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**Ambika Sharma**
Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India

**Rajesh Nigam**
Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India

**Dr. Ashish Kumar**
Ph.D., Scholar, Department of Biochemistry, All India Institute of Medical Sciences, Rishikesh, Uttarakhand, India

**Abhishek Pal**
Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India

**Vaibhav Tomar**
College of Biotechnology, DUVASU, Mathura, Uttar Pradesh, India

**Corresponding Author:**
**Dr. Ashish Kumar**
Ph.D., Scholar, Department of Biochemistry, All India Institute of Medical Sciences, Rishikesh, Uttarakhand, India

# SDS-PAGE analysis of urinary peptides in indigenous cow

**Ambika Sharma, Rajesh Nigam, Ashish Kumar, Abhishek Pal and Vaibhav Tomar**

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**Abstract**
Urinary proteins and peptides are biologically active molecules with diverse structural and antimicrobial properties for combating infections and microbial drug resistance. The aim of this study was to identify the proteins excreted in cow urine. In the present study, fresh urine samples were collected from ten healthy cycling indigenous cows and urinary proteins and peptides were extracted using ion exchange chromatography. Extracted urinary proteins and peptides of healthy cow were analyzed by a combination of SDS-polyacrylamide gel electrophoresis and coomassie brilliant blue stain. We have been able to identify multiple protein bands from cow urine with a molecular weight ranging from 22-153kDa.

**Keywords:** Urinary peptides, cow urine, SDS-PAGE, chromatography

## Introduction
Urine contains proteins and peptides which are either generated in the urinary tract and have specific functions there, or are the filtered or secreted by-products of physiological events taking place in the organism. With the exposure of the urinary tract to a variety of microbes, urine contains antimicrobial peptides which may play a role in local host defence [1, 2]. The urinary proteome is very dynamic and responsive not only to disease conditions but also to seasonality [3]. Because of the emergence of bacterial resistance to antibiotics, extensive search for alternatives to synthetic antibiotics has begun. Antimicrobial peptides represent a promising class of natural antibiotics that has not been extensively exploited yet. AMPs are expressed in many tissues and even urine. Urine of several domestic animals is of therapeutic value in Unani medicine [4] but Go-Mutra (Cow urine) is described as the best of all types of animal urine [5]. In ancient Indian literature, 'Sushrita Samhita' and 'Ashtanga Sangraha', cow urine has been described as the most effective secretion of animal origin with innumerable therapeutic values [6]. Nearly 1550 urinary proteins were identified and profiled from the Karan Fries cows [7]. The antibacterial activity of urinary antimicrobial peptides from cow urine was demonstrated against the *E. Coli* and *Staphylocoocus aureus* by [8]. The aim of the present study was to extract and characterize anionic proteins and peptides from the indigenous cow urine.

## Material and Methods
### Collection and preparation of urine samples
Early morning mid-stream urine was collected from ten healthy cycling indigenous cows from the Instructional Livestock Farm Complex of Veterinary College, Mathura, maintained under the intensive system. Freshly collected urine samples were filtered using 0.2µm membrane filter and diafiltered using 10 kDaAmicon Ultra-4 Centrifugal Filter Unit with the Ultracel-10 membrane (Millipore) by centrifugation at 4000g for 20 minutes.

### Extraction of anionic fractions by ion exchange chromatography
Peptide fractions were extracted from diafiltered urine using weak cation exchanger beads (Macro Prep®CM Resin, BIO-RAD, India) using method described by Valore *et al.* (1998) with slight modification. The anionic fractions were separated by washing the beads with approximately two bed volume of 25mM ammonium acetate(pH-7.5) by centrifugation at

200g for 10 minutes. This step of washing was repeated five times to ensure effective removal of anionic proteins. Protein quantification was done in extracted fractions using Lowry method (Lowry *et al*., 1951).

**SDS-PAGE**

Urine proteins were fractionated by SDS-PAGE using 12.5% resolving gel and 5% stacking gels [9], with a Mini Protean Tetra Cell system (Bio-Rad, USA). A molecular weight marker was run (Bangalore Genei, 250 kDa-10 kDa) along with urine sample. The amount of protein loaded was 5 µg per sample. Gel was stained with Coomassie brilliant blue R-250 staining solution kit (Bio-Rad, USA) and were analysed with a gel documentation system (Bio-rad, USA). The graphic representations and molecular weights of the bands for each lane were obtained using Image J software.

