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Esterase Polymorphism in Camel (*Camelus dromedarius*) Tissues and Comparison with other Mammalian Species

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Abstract

The activity of carboxyl esterase (CE) and the distribution of esterase isozymes in camel, cow, goat, rabbit, rat, and sheep tissue aqueous extracts were examined using methyl butyrate (MB) as substrate for measuring activity and non denaturant polyacrylamide gel stain after electrophoresis (PAGE) using α-naphthyl acetate (NA) as the substrate. The order of esterase activity was liver > lung > kidney > third stomach > intestine > spleen > heart > plasma > red blood cells. Camel tissues: heart, intestine, kidney, liver, lung, spleen, and stomach exhibited esterase specific activity (ESA) for MB, in µmol/min/g wet tissue, as: 0.67, 2.28, 4.61, 146.0, 29.2, 1.18, and 2.58, respectively. The esterase activity in these tissue extracts exhibited on the gel 3, 5, 1, 3, 2, 3, and 6 bands, respectively. Camel RBC and blood plasma showed no activity in gel electrophoresis. However, camel blood plasma yielded ESA of 0.42 using MB as substrate. Therefore it is concluded that camel serum albumin completely lacks serum esterase-like activity. The range of ESA values in tissue extracts of cow, goat, rabbit, rat, and sheep are as lowest to highest: Heart, 2.26 in goat to 3.75 in rabbit; intestine, 1.46 in cow to 12.8 in rat; kidney, 1.45 in cow to 14.6 in rabbit; liver, 73.4 in cow to 343 in rabbit; lung, 1.35 in goat to 14.6 in rat; spleen, 2.54 in cow to 3.44 in rat; stomach, 1.37 in cow to 3.13 in goat; RBC, 0 in all; plasma, 0.64 in cow to 3.99 in rat. The number of activity bands on the gel by each tissue ranged from one for cow kidney and spleen to nine for sheep intestine and stomach.

Keyworda: Camel esterase, camel esterase polymorphism, esterase polymorphism, mammalian esterase, mammalian esterase polymorphism, esterase distribution in mammalian tissues.

1. Introduction

Mammalian esterases are known to exhibit an extensive heterogeneity and they belong to the enzymes class hydrolases (EC.3). They hydrolyze simple and mixed ester bonds (Walker and Mackness, 1983). In studied mammalian systems, the highest esterase activity is found in liver (Satoh *et al.*, 2002). Also esterase activity has been found in rat kidney (Tsujita *et al.*, 1988), submandibular glands (Khullar *et al.*, 1986), intestine (Swell *et al.*, 1950), testis (Deimling *et al.*, 1985), skin (Prusakiewicz *et al.*, 2006), heart, muscle, and blood (Satoh *et al.*, 1998). Also, esterases were found in human breast (Banerjee *et al.*, 1991), serum (Tsujita *et al.*, 1979), erythrocytes (Lee *et al.*, 1986; Okuda and Wakabayashi, 1988), gallbladder (Kouroumalis *et al.*, 1984), intestine (Khanna *et al*., 2000), saliva (Finer and Santerre, 2004), and colon (Sanghani *et al.*, 2003). Mice esterases are found in liver, lung (Oehm *et al.*, 1982), kidney (Göppinger *et al.*, 1978), testis (Deimling *et al.*, 1985), and adipose tissue (Soni *et al*., 2004). The level of activity in the tissues varies according to the type of esterase. For example, human caboxylesterase-1, CE-1, (or hCE-1) activity is found in the order, liver>> heart> stomach> testis = kidney = spleen> colon; while hCE-2 is found in the order, liver> small intestine> colon> heart (Satoh *et al*., 2002). In addition to the type of esterase, the distribution order of esterases differs from one mammal to another. For example, in ox the level of activity was found in the order as liver, epididymis, lung, and kidney, while in sheep the order was liver, intestine, and epididymis (Holmes and Masters, 1968). Furthermore, esterases are present in various subcellular organelles. Mammalian CE is localized in ER lumen and lysosome (Satoh *et al.*, 2002; Tanaka *et al*., 1987). Other esterases are destined for export into the blood plasma; some others are present in the cytosol such as brain CE (Satoh and Hosokawa, 1998). Other esterases are associated with the cell membrane with its catalytic function directed extracellularly. Mentlein *et al.* (1988) found the non-specific esterases in subcellular fractions of rat liver. It was found that the relative levels of these enzymes were present in the cellular organelles and in the order of: smooth ER> rough ER> Golgi vesicles> plasma membrane> nuclei> lysosomes> peroxisomes> mitochondria> cytoplasm.

