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Studies for Urokinase-type Plasminogen Activator Isolated from Camel Urine and its Effect on Lipid profile

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ABSTRACT

Urokinase-type plasminogen activator (uPA) was isolated from the camel urine. One proteinous band had been isolated by gel filtration sephadex (G-100) and DEAE-Cellulose ion exchange from the proteinous supernatant produced by ammonium sulfate saturation (65%) after dialysis. The apparent molecular weight of the isolated uPA using gel filtration chromatography (55246 $\pm$ 350) Dalton and using SDS-PAGE giving two band (31002) and (24200) Dalton respectively.

Study the protective effect of uPA against atherosclerosis by measuring of lipid profile (Total cholesterol, HDL-C, LDL-C, VLDL-C and triglyceride) by intraperitoneal injection of 21 male albino white rats classified into 3 groups. The rats were treated with 0.5 mg/kg/day (Group II) and 1mg/kg/day (Group III) of isolated uPA injected intraperitonealy for 5 successive days. The results showed a significant reduction ($p \leq 0.05$) in the levels of total cholesterol, LDL-C, VLDL-C and triglyceride in comparison with the normal group (Group I) and significantly elevated in HDL-C for the group II and group III in comparison with the group I.

Conclusion, the uPA isolated from camel urine, its may be has pharmacological role of heart protection against lipids.

Keywords: Isolation, Urokinase-type plasminogen activator, Camel urine, Lipid profile.

دراسة لإنزيم اليوروكاينيز نوع البلازمينوجين المنشط المعزول من بول الإبل وتأثيره على صورة الدهن الكاملة

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الملخص

عزل إنزيم اليوروكاينيز نوع البلازمينوجين المنشط (uPA) من بول الابل حيث تم فصل حزمة بروتينية واحدة بتقنية الترشيح الهلامي سيفادكس نوع G-100 للراسب البروتيني الناتج من عملية الترسيب بكبريتات الامونيوم (65%) بعد عملية الديلزة، واعطت حزمة بروتينية واحدة ايضا عند امراها على عمود التبادل الايوني نوع DEAE-Cellulose، بعدها قدر الوزن الجزيئي للإنزيم باستخدام تقنيتي الترشيح الهلامي والهجرة الكهربائية نوع SDS-PAGE ووجدت أنها بحدود (55246 ± ٣٢٠) دالتون وتقنية SDS-PAGE اعطى حزمتين ذات اوزان جزيئية (٣١٠٠٢) و(٢٤٢٠٠) دالتون على التوالي.

درس تأثير إنزيم uPA ضد تصلب الشرايين بواسطة قياس صورة الدم الكاملة من الكوليستيرول الكلي والبروتين الدهني عالي الكثافة (HDL-C) والبروتين الدهني واطئ الكثافة (LDL-C) والبروتين الدهني واطئ الكثافة جدا (VLDL-C) والكليسرايد الثلاثي (TG)، اذ تم

اختيار ٢١ من ذكور الجرذان البيض والتي قسمت الى ٣ مجاميع، إذ أشارت النتائج إلى إن أعطاء إنزيم uPA المعزول وبجرعة (٠.٥ ملغم /كغم (للمجموعة II) و١ ملغم /كغم يوميا عن طريق البريتون (للمجموعة III) للجرذان لمدة خمسة ايام ، أدى ذلك إلى انخفاض معنوي بمستويات الكوليستيرول الكلي و LDL-C وVLDL-C وTG مقارنة بمستوياتها عند مجموعة الجرذان الطبيعية (المجموعة I)، وزيادة معنوية في مستوى HDL-C لمجاميع (II و III) مقارنة مع مجموعة I . استنتج أن الإنزيم uPA المعزول من بول الابل له يمكن ان يمتلك دور عقاقيري في حماية القلب من الدهون .

الكلمات الدالة: عزل إنزيم اليوروكاينيز نوع البلازمينوجين المنشط، بول الابل، صورة الدهن الكاملة.

INTRODUCTION

Urokinase (trade name Abbokinase), also called urokinase-type plasminogen activator (uPA), is a serine protease (EC 3.4.21.73) converts the inactive proenzyme, plasminogen into the active protease, plasmin (Figure 1)[1]. Plasminogen (PA) has been found in most animal tissues and fluids, and can be produced by normal and tumor cells in culture[2, 3]. PA has been classified into two types of PA, urokinase-like activator (uPA) and tissue plasminogen activator (t-PA). Urokinase was originally isolated from human urine, but is present in several physiological locations, such as blood stream and the extracellular matrix. The primary physiological substrate is plasminogen, which is an inactive zymogen form of the serine protease plasmin. Activation of plasmin triggers a proteolysis cascade that, depending on the physiological environment, participates in thrombolysis or extracellular matrix degradation. This links urokinase to vascular diseases and cancer [2].

