density of the livestock. Otherwise, the ammonia of urine may cause the health problem of livestock animals (Kumar, 2013).

In 2007, 6.52% and 7.99% population increased the female cattle and female buffalo above the premature census and the total number of female cattle and buffalo is 122.9 million and 92.5 million in 2012. The total goat population is an exhibit in India is 135.17 million in 2012, whereas the previous census, the goat population, reduced about 3.82% (Bhardwaj *et al.,* 2018). According to Pathak and Kumar, the cow is a mobile medical dispensary and just recently, during the recent research, scientists believe that cow urine is a panacea for most of the diseases (Pathak and Kumar, 2003). Cow urine is the mixture of water 95%, urea 2.5%, minerals, salts, hormones and enzymes 2.5% (Samvadsetu-Goumata, 2010). The amount of healthy gaumutra is 17-45 ml/kg/day, whose specific gravity can be up to 1.025-1.045. Due to weather changes, the pH of cow urine can range from 7.4 to 8.4. The difference between urea nitrogen and total nitrogen is approximately 23-28 ml/kg/day and 40-45 ml/kg/day (Pathak and Kumar, 2003). Urine contains several constituents like sodium, nitrogen, sulfur, vitamins, minerals, manganese, iron, silicon, chlorine, magnesium, citric acid, succinic acid, calcium salts, phosphate, lactose, carbolic acid, enzyme, creatinine and hormone, *etc*. After several biochemical tests, these components have been found in cow urine (Jain *et al.,* 2010).

Cow urine in the Indian tradition is of particular importance. It is said that cow urine also has a spiritual effect. Cow urine has described as the water of life or "Amrita" (beverages of immortality), the nectar of God. The mixture of cow urine, milk, dung, ghee and curd is called Panchgavya (Harshad *et al.,* 2017). Cow urine is used along with herbs to treat various diseases like fever, epilepsy, anemia, abdominal pain, constipation and so forth, by traditional healers all over India (Pathak and Kumar, 2003; Krishnamurthi *et al.,* 2004). Daily use of cow urine, as a result, excreted any malfunctioning of respiratory systems, hepatic gastrointestinal systems, cardiovascular systems and malignant neoplastic disease. It believed that Manu Smriti, Charak Samhita, is an ancient Indian Vedic scripture

[1]Department of Life Science, School of Basic Sciences, Arni University, Kangra-176 401, Himachal Pradesh, India.
[2]Animal Biotechnology Laboratory, DRDO-Defence Institute of High Altitude Research (DIHAR), Chandigarh-160 002, India.

Corresponding Author: Arup Giri, Department of Life Science, School of Basic Sciences, Arni University, Kangra-176 401, Himachal Pradesh, India. Email: arupsatadal@gmail.com

How to cite this article: Khushboo, Kumari, M., Kalotra, N. and Giri, A. (2021). Evaluation of Physico-Chemical and Antioxidant Properties of Dairy Cow, Goat and Buffalo Urine in Two Different Seasons in A Sub-Tropical Region of India. Indian Journal of Animal Research. 55(1): 25-30. DOI: 10.18805/ijar.B-3916.

Submitted: 04-09-2019 **Accepted:** 13-02-2020 **Online:** 17-03-2020

that is cited by current researchers (Achliya *et al.,* 2004). Panchagavya increases the quality and quality of fruits and vegetables in organic crops. Therefore, in the southern parts of India, some farmers use utensils made of Panchagavya (Sugha *et al.,* 2005). Cow urine therapy increases the transport of antibiotics such as Rifampicin tetracycline and ampicillin to 2-7 times, which is near the intestinal wall (Khanuja *et al.,* 2007). Cow urine is an antitoxic activity that is against the cure of cadmium chloride and antioxidant activity against this cadmium chloride toxicity can be used as a binomial of zinc (Randhawa *et al.,* 2007).