Furthermore, in a mammalian cell, esterases are localized in the endoplasmic reticulum (ER) and the cytosol of many tissues (Satoh et al., 1998). They are involved in the hydrolysis of a wide variety of endogenous

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and xenobiotic compounds and play an important role in drug metabolism (Redinbo and Potter, 2005). Little is known about the physiological roles of different esterase isoenzymes (Satoh *et al*., 1998). Nevertheless, esterases have been used as markers in a variety of population genetics and developmental genetic studies (Hart and Cook, 1976). Industrially, esterases are used in detergents, clarifying optically active materials, and reducing pitch problems during paper manufacturing process (Panda and Gowrishankar, 2005).

Esterases have been purified from several mammalian tissues. Carboxylesterase (CE) isoenzymes were purified from liver of camel (Ahmad and Abuerreish, 2011), liver mouse, hamster, guinea pig, rabbit, and monkey (Hosokawa *et al.*, 1990). An esterase called esterase 2B was isolated from mouse kidney (Lexow *et al*., 1980). Cholesterol esterase had been purified to homogeneity from porcine pancreas (Rudd *et al*., 1987). Prior to this study, no esterase polymorphism has been reported in camel tissues. However, it has been shown that camel has unique system among the mammals, for example, the immunoglobulin in the camel does not have light chains (Muyldermans, 2001). It may be worth mentioning that camel is considered as vanishing species. Therefore, it is important to define the biochemical system of this animal, and it is thought that esterase(s) from camel may contribute to the understanding of this aspect. In addition, studying camel esterases may be useful in certain aspects like evolution and veterinary sciences.

The aims of this study are identification of the distribution of esterase activity in various camel tissues and its comparison to the distribution in tissues of other mammals.

2. Materials and Methods

2.1. Materials

Chemicals- Bovine serum albumin, Fast Blue RR, sodium dodecyl sulfate (SDS), and triton X-100 were obtained from British Drug House (BDH), Chemicals Ltd., Poole, UK. Acrylamide, bisacrylamide (N, N`-methylenediacrylamide), glycine, and trichluoroacetic acid (TCA) were obtained from Merck, Darmstodt, Germany. Hercules, USA. Aminoantipyrine, chromotropic acid, Coomassie brilliant blue G-250 (CBB) were from Fluka, USA.. Tetramethylethylenediamine (TEMED) and trihydroxymethyl-amino-methane (Tris) were obtained from Riedel-de Haën, AG. Bromophenol blue was obtained from Matheson Coleman and Bell (MC & B), Division of the Matheson Company, Ohio, USA. Methyl butyrate was obtained from M and B, England. α-Naphthyl acetate was obtained from Peking Chemical Works, China. All other chemicals were analytical grades, and double distilled water was used throughout.

Instruments- Motor-driven glass-teflon tissue grinder (Griffin and George Company, Great Britain). pH meter (Pye Unicam Model 290 MK2, UK). Power supply (Power Pac Universal, Bio-Rad, USA). Refrigerated centrifuge (Martin Christ II, Germany). Vertical Electrophoresis Cell (Protean II xi cell, BioRad, USA). Water circulator and cooler LAUDA R3, Scientific technical Supplies, Germany. UV-9200 spectrophotometer, Biotech Engineering Management Company, UK.