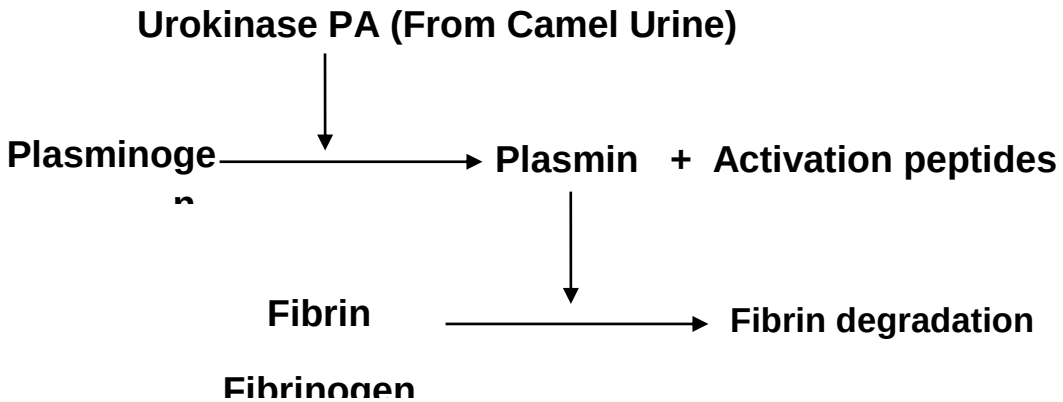

Figure 1: Schematic representation of uPA in fibrinolytic system for blood clot dissolution.

Plasminogen activators play a role, not only in fibrinolysis but also in events such as chemotaxis, collagen degradation, and cell spreading[4] . Urokinase is used clinically as a thrombolytic agent in the treatment of severe or massive deep venous thrombosis, pulmonary embolism, myocardial infarction, and occluded intravenous or dialysis cannulas. It is also administered intrapleurally to improve the drainage of complicated pleural effusions and empyemas. Increased expression of fibrinolytic system components and imbalance between plasminogen activators and its inhibitors (PAI) may be involved in the pathogenesis of severe allergic conjunctivitis, thus contributing to inflammatory cell migration and tissue remodeling [5] .

Cardiovascular diseases have become one of the biggest concerns all over the world[6]. Among these, thrombosis is the most widespread within the elderly population. The disease results from severe blood-clotting, which leads to obstruction of the blood flow circulation. In the physiological state, fibrin and platelets are utilized for clotting to prevent blood loss from injuries in a process called hemostasis [7].

Urine is not a waste product, but a purified sterile by-product of blood filtration, medically referred to as plasma ultra filtrate made by kidneys. It is rather an extraordinary valuable physiological substance [8].

It has been shown throughout the history of medical science till today that urine has a profound medical uses [8], such as effectiveness against allergies, psoriasis and all skin problems. Also Natalie (2002)[9] reported the effect of urine on fertility, fever, burns and tuberculosis. Camel urine, also a natural product, has been used traditionally in the treatment of many diseases in Arabic countries. Drinking camel urine was shown to be effective in treating numerous cancer cases[10] . Camel owners used its urine for treatment of various diseases, such as fasciolosis[11] and for correcting disorders in general, particularly hepatitis[9], Camel urine is efficacious in treatment of skin diseases such as ringworm, tinea and abscesses, sores that may appear on the body and ulcers [12]. Furthermore, it has been proved in vivo and vitro experiments using early camel pregnant urine acts effectively as hepatoprotective[13]. The objective of this study was to isolate uPA from camel urine and to study the effect of enzyme on lipid profile.

MATERIALS AND METHODS

Determination of urokinase activity with Chromogenic S-2444

The urokinase activity is determined by its amidolytic effect on the chromogenic substrate pyro-Glu-Gly-Arg-pNA (Chromogenic substrate S-2444). The rate at which p-nitroaniline (pNA) is released is measured photometrically at 405 nm.

This can be followed on a recorder (initial rate method) or read after stopping the reaction with acetic acid (Acid stopped method). The release of p-nitroaniline was measured at 37°C and 405 nm. A molar extinction coefficient of 10500 1/mol/cm was used for *p*-nitroaniline[14, 15].