Cattle urine has antibacterial activity as well as effective as a fungicide (Virender, 2009). Cow urine distillate serves as a potential tool as an antioxidant reported that the cow urine treated groups of imidacloprid-treated white leghorn cockerels had shown a significant difference in antioxidative parameters as lipid peroxidation and reduced glutathione level as compared to groups without cow urine treatment. Thus, it may be concluded that it serves as a source of antioxidative defense system by either decreasing the level of lipid peroxide generation or by enhancing the glutathione action to counteract the generated peroxide free radicals use of cow urine in today's society is not only for the prevention of dangerous diseases but also in the agricultural and sericulture areas for the antioxidative properties of urine (Tiwari, 2015).

Urine may act as a good biomarker for the health status of livestock animals. No such studies were conducted on the antioxidative properties of dairy cow, buffalo and goat urine. In this context, this study was conducted to determine the physics-chemical and antioxidative properties of dairy cow, buffalo and goat urine, which may establish urine as a strong biomarker to know the health status of livestock animals in Himachal Pradesh, India.

MATERIALS AND METHODS

Ethics statement

It has no requirements for any specific permits to conduct a field study as it is not related to any endangered or protected species. After the agreement of the owner/farmer, we have collected urine samples from dairy cow, buffalo and goat.

Study area and climatic condition

The present study was conducted in the village Badhera, tehsil Haroli, district Una in Himachal Pradesh, a sub-tropical region of India. The sampling sites located in Fig 1. The winter season is long in these regions from October to February. The heat starts from March and lasts till July. Due to the impact of monsoon in this region, it is mostly rain from July to September. It has found that in the winter season, the minimum temperature was 3.5°C and in the summer season, the maximum temperature was 48°C.

Experimental animals and management

Cow and buffalo were put under an iron shed in the summer season, which was open from all sides and in the winter season, cow and buffalo were placed under the roof of the grass, which was closed from all sides except a door. The waste material like urine and cow dung was arranged differently to go out. The experimental animal goat was placed under the roof of the grass pellet, which was half-open and half-closed. After the survey in the village, clinically healthy dairy cow, buffalo and goat were selected for the urine sample collection. Ten urine samples of each animal (30 nos) were collected in each season, which is summer and winter (total 60 nos).

Urine sample collection and physicochemical analysis

Urine sample (100 ml) was collected in a polypropylene bottle from the experimental animals, usually or the following stimulation through massaging the ventral commissural of the vulva. After the sample collection, all the samples were stored at -20°C in the refrigerator for further analysis. pH, electrical conductivity (EC) and TDS were measured immediately after sample collection by the pH meter, EC meter (S358236) and TDS meter (S358236), respectively. All the physico-chemical and biochemical analyses were performed in the Department of Life Science, Arni University, Himachal Pradesh, India.

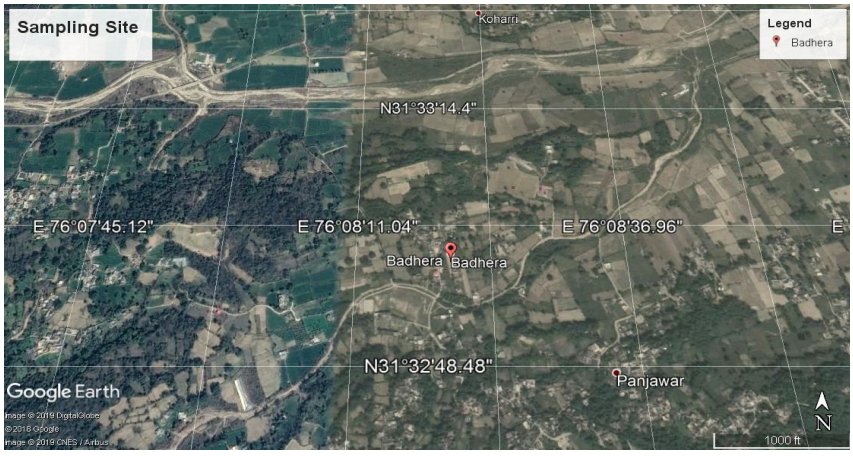


Fig 1: Urine sample collection sites; picture by Google 3D Earth Pro software.

