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ORIGINAL ARTICLE

Metabolomic and elemental analysis of camel and bovine urine by GC–MS and ICP–MS

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Abstract Recent studies from the author's laboratory indicated that camel urine possesses antiplatelet activity and anti-cancer activity which is not present in bovine urine. The objective of this study is to compare the volatile and elemental components of bovine and camel urine using GC–MS and ICP–MS analysis. We are interested to know the component that performs these biological activities. The freeze dried urine was dissolved in dichloromethane and then derivatization process followed by using BSTFA for GC–MS analysis. Thirty different compounds were analyzed by the derivatization process in full scan mode. For ICP–MS analysis twenty eight important elements were analyzed in both bovine and camel urine. The results of GC–MS and ICP–MS analysis showed marked difference in the urinary metabolites. GC–MS evaluation of camel urine finds a lot of products of metabolism like benzene propanoic acid derivatives, fatty acid derivatives, amino acid derivatives, sugars, prostaglandins and canavanine. Several research reports reveal the metabolomics studies on camel urine but none of them completely reported the pharmacology related metabolomics. The present data of GC–MS suggest and support the previous studies and activities related to camel urine.

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1. Introduction

Urine is produced in mammals by the kidneys. It is transparent, sterile and slight yellowish in nature. The urine contains urea, amino acids, creatinine, organic acids, ammonia, toxins and inorganic salts. All these components are water soluble so they are easily excreted. The medical use of human urine and its extracts has been known for centuries (Armstrong,

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1971; Burzynski et al., 1977). Urine analysis can be performed to study various types of renal disorders like bladder, ovary and kidney diseases. To achieve and identify the polar metabolites of the camel and bovine urine, we widely used the GC–MS technique.

The milk and urine of camel are reported to be useful in treating several diseases (Al-Haider et al., 2011). Camel urine has a lot of chemical constituents which can act as antibacterial, antifungal, antiviral and anticancer agents (Al-Yousef et al., 2012). In the Arabian region, people usually wash their hair with urine. Camel urine is used in Asian countries to treat diabetic neuropathy (Agarwal et al., 2009). Camel milk has been studied for its medical value in relation to chronic diseases like hepatitis (Sharmanov et al., 1982), peptic ulcer (Sharmanov et al., 1981) and food allergies (Shabo et al., 2005). Camel milk also shows antimicrobial and antiviral activities which are mainly due to the presence of lysozyme and lactoferrin, respectively (Ikeda et al., 2000; Benkerroum et al., 2004; Redwan et al., 2014). The published data for camel urine is less and some reports support that it has anticancer and antiplatelet activities.

The therapeutic efficacies from clinical studies of camel urine were investigated by some of researchers (Ohaj, 1993; Ohag, 1998). Profiling from NMR and GC–MS of urinary acids and metabolites is very useful tool in these days. These days, there is an urgent need to explore, identify and characterize the components present in camel urine.

Camel urine showed potent platelet inhibition activity (Al-Haider et al., 2011) and anticancer (Al-Harbi et al., 1996; Al-Kabarity et al., 1987; Al-Haider et al., 2014) activity which is not detectable in bovine and human urine. The aim of the current study was to identify and characterize the camel urine components that contribute to the anticancer and antiplatelet effects. The results and data of this work could be useful in linking the composition of bovine and camel urine with various biological activities.

2. Materials and methods

2.1. GC–MS

2.1.1. Sample collection

Camel urine was obtained from healthy, virgin and lactating domesticated camels (camel dromedaries). All animals were female and aged between 2 and 10 yr. All the animals were raised on a private farm, were disease free and were provided free access to water and feed. Camel urine was obtained during feeding with the help of experienced camel attendants. Approximately 250–300 ml of urine sample from each of the animals was collected. Urine was first collected directly into stainless steel containers and then transferred to suitable glass vials. Urine samples were then carried out to the laboratory and stored at −80 °C till further use. Bovine urine was also collected by the same process.

2.1.2. Sample preparation

Various solvents like ethyl acetate, methanol, chloroform, petroleum ether and dichloromethane (DCM) were selected and tried for extraction of the urinary components. DCM was selected on the observation that it has a low boiling point and most of the urinary components were easily dissolved in DCM and the derivatization reaction was completed within 20 min.

2.1.3. Derivatization

Freeze dried samples were dissolved in a suitable amount of DCM. The samples were transferred to GC vials in an appropriate aprotic solvent such as DCM. Traces of methanol were removed because it could react with the reagent. About 80 μL of BSTFA and 50 μL of pyridine were added to the sample. This amount is enough for a sample containing 100 μg of total derivatizable material and dissolved in 100 μL of solvent.

The vials were capped tightly which were heated at 65 °C for 20 min. The heating step was performed to ensure the completion of reaction. After heating, the samples were allowed to cool down at room temperature and injected on the GC/MS. Derivatized samples were stored in the freezer in order to extend their lifespan. BSTFA is reported to be very corrosive for metal syringe needles and plungers. Therefore, the rinsing solvents were properly labeled and all syringes that come in contact with BSTFA were cleaned thoroughly, first using methanol and then DCM. This also includes the autosampler syringe on the GC/MS. Finally the vials were then tightly screwed and stored in the refrigerator until GC–MS analysis.

The GC–MS analysis was performed in a Perkin Elmer Clarus 600 gas chromatograph linked to a mass spectrometer (Turbomass) available at Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. An aliquot of 2 mL of extract was injected into the Elite-5MS column of 30 m, 0.25 μm film thickness, and 0.25 mm internal diameter.

2.1.4. Capillary column using the following temperature program

The GC–MS system starts with the initial oven temperature of 60 °C for 5 min, increasing to 240 °C at a rate of 15 °C for 5 min, and then to 300 °C at a rate of 15 °C for 5 min. The injector temperature was maintained at 200 °C. The interface temperature was 250 °C. Helium was used as a mobile phase at a flow rate of 1.0 mL/min. Mass spectral detection was carried out in electron ionization mode by scanning at 40–600 (m/z). Finally, unknown compounds were identified by comparing the spectra with that of the National Institute of Standard and Technology library. The total time required for analyzing a single sample was 31 min.

2.1.5. Metabolites detected by GC–MS

The metabolomics study of urine by GC–MS gives a lot of information regarding the volatile products. In this experiment the comparison between the cows (Table 1) and camel urine (Table 2) showed entirely different components depending on the climate, food and categories. A total of 33 detectable peaks were selected for metabolite identification in the National Institute of Standard and Technology (NIST) 2005 Library and Wiley Access Pak v7 May, 2003 library. Approximately 14 components are identified in bovine and 20 components are identified in camel urine on the basis of Match factor. Most of the volatile components are identified are alcohols, alkanes, acids, amines, sugars and ketones.