Purification of uPA from camel urine

The method given here has yielded enzyme preparation acceptable camel urine. All steps were performed at 4 ^{o}C unless stated otherwise.

Step I: Collection of camel urine

Eight samples of camel urine were aseptically collected from Al-Khderr region located in the south of the Nenvia government, Iraq (Figure 2). The camel's urine samples is extracted from female an male camel with age around 4-5 years, early in the morning. The surface of the bladder was swabbed with 70% alcohol before five mL amounts of urine were transferred aseptically into sterile bijous bottles, kept in insulated boxes using freezing packs, and transferred to the laboratory and centrifuged immediately at 6000 rpm for 5 min [16].

Figure 2: Type of camel used in the research.

Step II: Ammonium sulfate fractionation

A supernatant was obtained from the camel urine and protein was precipitated using 65% ammonium sulfate $(NH_4)_2SO_4$ saturation (Protein salting out) [17, 18].

Step III: Cooling ultracentrifuge separation

The suspension was centrifuged at 9000 Xg for (45) min at -4°C. The protein in precipitate and supernatant are determined using the modified Lowry method[19], uPA activity determined in each fraction [14].

Step IV: Dialysis

Dialysis was made using a semi permeable cellophane dialysis membrane with M.wt. cut off (<10000) Dalton. The dialysis sac containing the suspension in (Step III) was dialyzed against (0.1M sodium phosphate, pH 7.0 contained 0.1% EDTA) was stirred with a magnetic stirrer overnight at 4 ^{o}C . The solution of dialysis was changed three times only per 3 hours during dialysis for 24 hour [20]. The protein of the dialyzed enzyme was estimated by modified Lowry method [19] and uPA activity determined [14].

Step V: Gel filtration chromatography using Sephadex G-100

The sephadex gel G-100 supplied as a powder was suspended in adequate distilled water so that when it was stirred incorporated air bubbles that escape rapidly to the surface [18, 21]. It was then allowed to swell for 3 hours at 90 ^{o}C in a complete swelling. This procedure was used for column packing and sample application. In the present study, the column of dimension (2.0 × 125) cm which contained a gel sephadex height of (120) cm. The exclusion limit for sephadex G-100 is (150000) [20]. A concentrated sample (5) mL of the protein material (by freeze-dryer technique), which was obtained in (Step IV), was applied on the top of a bed sephadex G-100 followed by 0.1 M sodium phosphate, pH 7.0 .

Elution of the protein materials was carried out at a flow rate for G-100 a flow rate (62) mL/ hour with a definite time (5) min. was used 0.1 M sodium phosphate, pH 7.0 as eluant. The fractions were collected by using a fraction collector apparatus which was worked on minute system. The protein compounds in each fraction collected were detected by following the absorbance at wave length (280) nm by using UV/Visible Spectrophotometer. Peak was combined separately from the plot of an

absorbance versus elution volumes with determined of uPA in each fraction[14, 22].

Step VI: Freeze-dryer (Lyophilization) technique

The enzyme fraction which was obtained from gel filtration was dried using a freeze-dryer (Lyophilization) technique to obtain a powder or a concentrated protein. The enzyme was kepted in a deep freeze at -20^{o}C in a tight sample tube to be used in further investigations.

Step VII: Ion-Exchange Chromatography

A concentrated sample (10) mL of the enzyme fractions, which was prepared in step (VI), was applied to the top of a column (3 × 60) cm which contained DEAE-Cellulose (Diethyl amino ethane – cellulose) anion exchange to (55) cm height, which has been equilibrated with gradient sodium phosphate buffer (50-250) mM of pH=7 fractions of (5)mL volume were collected. Flow rate was approximately 72 mL/hr. The enzyme in each fraction collected was detected by following the absorbance at wave length (280) nm by using UV/Visible Spectrophotometer. Peak was combined separately from the plot of an absorbance versus elution volumes with determined of uPA in each fraction [14, 22].

Step VIII: Electrophoresis

Electrophoresis is the movement of charged molecules in an electric field. It is a rapid and often employed technique for the determination of the molecular weight of protein and for the separation of biological molecules such as nucleic acid, nucleotides, amino acids and protein [20]. Step VI which was applied on Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) using slab electrophoresis unit quick fit instrumentation[23].