Antioxidant parameter analysis

DPPH (2, 2-diphenyl-1-picrylhydrazyl)

This method used for the detection of total antioxidant status in urine (Abe *et al*., 2000). This assay determines the antioxidant of low molecular weight present in urine. The assay is 765 based on the reduction of relatively stable DPPH radicals and the formation of 1, 1-diphenyl-2-picrylhydrazine. The solution of DPPH (0.2 mM) in methanol was prepared by dissolving 1.9 mg of DPPH in Methanol and volume was made up to 50 ml with methanol. 0.1ml of urine was deproteinated by the addition of 1 ml of methanol, vortexes for 30 s then centrifuged at 3000 RPM for 30 min to separate the proteins. Then, 1.5 ml of methanol and 0.5 ml of DPPH solution were added to the clear supernatant and mixed thoroughly. After that, absorbance was read at nm against the blank, prepared identically, but without the addition of urine by the help of micro titer plate reader (Spectra Max M2e Softmax R Pro 5 serial no. SMP500-16397-KZSS) and concentration of test, samples were determined by plotting the standard curve. For the percentage of absorbance at 517 nm wavelength decrease concerning negative blank. Scavenging activity of DPPH was calculated by using the formula as bellow as %.

Scavenging activity (%) =

$$\frac{\text{Reagent Blank- Sample O.D.}}{\text{Reagent Blank}} \times 100$$

FRAP (Ferric Reducing Ability of Plasma)

The total antioxidant capacity of urine was determined by the ferric reducing ability of plasma (FRAP) assay, as described by Benzie and Strain (1996) with some modification. The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-striazine [Fe (III)- TPTZ] complex to the ferrous 2,4,6-tripyridyl-s-triazine [Fe (II)-TPTZ] complex in the presence of antioxidants, which has an intense blue color, can be monitored by measuring the change in absorption at 593 nm. The standard curve was prepared after using the $FeSO_4.7H_2O$. For the preparation of 1000 µM solution of $FeSO_4.7H_2O$, 13.90 mg $FeSO_4.7H_2O$ was dissolved into 50 ml of water. Five different dilutions (200, 400, 600, 800 and 1 µM) were prepared by using formula.

$$C_1V_1=C_2V_2$$

(C was the concentration of the solution and V was the volume of solution).

ABTS assay

The ABTS assay of our samples was determined by the modified method proposed by Turoli *et al*. (2004). The stock solution of the 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) radical was prepared by dissolving 76.8 mg of ($ABTS^{\bullet+}$) in 20 mL of a sodium persulphate solution (2.45 mM) and the solution was dark stored for overnight. The working solution was obtained by diluting the stock solution of the $ABTS^{\ddagger+}$ radical cation with methanol to obtain an absorbance of 0.7 ± 0.005 at 734 nm. A proper amount of samples (10 mL of urine) was mixed with 1 mL of the $ABTS^{\bullet+}$ working solution for 10 min at room temperature. After centrifuging, (5 min, 8500 rpm) absorbance was measured at 734 nm against the reference sample of methanol by using ELISA plate reader (Spectra Max M2e Softmax R Pro 5 serial no. SMP 500-16397-KZSS). The antioxidant capacity of the biological samples was expressed in milligrams of the Trolox Equivalent (TE) per decilitre of urine based on the calibration curve.

Statistical analysis

Statistical analysis was performed using the IBM Statistical Packages for Social Sciences (SPSS) version 22.0. to determine the level of significance between the two different seasons (summer and winter) of Cow, Buffalo and Goat urine properties. All the data set has been executed for the independent 't' test and the level of significance has been determined at the $p<0.05$ level.