2.2. Methods

2.2.1. Collection of Mammalian Tissues

Tissues of mammals, camel, cow, goat, sheep (Order *Artiodactyla*), rabbit (Order *Lagomorpha*), and rat (Order *Rodentia*) were obtained immediately after killing the animals. The tissues were washed with cold saline solution and cut into small pieces. If not used immediately, the tissue pieces were packed into plastic bags and stored at –26˚C until use (Bashir, 1981; Abu-Harfeel and Abuerreish, 1984; Ahmad, 2008; and Ahmad and Abuerreish, 2011). The blood was collected in tubes containing EDTA as anticoagulant and was kept in ice.

2.2.2. Preparation of tissue crude extract, blood plasma, and red blood cells (RBC) lysate:

The tissues (heart, intestine, kidney, liver, lung, spleen, and third stomach) was homogenized in Tris/HCl buffer (10 mM), pH 8, in 1:1 ratio (w:v) with intermittent cooling. The homogenate was centrifuged at 12,000 rpm, for 30 min at 4 °C, in Martin Christ II refrigerated centrifuge. Blood was centrifuged at 12,000 rpm for 20 min at 4 °C. The plasma (supernatant) was separated from the RBCs in the pellet. The RBCs lysate was obtained by suspending the cells into an equivalent volume of cold double distilled water (Ahmad, 2008; and Ahmad and Abuerreish, 2011).

2.2.3. Protein assay

Protein concentration was determined according to Bradford (1976) using bovine serum albumin (BSA) as standard and Coomassie Brilliant Blue as protein-binding dye in which the light absorbance was measured at 590 nm using 1-cm light path cuvette.

2.2.4. Esterase assays

The esterase assay was adopted from the method of Tsujita and Okuda (1983). Samples were incubated at 37°C in water bath with Tris/HCl buffer (10 mM, pH 8). Methyl butyrate in Tris buffer (20 mM total concentration) was added to the sample to initiate the reaction. The reaction mixture (0.5 mL) was incubated for an additional time period (this was within the linear range of the reaction, depending on the sample source) and then was stopped with 0.5 mL of cold 10 % (w/v) TCA. The mixture was centrifuged at 8000rpm for 5 min to remove denatured proteins. $KMnO_4$ solution (0.1 mL of 2 %, w/v) was added to 0.5 mL of the above supernatant and incubated for 5 min at room temperature. After that, 0.1 mL of 10 % (w/v) $NaHSO_3$ was added followed by addition of 4 mL chromotropic acid (0.4 g/ 5 mL water with 95mL of 76% (v/v) H_2SO_4) and boiling for 15 min in water bath. Finally, the mixture was cooled and was read at 570 nm light wavelength. One unit of the enzyme activity (U) is defined as the amount of the enzyme that produces 1µmole of methanol per minute under the conditions of the assay.

2.2.5. Localization of esterase activity on polyacrylamide gel

Native polyacrylamide gel electrophoresis (PAGE), 7.5%, using α-naphthyl acetate as substrate and Fast Blue RR was performed according to published methods (Tsujita and Okuda, 1983; Pond *et al.*, 1998; Kouroumalis *et al.*, 1984).

PAGE was applied (Weber and Osborn, 1969; Laemmli, 1970; Welner *et al.*, 1972) as follows: The sample was electrophoresed in the gel (2 mm x 16 cm x 20 cm) at constant voltage of 100 V, at 4 °C, for 12 h. After electrophoresis, the gel was removed from the plate and immersed in 400 mL Tris/HCl buffer containing 1 mM $CaCl_2$ for 5 min. The gel was stained for esterase activity by adding α-naphthyl-acetate, 5mM final concentration in the reaction mixture (the substrate was dissolved in acetone) of 0.05 M Tris/HCl buffer (pH 7.4) containing 0.05% (w/v) Fast Blue RR. Staining continued to develop the bands.