Animals and Experimental protocol

Twenty one white Albino rats males, weighing 200- 300 gm were used in this study; they were obtained from and maintained in the Animal House of the Veterinary College, University of Mosul under conditions of controlled temperature. Rodent food rich in nutrient and tap water were used as bedding. The animals were divided into five groups of seven animals and treated as follow: **Group I**- received single intraperitoneal(IP) dose of normal saline, this group served as a negative control . **Group II and Group III** - received single IP dose of uPA isolated alone (0.5 mg and 1mg /kg/day respectively) for 5 successive days . Animals have been anesthetized by ether, blood was collected directly from orbital sinus puncture (Intraocularly) and poured into plain tubes[24], the clot was dispersed with glass rod and then centrifuged at 3000 X g for 15 minute ; the serum was used within 2 days for the estimation of total cholesterol [25], high density lipoprotein(HDL)[26], low density lipoprotein (LDL) [27], very low density lipoprotein (VLDL)[28] and triglyceride (TG)[29] by using standard kits (BioLab). The data presented as Mean $\pm$ SD. The significance of differences between the mean values were calculated using ANOVA test. p-values equal or less than 0.05 were considered to be significantly different[30].

RESULTS AND DISCUSSION

Urokinase was identified in human urine more than 60 years ago[31] and has been purified from this source for use as a thrombolytic agent[32, 33]. Most efforts he pharmaceutical industry in this area have been targeted at increasing yields of uPA isolated. Camel's urine (CU) can be classified as environmentally friendly inhibitor, because microbiological study on CU proved its high efficiency against a number of pathogenic microbes when compared with some antibiotics. Camel urine possesses potent antiplatelet activity, not found in human or bovine urines, suggesting a possible role for camel urine in inhibiting platelet function[34]. Moreover, the effective constituent of CU was isolated and tested as anticancer agent which is labeled as PM 701[35]. Alhaider *et al*. examined the ability of three different camel urine samples (virgin, lactating, and pregnant sources) to modulate a well-known cancer-

activating enzyme, cytochrome P450 in the murine hepatoma Hepa 1c1c7 cell line([10]). Camel urine, milk and meat have been used for medicinal purposes in many countries. The medicinal properties of camel products were known to Arab physicians centuries ago[36]. Early in the sixteenth century one of most well-known medicinal encyclopaedias in China recorded in detail the medicinal value of camel products [37]. This traditional knowledge has been respected, improved, and applied in modern medical practice. A large number of studies have been conducted into the medicinal value of camel products. Because of there is no information available on the uPA purification from camel urine and effect of uPA on lipid profile.

Enzyme purification

The results predicted that the enzyme activity was found in the (65%) of a saturation ammonium sulfate precipitates for Camel urine. As shown in Table (1), the specific activity was increased after dialysis and giving four folds of purification. This might be due to the removal of the small molecules (Below 14000 Dalton) and increasing the purification of uPA.

Fibrinolytic enzymes (for example uPA) were identified and studied among many organisms including snakes, earthworms, and bacteria: *Streptococcus pyogenes*, *Aeromonas hydrophila*, *B. natto*, *Bacillus amyloliquefacens*, Actinomycetes and fungi: *Fusarium oxysporum*; *Mucor sp, Armillaria mellea* [38]. Fibrinolytic enzymes can be found in a variety of foods, such as Japanese Natto, Tofuyo, Korean Chungkook- Jang soy sauce and edible honey mushroom. For uPA induction, preferable compounds are saccharides such as glucose, inositol, ribose and deoxyribose, hormones such as adrenaline[39, 40].

Gel filtration separations

This technique was applied to separate the protein as a source of enzyme, which was obtained after dialysis and by using a column containing sephadex G-100 gel as shown in (step V). The result (Figure 3) indicated that there was mainly one peak . The elution volume of peak

was (180.4) mL. The specific activity of the enzyme peak was (282621.6 U/mg protein) and 11 folds of purification compared to initial extract Table (1). Usually UK production by mammalian cells depends on the following factors: (i) regulation of UK expression (ii) supply of amino acid building blocks for UK synthesis. Moreover some amino acids like glycine have been known to bring about stabilization of proteins [41], while arginine is known to induce UK by acting as precursor of nitric oxide, which induces UK production [42].

Table 1: Partial purification steps of urokinase (uPA) from the camel urine.