RESULTS AND DISCUSSION

In all the urine samples, the application of t-test statistics showed that the pH value is the same in both the summer and winter seasons. In the case of EC, both goat and buffalo urine samples, it was insignificantly ($p>0.05$) lower in winter season than the summer season, But in the cow urine samples, it was decreased in the summer season than the winter season. In the case of TDS, results showed that TDS were insignificantly ($p>0.05$) lower in winter season than the summer season and in the Goat group, it was insignificantly higher in the winter season (Table 1). Usually,

Table 1: Difference of pH, TDS and EC value in summer and winter season.

Animals	Parameter	Summer	Winter
Cow	pH	8.70 ± 0.08	8.41 ± 0.16
	EC (µs/cm)	81.00 ± 13.55	109.10 ± 12.96*
	TDS ($mg{\cdot}L^{-1}$)	135.50 ± 16.57	117.50 ± 12.98***
Buffalo	pH	8.51 ± 0.11	8.54 ± 0.13
	EC (µs/cm)	114.80 ± 13.05	89.30 ± 8.42***
	TDS ($mg{\cdot}L^{-1}$)	124.00 ± 14.27	90.90 ± 17.02***
Goat	pH	8.41 ± 0.16	8.07 ± 0.08
	EC (µs/cm)	122.10 ± 13.62	109.30 ± 9.77***
	TDS ($mg{\cdot}L^{-1}$)	113.30 ± 18.78	121.30 ± 16.43*

Note: EC-Electrical Conductivity; TDS-Total Dissolves Solids * and *** signifies the difference in a row (*$p<0.05$ and $p<0.01$*).

Table 2: Difference of DPPH, FRAP and ABTS value in summer and winter.

Animals	Parameters	Summer	Winter
Cow	FRAP (µmol/L)	1.35 ± 0.28	0.71 ± 0.02*
	DPPH (%)	94.53 ± 1.36	8.16 ± 0.03***
	ABTS (mg TE/100 cm^3)	33.38 ± 2.39	48.64 ± 1.15
Buffalo	FRAP (µmol/L)	1.25 ± 0.32	0.64 ± 0.04
	DPPH (%)	58.99 ± 0.76	8.75 ± 0.65***
	ABTS (mg TE/100 cm^3)	37.39 ± 0.84	49.73 ± 0.01
Goat	FRAP (µmol/L)	1.29 ± 0.30	0.75 ± 0.01
	DPPH (%)	77.23 ± 12.35	9.00 ± 0.91***
	ABTS (mg TE/100 cm^3)	32.12 ± 5.23	17.43 ± 6.14

Note: Means bearing * and *** signifies the difference in a row (*$p<0.05$ and $p<0.01$*).

in the case of urine, low pH is caused by unbuffered organic acids and is caused by high pH unbuffered ammonium ion. But both the summer and winter season ph value is the same due to organic acids and ammonium ions are normal (Putnam, 1971). The electrical conductivity value in Buffalo and Goat urine show high variability in the summer season due to influences such as stage of lactation, breed milking interval or due to factor related in general cow status (Fazil-Marickar, 2008).

In all the urine samples, the application of t-test statistics showed that DPPH was significantly ($p<0.05$) lower in the winter season than in the summer season. In the case of ABTS, both cow and buffalo urine samples, it was insignificantly ($p>0.05$) lower in (33.38 ± 2.39 mg TE/100 cm^3), (37.39 ± 0.84 mg TE/100 cm^3) summer season than the winter season. But in the goat urine sample, it was insignificantly ($p>0.05$) lower (17.43 ± 6.14 mg TE/100 cm^3) in the winter season than the summer season (Table 2). In the case of FRAP, application of t-test statistics showed that FRAP were significantly ($p<0.05$) lower (0.71 ± 0.02 µmol/L) in winter season than the summer season and in the goat and buffalo group, it was insignificant lower (0.64 ± 0.04 µmol/L), (0.75 ± 0.01 µmol/L) in winter season (Table 2). In the animal body, lead to the production of reactive oxygen species, many types of stress help with cold stress. Despite the formation of ROS in normal cells, its level is also low. The cause of oxidative stress in the cell is the accumulation of ROS which causes many disruptions in the cell and ultimately, the cell goes to die. As a result of this process, many types of health-related diseases were caused and production levels decreased in livestock animals, including cows (Boveris and Chance, 1973; Bharti, 2016; Giri *et al*., 2018). The homeostatic mechanism will create the oxidant / antioxidant system to attract oxygenates stress in the cell. There are several enzymatic and non-enzymatic defense mechanisms in the body to reduce stress. To study the oxidative stress in the animal body, it is necessary to determine the level of oxidative stress and its anti enzyme level (Sahin and Gumuslu, 2004). Therefore, this study reveals the level of FRAP, DPPH and ABTS in livestock urine.