Distaining the Gel- The excess stain in the gel was removed by immersing it in distaining solution containing methanol: acetic acid: water as 5:1:5, respectively.

3. Results

3.1. Esterase activity and its isoenzymes distribution in tissues of camel and other mammals

The homogenates of mammalian tissues were prepared as described in methods. Esterase activity was monitored using methyl butyrate (see methods). The enzyme activity in the tissues of camel, cow, goat, rabbit, rat and sheep is presented in Table 1. This activity represents total activity of combined isoenzymes.

The activity in these species was lowest in RBC lysate ~ 0 (activity was not detected under the conditions of the assay). Table 2 summarizes the esterase activity in the tissues of the animals used in order of its abundance as shown in the left column. The order of the enzyme activity relative to its abundance in the animals is given horizontally. For example, in liver: rabbit > goat > sheep > camel > rat > cow which showed the enzyme specific activity, in μmol / min/ g wet tissue, 343, 245, 184, 146, 108, and 73, respectively.

The profile of activity among isoenzymes towards α-naphthyl acetate on PAGE is shown in Figures 1-9. The number of phenotypes (isoenzymes) in camel, cow, goat, rabbit, rat, and sheep are, respectively, 12, 11, 15, 18, 14, and 16. The names of tissues are abbreviated as given in the legend of Figure 1.

Table 3 shows the number of bands and their migration order. The bands are numbered according to their anodic (+) migration. The slowest band among the nine profiles, Figure 1 to Figure 9, is assigned number one. The fastest anodic band among the profiles is assigned number 18 which is the maximum number of total phenotypes (variants) found in these mammalian species. Since most of the plasma proteins are synthesized in the liver, one would not expect to have all the genetic phenotypes present in the plasma as observed in Figure 7.

Table 1. Esterase activity in the mammalian tissues. Tissue homogenization and crude extract preparation were as described in "Methods". Methyl butyrate was the substrate in the assay. The incubation times were 4 min for liver, 30 min for kidney, lung, plasma, spleen, heart, RBC lysates, and intestine, and 50 min for stomach. One unit of enzyme activity is equal one μmole methanol produced / min / g tissue under the conditions of the assay.

	Specific activity of esterase, units / g wet tissue					
	Camel	Cow	Goat	Rabbit	Rat	Sheep
Heart	0.67±0.16	2.35±0.00	2.26±0.00	3.75±0.39	2.45±0.04	2.36±0.41
Intestine	2.28± 0.00	1.46±0.10	3.63±0.00	7.42±0.43	12.8±2.11	4.60±0.55
Kidney	4.61± 0.87	1.45±0.35	7.08±0.25	14.6±1.25	2.78±0.23	1.92±0.01
Liver	146±7.10	73.4±10.5	245±20.0	343±19.5	108±21.2	184±0.00
Lung	29.2± 0.74	3.06±0.16	1.35±0.40	14.2±3.20	14.6±0.00	2.56±0.08
Plasma	0.42 ± 0.00	0.64±0.20	1.64±0.05	0.85±0.11	3.99±0.82	1.37±0.0
RBC*	0.00± 0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.0±0.0	0.00±0.00
Spleen	1.18± 0.12	2.54±0.13	3.20±0.15	2.88±0.00	3.44±0.72	2.54±0.28
Stomach	2.58 ±0.1	1.37±0.14	3.13 ±0.21	2.21±0.65	1.61±0.30	1.72±0.00

* RBC lysate. Statistical analysis- Data averaging was performed on six tissue samples of each animal. The standard deviation (±) was calculated from a computer-run program.

Table 2. Esterase specific activity in tissues of mammals that were studied in this work. The tissues are arranged vertically according to their highest enzyme activity, followed by the arrangement of the animals that have the highest to lowest values, μmol / min /g wet tissue, horizontally.