Purification stage	Volume taken (mL)	Protein conc. (mg/mL)	Activity (U*/mL)	Total activity (U)	Sp. activity (U/mg protein)	Folds of Purification	Recovery %
Camel urine	500	0.08	2099	1049500	26237.5	1	100
Precipitate by $(NH_4)_2SO_4$(65 %)	55.5	0.162	9956	552558	61456.8	2	52.7
Dialysis	62.3	0.083	8351	520267	100614.5	4	49.6
Sephadex G-100 (Fractions)Peak	45	0.037	10457	470565	282621.6	11	44.8
DEAE-cellose (Fractions) Peak A	27	0.021	15785	426195	751666.7	29	40.6

U*: a mount of urokinase (uPA) catalyzing the formation of one micromole of product per min under optimum conditions.

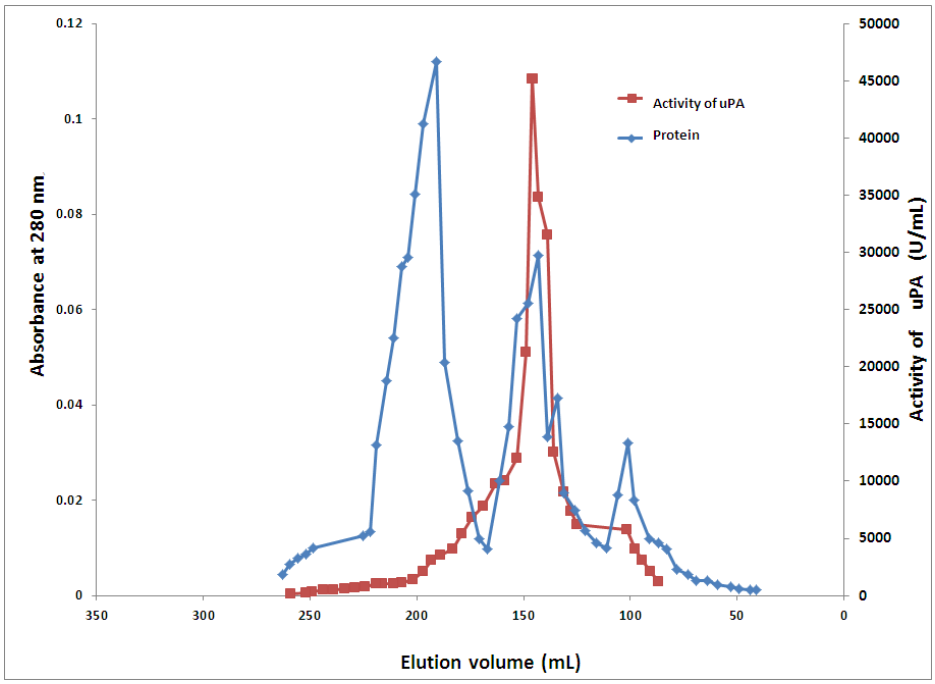

Figure 3: Elution profile of uPA for camel urine on sephadex G-100.

Ion-Exchange Chromatography

Selective adsorption and elution of proteins from the polydextran derivatives anion exchange diethylaminoethyl (DEAE-Sephadex) has also been extremely successful for extensive and rapid purification. Figure (4) explains the elution profile of purified uPA by ion exchange chromatography. I obtained a one peak at elution volume (150-300) mL with a specific activity (751666.7) U/mg protein and (29) folds of purification.