FRAP assay can measure the antioxidant level in the animal body. It contributes to total plasma antioxidants like uric acid, ascorbic acid, vitamin E, bilirubin, albumin, *etc*. Mainly it reduces ferric ions to ferrous ions. Antioxidant capacity by the aqueous phase of plasma is reflected by FRAP concentration (Wood *et al.,* 2003; Yeum *et al.,* 2004). In the present study, FRAP concentration was significantly ($p<0.05$) lower in the winter season than the summer season in urine samples. The mean value of FRAP Concentration in summer and winter is 1.35 ± 0.28 µmol/L (Cow), 1.25 ± 0.32 µmol/L (Buffalo), 1.29 ± 0.30 µmol/L (Goat) and 0.71 ± 0.02 µmol/L (Cow), 0.64 ± 0.04 µmol/L (Buffalo), 0.75 ± 0.01 µmol/L (Goat) respectively (Table 2). The resulted values might be due to the more oxidative stress in the winter season (Charan, 2013; Kumar, 2015; Mann *et al.,* 2016; Giri *et al.,* 2018; Kumar *et al*., 2019).

To measure the activity of radical scavengers and the antioxidant capacity in biological samples, 2, 2-diphenyl-1-picryhydrazyl (DPPH) is a crucial test. DPPH is a stable nitro-gene radical which does not have any similarity in the highly reactive and transient Peroxyl radicals involved in lipid peroxidation (Burda and Oleszek, 2001). In the present study, the scavenging activity of DPPH was significantly ($p<0.05$) lower in the winter season of urine samples. The mean value of DPPH concentration in summer and winter is 94.53 ± 1.36% (Cow), 58.99 ± 5.76% (Buffalo), 77.23 ± 12.35% (Goat) and 8.16 ± 0.033% (Cow), 8.75 ± 0.65% (Buffalo), 9.00 ± 0.91% (Goat) respectively (Table 2). Very harsh conditions characterize the winter season; it can be very effective for the production of free radicals on the body of the animal. As the high level of free radicals, the activity of scavengers will be less (Charan, 2013; Kumar, 2015; Mann *et al.,* 2016; Giri *et al.,* 2018; Kumar *et al*., 2019).

2, 2-azinobis radical cation ($ABTS^+$) is an assay by which not only antioxidant activity measures but also measures the biologically inactive antioxidants in the biological samples (Rice-Evans and Miller, 1995; Salah *et al*., 1995). The mean value of ABTS concentration in summer and winter is 33.38 ± 2.39 mg TE/100 cm^3 (Cow), 37.39 ± 0.84 mg TE/100 cm^3 (Buffalo), 32.12 ± 5.23 mg TE/100 cm^3 (Goat) and 48.64 ± 1.15 mg TE/100 cm^3 (Cow), 49.73 ± 0.01 mg TE/100 cm^3 (Buffalo), 17.43 ± 6.14 mg TE/100 cm^3 (Goat) respectively (Table 2). In this study, insignificantly ($p>0.05$) lower level of the ABTS has been found in the winter

season than the summer season of urine samples. In it might be due to the more oxidative stress in the winter season (Charan, 2013; Kumar, 2015; Mann *et al.,* 2016; Giri *et al.,* 2018; Kumar *et al*., 2019).