**Results and Discussion**

The aim of this study was to identify the proteins excreted in the urine. Since sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) allows excellent separation of proteins according to their molecular weights [10], this technique was used for analysing the urinary proteins in healthy cows. Although urine is abundant and easy to collect, it is a challenging sample for proteomics studies. The interfering salts and other contaminants can reduce the efficiency of extraction. Obtaining pure protein samples from urine, without sample loss can be difficult and a major impediment to a successful gel-free approach [11]. Numerous protocols have been employed to concentrate and purify urinary proteins; e.g. Lyophilisation [12], precipitation [13], ultracentrifugation [14] and centrifugal filtration [15]. We developed a protocol in our laboratory by combining ultrafiltration and ion exchange chromatography for extracting cow urinary protein in good amount.

The urinary protein concentrations were distributed normally in the extracted anionic fractions. The lowest protein concentration measured was 1.56 µg/µl, the highest, 1.8 µg/µl. SDS-PAGE separated protein primarily on the basis of mass. Cow urinary proteins were subjected to SDS-PAGE and compared with the molecular markers ranged from 250 kDa to 10 kDa. The gels were analyzed in gel documentation system to determine the relative molecular weight of protein bands of sample appeared on the gel. Representative gels and pherograms of anionic fraction of urine samples from cows are reported in Figure 1. We separated 12 protein bands in the urine of cows. The exact molecular weights were 153.4, 84.0, 72.0, 66.6, 62.7, 58.2, 42.6, 38.1, 28.2, 22.1, 18.1 and 16.8. The majority had a molecular weight (MW) between 22 and 75 kDa. Similar study reported in cows found 13±5 protein bands in the urine [16]. Two proteins between 16-18 kDa was also identified but it has been reported that the proteins with molecular weight less than 20 kDa cannot be reliably separated by SDS PAGE [17, 18], therefore we have not considered those bands in our study.

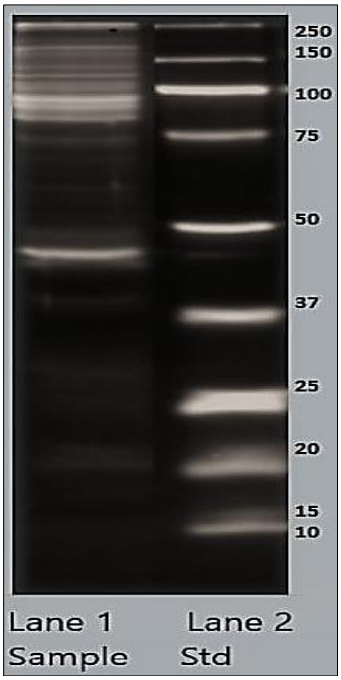


**Fig 1:** SDS-PAGE of anionic fractions of cow urine

For healthy cattle the urinary protein pattern depended neither on the urine sampling technique (catheterization, spontaneous urine) nor on the reproductive stage [17]. In a study with non-pregnant cow urine, three protein bands were found with molecular weights of 67.57, 62.27 and 55.04 kDa [19]. A similar study was carried out in non-pregnant cow urine by [20], where she found bands of 69, 63, 50, 38, 34, 25 and 13 kDa. A 12 kDa molecular weight protein was separated as a single band on SDS-PAGE from the cow urine (Kawamura *et al*., 1990).

By performing urinary electrophoresis, the proteins with different molecular weight could be identified. The mechanism of excretion of high molecular weight proteins (Albumin, Tf, IgG) and proteins smaller than the albumin in urine has been well established as a consequence of which only small amounts of serum proteins are excreted in the urine. The highest percentage of protein found in the urine had molecular weight of approximately 84kDa. Other LMW proteins in the cow urine were at 22, 28, 38, 42 and 58kDa while the HMW protein was at 153 kDa. The 55-65 bands may be α1- antitrypsin while the band near 45 kDamay be heavy chain IgG or IgA (Outteridge, 1985). Analysis of protein precipitated by sodium chloride using SDS-PAGE revealed a prominent high molecular weight band at the level

of 85 kDa (Uromodulin) in coomassie stained gels in Buffaloes [21]. The total protein yielded from SDS-PAGE and expressed as electrophoretic urinary total protein creatinine (E-UTPC) ratio was determined in urine of dogs [22] and they found that the urine proteins are distributed from molecular weight 10 kDa to 80 kDa.

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