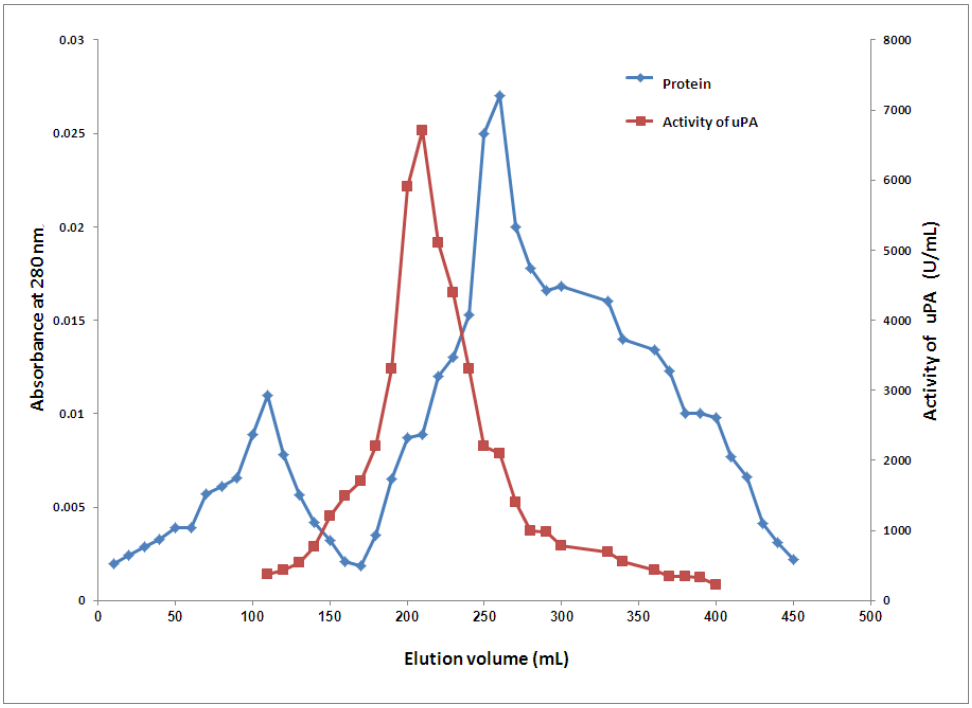

Figure 4: Purification of uPA by DEAE-cellulose chromatography.

Urokinase(UK) is secreted from cells as a single chain proenzyme (scu-PA) from which the active two chain enzymatic plasminogen activator (tcu-PA) is derived by proteolysis, the two chains remaining linked by a disulphide bond. The UK that is used clinically is tcu-PA type. UK is enzymic and acts directly as a plasminogen activator and it is not antigenic. The amino terminal fragment of urokinase-type plasminogen activator (u-PA) is 130 amino acid residues long. It consists of 2-α-helicles and two antiparallel strands[(3)].

Molecular weight determination of uPA by gel filtration

The molecular weight of peak as a source of uPA was determined by gel filtration chromatography using sephadex G-100 column (2 × 125) cm calibrated with known molecular weight proteins that were listed in Table (2).

Table 2: Elution volumes of known molecular weight materials on sephadex G-100.

Materials	Molecular weight (Dalton)	Elution volume (mL)
Blue dextran (Void volume(V_o))	2000000	138.5
Bovine serum albumin	67000	143.2
Egg albumin	45000	188.3
Pepsin	36000	288.3
Papain	23000	320.5
Tryptophan (Internal volume(V_i))	204	446.4
Unknown (peak A)	55246	169*

*This value was obtained from Figure (3).

A plot of logarithmic molecular weight of each material versus the elution volumes indicated in Table (2) gives a straight line as illustrated in Figure (5).

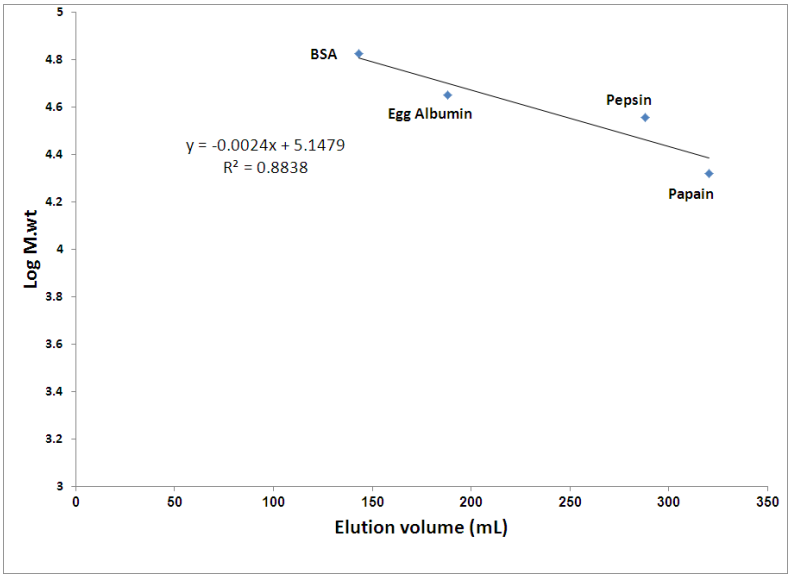

Figure 5: A plot of the logarithm molecular weights of known proteins versus elution volume on a sephadex G-100.

The molecular weight of unknown protein compound separated by the column chromatography as shown in (step V) was determined from the standard curve, which was represented by Figure (5). The comparative molecular weight of peak as a source of uPA is approximately equal to (55246 $\pm$ 320) Dalton.