CONCLUSION

The study was designed to evaluate the level of stress in livestock animals like cows, goats and buffalo. After study for two different seasons of urine samples for the Physico-chemical and antioxidant properties of cow, goat and buffalo, results showed that during the summer season in District Una, Himachal Pradesh; there is an increased value of antioxidant parameters in cattle, buffalo and goat. DPPH and FRAP levels were significantly ($p<0.05$) higher in the summer season compared to the winter season. Altogether, it may be concluded that heat stress in the summer season prevailed in all the experimental animals. Therefore, in the summer season, microenvironment management should be given the utmost importance to reduce the heat stress in the animal body for better livestock health management and a better production level.

ACKNOWLEDGEMENT

The authors are thankful to Vice-Chancellor of Arni University, Himachal Pradesh, India, for financial support. We are very grateful to all the dairy farmers for allowing and helping us during the sample collection. We are also thankful to Dr. Rajesh Kumar (HOD of Life Science Department) and Mrs. Indu Kumari (Research Associate, Life Science Department) for their continuous support during the entire lab work.

Conflict of Interest

The author(s) declared that there was nothing of potential conflicts of interest with any consequences of this article.

REFERENCES

Abe, N., Nemoto, A., Tsuchiya, Y., Hojo, H. and Hirota, A. (2000). Studies on the, 1-diphenyl-2-picrylhydrazyl radical scavenging mechanism for a 2-pyrone compound. Bioscience, Biotechnology and Biochemistry. 64: 306-313.

Achliya, G.S., Meghre, V.S., Wadodkar, S.G. and Dorle, A.K. (2004). Antimicrobial activity of different fractions of Cow Urine. Indian Journal of Natural Products and Resources. 20: 14-16.

Benzie, I.F. and Strain, J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry. 239: 70-76.

Bhardwaj, J.K., Kumar, V., Saraf, P., Kumari, P. and Mittal, M. (2018). Current status and changing national scenario of goat population: A Review. Agricultural Reviews. 39: 91-103.

Bharti, V.K. (2016). Studies on Sea Buckthorn plant produce as a novel feed additive for animals at high altitude. Defence Research and Developlopment Organization Science Spectrum. 135-142.

Boveris, A. and Chance, B. (1973). The mitochondrial generation of hydrogen peroxide General properties and effect of hyperbaric oxygen. Biochemistry Journal. 134: 707-716.

Burda, S. and Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. Journal of Environmental, Agricultural and Food Chemistry. 49: 2774-2779.

Charan, G. (2013). Studies on certain essential minerals status and heavy metals presents in soil, plant, water and animal at high altitude cold arid environment [Ph.D. Thesis], Waknaghat, Solan, India: JAYPEE University of Information Technology.

Chauhan, R.S., Singh, B.P. and Singhal, L.K. (2001). Immunomodulation with Kamdhenu Ark in mice. Journal of Immunology and Immunopathology. 3: 74-77.

Fazil-Marickar, Y.M. (2008). Electrical conductivity and total dissolved solids in urine. Urological Research. 36: 157-232.

Giri, A., Bharti, V.K., Kalia, S., Raj, T. and Chaurasia, O.P. (2018). Evaluation of antioxidant status in serum and milk of Jersey cross bred in different seasons reared under high altitude stress condition. Biological Rhythm Research. 49: 1-13.

Hall, J.E. (2016). Guyton and Hall Textbook of Medical Physiology (13th edition.). Elsevier. Philadelphia, PA.

Harshad, G., Amit, N., Nilesh, M., Sunanda, B. and Amrut, S. (2017). Gomutra (Cow urine): A multidimensional drug review article. International Journal of Research in Ayurveda and Pharmacy. 8: 1-6.

Jain, N.K., Gupta, V.B., Garg, R. and Silawat, N. (2010). Efficacy of cow urine therapy on various cancer patients in Mandsaur District, India - A survey. International Journal of Green Pharmacy. 4: 29-35.

