RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES
PRINT ISSN 2221-1896, ONLINE ISSN 2223-0343
www.roavs.com

Thin layer chromatography of camel urine

Tarig Hab[1], Samia H. A[2], Baragob A. E. A[3] and Khojali S. M. E[2]

[1]Department of Biochemistry Sharg Alneel College; [2]Central Veterinary Research Laboratory, Khartoum, Sudan; [3]Department of pharmaceutics, Karai University, Omdurman, Sudan

Abstract

General screening of 10 camel urine samples was carried out to determine the most common constituent of camel urine. Samples used were crude, ethanolic and chlorofermic extracts and compared with their lyophilysed urine. The study revealed the presence of alkaloids and triterpene. The objectives of this investigation are to verify camel urine major chemical constituents which are extremely valuable information for detecting new drugs of natural origin.

Introduction

Chromatography is a method used for separation, purification and identification of crude substances that consist of many compounds on the basis of physiochemical and physical methods of separation. In this investigation, lyophilized camel urine and its ethanolic and chloroformic extracts were separated to thin layer chromatography.

The reliable results obtained from clinical trials, hepatoprotein therapeutic and *in vitro* tests using tissue culture cells. It was felt that it is necessary to screen camel urine to know its chemical constituents. In our previous work, it was showed that camel urine and its extracts prevented liver injury induced by carbon tetrachloride through inhibition of increased hepatic transaminases (khojali, 2005).

Camel urine is claimed in our honourable Sunna as a remedy and medicament of a variety of ailments Muslim, (1987) and Bukhari, (1987). Only few studies have been made on the animal urine (Guddum, 1953). However very few previous studies on chemical analysis were reported. Philpot (1970) studied the separation of trinitrophinyl deravitives of camel serum and urine using thin layer chromatography. So an attempt was made to investigate the chemical substance(s) found in camel urine and its extracts.

Material and Methods

Camel urine samples were collected from Gezera and Butana area in Sudan. Crude samples, methanolic and chloroformic extracts were used. 1gram of the lyophilized urine of camel was boiled with 100 ml of 80% of ethanol for ½ an hour. The cold solution was filtered and enough ethanol was passed through the volume of filtrate and kept as stock solution.

5 ml of the stock solution was evaporated to dryness on the water bath and the cooled residue was stirred several times with petroleum ether. The residue was then extracted with 20 ml of chloroform. The chloroformic solution was dehydrated over sodium sulphate anhydrous.

5 ml portion of the chloroformic solution was mixed with 0.5 ml of acetic unhydride followed by 2 drops of conc. Sulphuric acid. The gradual appearance of green, blue pink to purple colour was taken as an evidence of the presence of sterols (green to blue) and/or triterpenes (pink to purple).

7.5 ml of the prepared extract was evaporated to dryness on a water bath 5% of 2N HCl was added and stirred while heating on the water both for 10 minutes, cooled filtered and divided into two test tubes.

To one test tube few drops of Mayer's reagent was added while to the other tube, few drops of Valger's reagent was added. A slight turbidity or heavy precipitate in either of the two test tubes was taken as presumptive evidence for the presence of alkaloids.

Mayer's reagent (1.3g of mercuric chloride were dissolved in 60 ml of water, and 5g of potassium sulphite (Ks) in 10ml of water. The two solutions are mixed and diluted to 100 ml with distilled water.

Thin layer (0.1-0.5 mm) of the grained silica gel (kessel gellG; 30 gram) was dissolved in 60 ml of D.W, spread on 20x20 cm carrier plate made of glass. 1-5µl of sample was applied as spots and the stating line about 10-15 mm from the bottom edge of the plate.

Calculation: R_F = Distance traveled by sample / Distance traveled by solved

Results

Ethanolic extracts of lypholysed urine, chlorofomic extract, protein precipitate and their positive reactions to unsaturated sterols, triterpenes and alkaloids were represented in table 1.

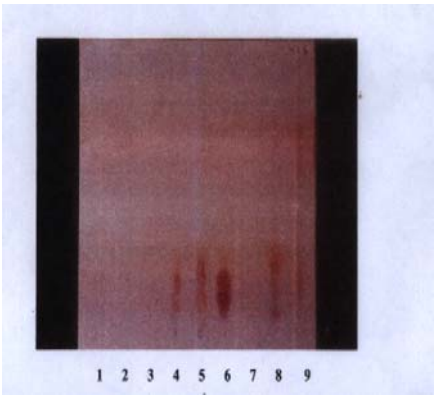

Fig. 1: Thin layer chromatography of crude urine ethanolic and chloroformic extracts of camel urine by using BAW system at ratio of 4:1:5

Thin layer chromatography of butanolic chloroformic, methanolic and ethanolic extract was shown in Figure 1, using BAW system at a ratio 4:1:5. The spots appeared in the figure indicates that the different extracts used, which contain the resulted compound are similar.

The chloroformic extract and crude urine were hydrolyzed by 6N Hcl then chromatographed on silica gel using propanol and water 70:30 one spot of each was read with R_F 0.81 and 0.79 respectively. As shown in figure 2 where the migration of the compound was found in both chloroformic extract and crude urine.

The chloroformic extract chromatogram on using methanol in ammonia water 100:1.5 (two plates) revealed one spot of orange colour after dried off reagent and potassium iodinate vapour as shown in Fig.3. The orange colour indicates that there is alkaloid substance.

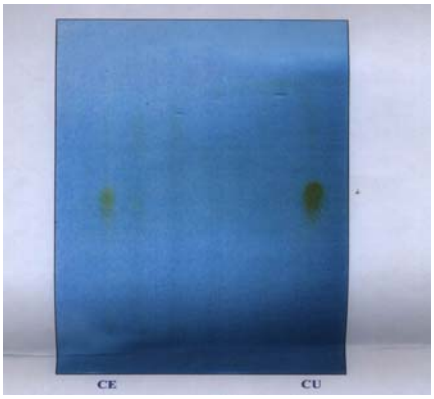

Fig. 2: Chromatogram of chloroformic extract (E) and crude camel urine (CU) treated with 6 (NHCL).

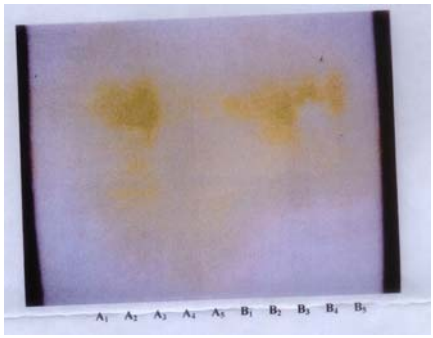

Fig. 3: Dragndoff detection of alkaloid in camel urine and chloroformic extract. Spot represent chloroformic erxtract of camel urine dragndoff reagent revealed positive reaction (orange colour).

Discussion

No previous screening was available for camel urine on the bases of TLC results. We could say that chloroformic, ethanolic and crude urine deprocess alkaloid materials which was similar to the observations obtained by umbeto et al. (1987) using HpLC analysis of liver homogenate in Zebo cattle. Wasfi et al. (1998) found flunixin metabolite in camel urine. Much more investigation should be done to purify and identify these competent.

Table 1: Represent the positive tested groups and yield recoveries of the different urine extracts

Treatment	Yield recovery	Test		
		Unsatislered	Trilopene	Alkaloids
Ethanolic extract	80 ml	-	+ (purple+ pink colour aradd off	+ (Mayer's reagent)
Chloroformic extract	0.5 gm	-	-	+ minhydrium and (Diagudoff reagents
Protein precipitate	0.003 gm	-	-	+

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