It had been known uPA could be found in multiple molecular sizes. There appeared to be two major forms: low molecular weight (33000 Dalton) and high molecular weight (57000 Dalton)[3] and the M.wt. for uPA equal (54000) Dalton have been isolated from plasma [43, 44] that are similar of urinary urokinase. Approximately 25% of the urinary urokinase-related antigen represents a single-chain molecule with M.wt. 54000 Dalton[45] and M.wt.= 54000 Dalton from cultured human endothelial cell indistinguishable from urinary urokinase[46]. this result were in a agreement with other reported results for Ravindra Vijay *et al.*, result appeared 59000 Dalton in Cow urine [18].

Molecular weight determination by SDS-PAGE

The electrophoretic mobility of uPA in SDS gels, the enzyme migrated as two bands in camel urine only as shown in Figure (6) with an apparent molecular weights (31002) and (24200) Dalton respectively was determined by using known molecular weight compounds as shown in Figure (7).

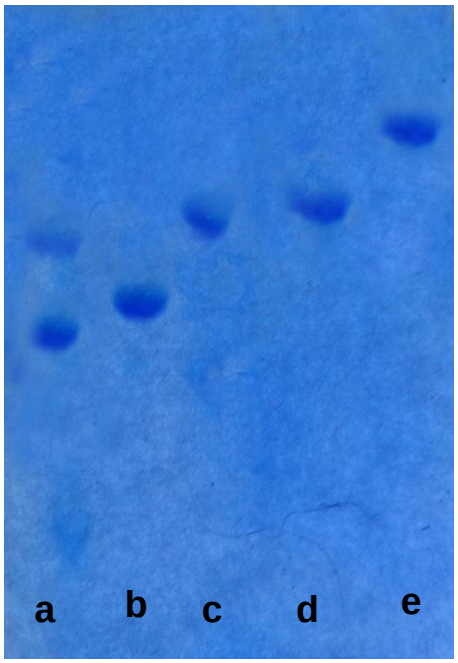

Figure 6: Protein patterns obtained by SDS gel electrophoresis. The tubes from left to right contained (50)µg of standard protein employed to calibrate the columns were:

a. uPA separated. **b.** Papain (M. wt. 21000). **c.** Pepsin (M. wt. 36000). **d.** Egg albumin (M. wt. 45000). **e.** Bovine serum albumin (BSA) (M. wt. 67000).

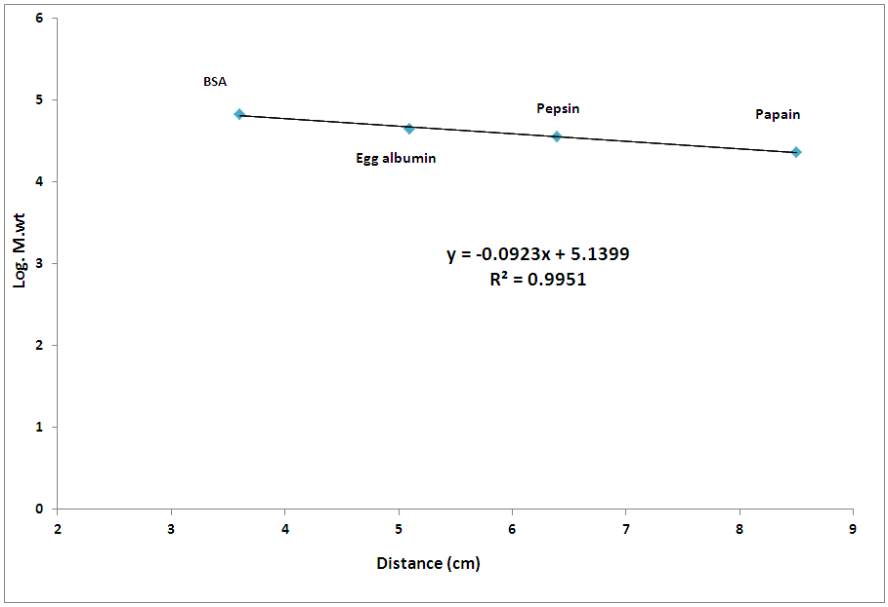

Figure 7: Calibration plot for molecular weight estimation of uPA by (SDS) gel electrophoresis using known molecular weight proteins.

To confirm the purity of the enzyme, SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed and a two band was observed at approximately M.wt (31002) and (24200) Dalton respectively this result were in a agreement with other reported results for Soberano *et al*. the 47 000 M.wt. form had two chains (33100 and 18600) Dalton linked by disulfide bonds[47], and uPA, first identified in human urine[43] is a two-chain polypeptide (34000 and 20000) Dalton with a single disulfide bridge.