Khanuja, S.P.S., Kumar, S., Shasany, A.K., Arya, J.S. and Darokar, M.P. (2007). Use of bioactive fraction from cow urine distillate ('Go-mutra') as a bioenhancer of anti-infective, anti-cancer agents and nutrients U.S. Patent US 7235262.

Krishnamurthy, K., Dutta, D., Devi, S.S. and Chakravarty, T. (2004). Protective effect of distillate and redistillate of cow's urine in human polymorpho nuclear leukocytes challenged with established genotoxic chemicals. Biomedical and Environmental Sciences. 17: 57-66.

Kumar, P. (2015). Studies to characterize physio-biochemical, transcriptional and phenotypic parameters in different goat breeds (*Capra hircus L*) exposed to high altitude stress [Ph.D. Thesis]. Coimbatore, India: Bharathiar University.

Kumar, P., Giri, A., Bharti, V.K., Kumar, K. and Chaurasia, O.P. (2019). Evaluation of various biochemical stress markers and morphological traits in different goat breeds at high-altitude environment. Biological Rhythm Research. DOI:10.1080/09291016.2019.1594123.

Kumar, S. (2013). Analysis of cow's urine for detection of lipase activity and anti-microbial properties. International Journal of Pharma and Bio Sciences. 7: 01-08.

Mann, S., Shandilya, U.K., Sodhi, M., Kumar, P., Bharti, V.K., Verma, P., Sharma, A., Mohanty, A. and Mukesh, M. (2016). Determination of antioxidant capacity and free radical scavenging activity of milk from native cows (*Bos indicus*), exotic cows (*Bos taurus*) and Riverine buffaloes (*Bubalus bubalis*) across different lactation stages. International Journal of Dairy Science. 3: 66-70.

Pathak, M.L. and Kumar, A. (2003). Cow praising and importance of Panchagavya as medicine. Sachitra Ayurveda. 5: 56-59.

Putnam, D.F. (1971). Composition and concentrative properties of human urine. National Aeronautics and Space Administration, NASA CR- 1802. http//ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/19710023044.pdf

Randhawa, G.K., Kullar, J.S. and Rajkumar. (2011). Bioenhancers from mother nature and their applicability in modern medicine. International Journal of Applied and Basic Medical Research. 1: 5-10.

Rice-Evans, C.A. and Gopinathan, V. (1995). Oxygen toxicity, free radicals and antioxidants in human disease: biochemical implications in atherosclerosis and the problems of premature neonates. Essays in Biochemistry. 29: 39-63.

Sahin, E. and Gumuslu, S. (2004). Alterations in brain antioxidant status, protein oxidation and lipid peroxidation in response to different stress models. Behavioural Brain Research. 155: 241-248.

Salah, N., Miller, N.J., Paganga, G., Tijburg, L. and Rice-Evans, C.A. (1995). Polyphenolic flavonols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Archives of Biochemistry and Biophysics. 322: 339-346.

Samvadsetu-gaumata. Home page on internet (cited 2017 April 19) Available from-http://samvadsetugaumata.blogspot.in/2010/12/cow-urine-content.html

Sugha, S.K. (2005). Antifungal potential of Panchagavya. Plant Disease Research. 20: 156-158.

Tiwari, S. (2015). Studies on clinic pathological and immuno--pathological effects of induced imidacloprid toxicity in white leghorn cockrels. Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.

Turoli, D., Testolin, G., Zanini, R. and Bellu, R. (2004). Determination of oxidative status in breast and formula milk. Acta Paediatrica. 93: 1569-1574.

Virender, K.J. (2009). Cow urine can cure many diseases. Articles on Ayurveda, Indore Publishers.

Wood, Z.A., Schroder, E., Harris, J.R. and Poole, L.B. (2003). Structure, mechanism and regulation of per-oxiredoxins. Trends in Biochemical Sciences. 28: 32-40.

Yeum, K.J., Russell, R.M., Krinsky, N.I. and Aldini, G. (2004). Biomarkers of antioxidant capacity in the hydrophilic and lipophilic compartments of human plasma. Archives of Biochemistry and Biophysics. 430: 97-103.