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OriginalArticle

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# Qualitative and Quantitative Estimation of Keratin in some Sudanese Camel Urine samples

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## ABSTRACT

Five samples of Sudanese camel urines were analyzed separately. Protein of camel urine was hydrolyzed using HCl to extract amino acids. When lead acetate was added, lead sulphide precipitate was formed(1). This proved the presence of amino acids containing sulphur (cysteine and methionine). The ninhydrin test showed the presence of α amino acids. Alkaline Copper sulphate showed the presence of proteins, (Buirt test). The absorbance peak of protein in the UV appeared at 350 nm indicating the presence of Keratin. The presence of keratin was confirmed by TLC technique, where the standard camel urine samples had the same $R_f$ value. The average concentration of Keratin in camel urine was 0.037 M (5500ppm), which indicted that camel urine contained a high concentration of keratin.

**KEY WORDS:** *Qualitative. Quantitative, Keratin, Camel urine*

## INTRODUCTION

Camel milk contains a high amount of minerals(1), like Fe, Ca, and P. Urine of camel can be administered to patients, who complain from liver cancer. Camel urine can, also, be used in increasing the length and strength of hair, for there are lipid glands surrounding hairs. These glands secrete lipids for smoothing the hair, so dry or fatty hair can be found according to the secretions of these glands. Melanin pigment cells were also found around hairs, these pigments provided the colour of hair and depending on genetic factors(2). Proteins are important for growth of hair(3) because they contain amino acids containing sulphur and these amino acids were checked by lead acetate test(4). Lipids provide a silky and a glittering texture to hair.

Besides also they absorb fat soluble vitamins like vitamin A (β carotene) and Vitamin E (Totopherol), and contain essential fatty acids which help the growth of hair[5]. Minerals like iron, silicon, sulphur and manganese which are very important for the growth and strength of hair. Vitamins in general prevent growth of fatty hair; increase the absorption of oxygen in the case of head edema, and make cortisones circulate in the blood stream as to cycle around the hair follicles of edematous tissue[3].

## MATERIALS & METHODS

### Preparation of urine samples

Five samples of camel urine were filtered using filter paper. Then they were transferred into dark flask bottles and kept at room temperature.

### Preparation of standard keratin

A solution of standard keratin was prepared by mixing 60 ml containing 0.015g, and 40 ml of standard keratin. Distilled water and acetic anhydride were added respectively in a cleaned glass beaker. The mixture was transferred quantitatively into a 100 ml volumetric flask.

### Copper sulphate solution (2M)

Copper sulphate solution was prepared by dissolving 16 g of copper sulphate pentahydrate ($CuSO_4.5H_2O$) in a sufficient amount of distilled water, then transferred quantitatively into a 100 ml volumetric flask. The volume was completed to the mark with distilled water. Accurate 10 ml of the prepared solution was taken and poured into a 50 ml glass beaker. Then 2 ml of 1M NaOH was added.

### Lead acetate (2)

A 13.3 grams of lead acetate were weighted, some distilled water was added and the solution was completed to 100 ml in a volumetric flask.10 ml were taken from the solution (using a measuring cylinder) and transferred to a 100 ml volumetric flask. 2 ml of 1M NaOH were added to the solution[6].

### Methodology

### Analysis techniques

### Protein hydrolysis

Camel urine protein was hydrolyzed by taking 1ml of sample, transferring it into a test tube and adding 1ml of conc HCl.

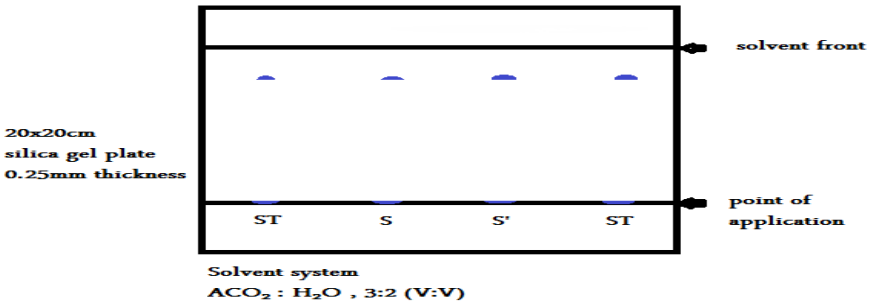
### Ninhydrin test

1ml of camel urine was taken by a measuring cylinder and 1ml of ninhydrin was added. The appearance of a blue colour indicated the presence of amino acids.