The effect of uPA isolate on lipid profile

The results effect uPA isolation on the serum levels of cholesterol, HDL-C, LDL-C, VLDL-C and TG for the treated animals were listed in Table (3).

Table 3 : The effect of uPA isolation on the serum levels of cholesterol, HDL-C, LDL-C, VLDL-C and TG for the treated animals .

Groups N=7 each group	Control (Normal saline) (Group I)	uPA(0.5 mg/kg) (Group II)	uPA(1 mg/kg) (Group II)
Total cholesterol (mg/100mL)	119 ± 11.8 **C**	99.2 ± 10.2 **B**	88.8 ± 9.5 **a**
HDL-C (mg/100mL)	41.35 ± 3.44 **A**	49.89 ± 2.5 **B**	51.81 ± 3.7 **c**
LDL-C (mg/100mL)	76.11 ± 9.21 **C**	59.33 ± 7.74 **B**	49.78 ± 7.9 **a**
VLDL-C (mg/100mL)	7.89 ±01.46 **B**	6.73 ± 1.02 **A**	6.55 ± 1.33 **a**
TG (mg/100mL)	39.37 ± 4.41 **B**	32.22 ± 5.3 **A**	29.1 ± 3.3 **a**

- Each value represents Mean $\pm$ SD.
- Different letters horizontally a, b, c indicate that the mean are different significantly at $P \leq 0.05$.

The results indicated that serum levels of total cholesterol, LDL-C, VLDL-C and TG were significantly reduce ($p \leq 0.05$) in the group II and group III in comparison with the group I, and significantly elevated ($p \leq 0.05$) in HDL-C in the group II and group III in comparison with the group I.

The decreased level of total cholesterol and triglycerides in groups (II and III) compared to control group (Group I) may be explicated by reducing activities of fat splitting enzymes such as, lecithin: Cholesterol acetyltransferase (LCAT) and lipoprotein lipase. LCAT is responsible for

an esterification of free cholesterol in plasma, and it indirectly controls the levels of free cholesterol in various cells and tissues. Lipoprotein lipase is the clearing factor for triglyceride in plasma and cleaves triglycerides into free fatty acids and glycerol [48].

Serum total cholesterol and serum LDL-C significant decreased with increased uPA dose in treated animals which can be explained by the work of Zhang and his colleagues who showed that although uPA was involved in the release and disaggregation of LDL in macrophages as the resulted plasmin is protected from the action of the serum inhibitors, it doesn't cause degradation of native (Monomeric) LDL owing to limited expression of LDL receptor on macrophages [49, 50]. However, some investigators suggested that over expression of Apo A in mice (a component of HDL) increases susceptibility to diet-induced atherosclerosis by decreasing cell associated plasminogen activation of the vessel wall (١).

Low-density lipoproteins (LDL) are the most atherogenic type of lipoproteins both in plasma and in the vessel wall[51] and retention and aggregation of LDL (agLDL) in the arterial intima, facilitated by the proteoglycans that conform the extracellular matrix, are key events in atherosclerotic plaque formation[52, 53].

A study reported a novel role for uPA in decreasing cholesterol biosynthesis in human macrophages through the mevalonate pathway, via induction of 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) by intracellular signals induced by uPA[54]. The mevalonate pathway contributes highly to cholesterol biosynthesis, and HMGCR is a rate-limiting enzyme in the pathway.

Since, the reduction in HDL cholesterol is mostly compensated by an increment in LDL cholesterol, and vice versa[55]. The decreased synthesis of very low density lipoprotein-cholesterol observed in groups II and III could have led to the decreased triglyceride levels in animals.

Low-density lipoproteins (LDLs) are the most atherogenic type of lipoproteins both in plasma and in the vessel wall[56] and retention and aggregation of LDL (agLDL) in the arterial intima, facilitated by the proteoglycans of the extracellular matrix,[53, 57] are key events in atherosclerotic plaque formation[58] .

uPA is a multi-functional multi-domain protein. Besides its relevant role in fibrinolysis, uPA is also associated with several acute and chronic pathological conditions including vascular disease[54, 59] . Different studies in human and in animal models have suggested that uPA plays a role in the initiation and development of the atherosclerosis[60] . As proved by other investigators who showed that both uPA and its receptors have been detected in human atherosclerotic lesions[61] and uPA decreases the removal of HDL in the liver[62].

Conclusions: Camel urine has potent anti atherosclerosis by uPA isolated and improved lipid profile.

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