### Buiret test

1ml of camel urine was taken by a measuring cylinder. It was transferred into a test tube. 2ml of alkaline copper sulphate was added. The appearance of violet or blue colour showed the presence of proteins in the sample[7].

**Comment [A1]:** This is sheer information ar no reference to it in the discussion or results!




**Thin layer chromatography test**

20X20 cm silica gel plate was used. $ACO_2$ (40 ml) was added to 60 ml distilled water. (This is the solvent system).

Two protein samples of standard keratin (0.01M) were applied at the application point. Two protein samples of camel urine were applied at the application point. The plate was soaked in the solvent system tank and adsorption of standard and sample were left to migrate till they reached the solvent front. The $R_f$ was calculated.

$$R_{fsample} = \frac{Distance\ migrated\ by\ sample}{distance\ migrated\ by\ solvent}$$

$$R_{fstandard} = \frac{Distance\ migrated\ by\ standard}{distance\ migrated\ by\ solvent}$$

$$R_{fsample} = R_{fstandard}$$

This result confirmed that camel urine sample was keratin. The plate was taken from the tank and dried at 100 °C for 10 minutes and ninhydrin solution was sprayed onto the plate. The standard and camel urine sample showed at the same horizontal level.

**Visible spectrophotometer**

When the camel urine sample keratin detected by the spectrophotometer, it read at the spectral wave of keratin, (350nm).

This is confirmed that camel urine sample contained keratin. The spectral analysis showed that the keratin concentration of this sample was (0.037M), which was equivalent to (5500ppm).

**Results and discussion**

**Table1: Qualitative analysis of collected camel urine samples**

| Test name | Result |
|---|---|
| Hydrolysis of protein by HCl | +ve |
| Ninhydrin test | +ve |
| Buiret test | +ve |
| Sulfur Sulphur test (lead acetate test) | +ve |
| Thin layer chromatography | +ve |
| Spectrophotometer | +ve |

Urine of camel contains proteins. This was checked by Biuret test[4]. Hydrolysis of camel urine proteins by concentrated HCl resulted in the formation of amino acids. Amino acids were checked by ninhydrin test and the blue colour appeared[2].

Presence of amino acids containing sulphur were checked by lead acetate test which gave a black precipitate of lead sulphate[6].The sulfur strengthens hair. In addition to the presence of amino acids containing sulphur join together by peptide bond to form keratin.

The keratin is a simple protein which is fibrous protein. It does not dissolve in water and dilute salt solution. Keratin is present in the skin, convective tissue, hair, nails and feathers of birds and blood vessels keratin contains collagen and elastin[3].

Comment [A2]: This clause is irrelevant!

Another type of simple protein is globular protein, which is soluble in water. This protein folds together like a ball. Albumin, globulin and protamins are important in dynamic activity of the cell[2].

is a fibrous protein, in addition to its presence in hair, nails, and feathers. It is also present in meat and fish in a high percentage (59%). The daily intake of keratin is (95%) from meat and about 19 grams from fish. Keratin can be stored in the muscles of the heart, kidney and testes, (5%). The main amino acids of keratin are glycine, arginine and methionine. The α keratin contains amino acid cysteine, which represents a high percentage of the protein, (20%).

Keratin can be separated using TLC (Thin Layer Chromatography) employing the solvent system (ACO2:$H_2O$, 3:2 (v/v)). The locating reagent was ninhydrin solution. 20x20 cm silica gel plate was used to separate the keratin standard and camel urine keratin. They have the same $R_f$ value for they were in the same horizontal line as the silica gel plate. Applying the technique of (TLC) the camel urine of the keratin sample was eluted by

$ACO_2$ and water through silica gel and separated by centrifugation. Then the sample was introduced to the spectrophotometer. The maximum absorbance peak of the sample was 350nm, which is the absorption beak of the standard keratin. Further analysis on the concentration of keratin in the camel urine sample was attempted and was found to be 0.037M that equals (5500ppm), which is a high concentration.

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