Tissue						
1. Liver	R	> G	> S	> Ca	> Ra	> Co
	343	245	184	**146**	108	73
2. Lung	Ca	> Ra	> R	> Co	> S	> G
	29	14.6	14.2	3.0	2.56	1.35
3. Kidney	R	> G	> Ca	> Ra	> S	> Co
	14.6	7.08	**4.61**	2.78	1.92	1.45
4. Intestine	Ra	> R	> S	> G	> Ca	> Co
	12.8	7.42	4.60	3.63	**2.28**	1.46
5. Plasma	Ra	> G	> S	> R	> Co	> Ca
	3.99	1.64	1.37	0.85	0.6.4	**0.42**
6. Heart	R	> Ra	> S	> Co	> G	> Ca
	3.75	2.45	2.36	2.35	2.26	**0.67**
7. Spleen	Ra	> G	> R	> S	= Co	> Ca
	3.44	3.20	2.88	2.54	2.54	**1.18**
8. Stomach	G	> Ca	> R	> S	> Ra	> Co
	3.13	**2.58**	2.21	1.72	1.61	1.37
9. RBC	nd*	nd	nd	nd	nd	nd

* not detected. Ca, Co, G, R, Ra, and S stand for camel, cow, goat, rabbit, rat, and sheep, respectively.

Table 3. Summary of migration order of esterase isoenzymes in the gel as shown in figures

			8, (10–11)		(13-15)	
LE	(3,4,6)	(4,5,10)	(5,7,8,10,12)	(3,10,11,12)	(1,2) (8–13)	(1,3,6)
LuE	(4,7)	(1,4,8,9)	(1,6,7)	(1,3,7,9)	(2,4,11,13)	(2,5,6,8,10)
RbE	-	(3,4)	2	4	1	4
PE	-	(3,4,5)	(2,6)	8	(1,2,10)	2,(3–6),9,10
SpE	(3,5,6)	5	(4–6)	1,(4–6)	(6–8)	(2,3,5,7)
StE	(7,8),(10–13)	(13,15,17)	2,(5–7) (11,12,15,16)	(1,3,4),(8,9) (12,13,15,16)	(5,12,14,16, 18,19)	(9,10,12, 15,18)

* TE is Tissue Esterase. Abbreviations are for Heart, Intestine, Kidney, Liver, Lung, Red blood cells, blood Plasma, Spleen, and Stomach, respectively.

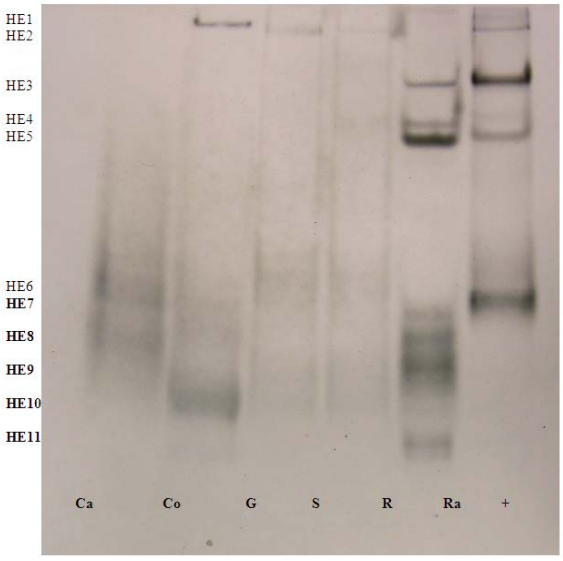

Figure 1- Heart esterase (HE) isoenzymes in mammals: (Ca), Camel, 0.5 mg protein. (Co), Cow, 0.5 mg protein. (S), Sheep, 0.29 mg protein. (G), Goat, 0.14 mg protein. (R), Rabbit, 0.14 mg protein. (Ra), Rat, 0.14 mg protein.

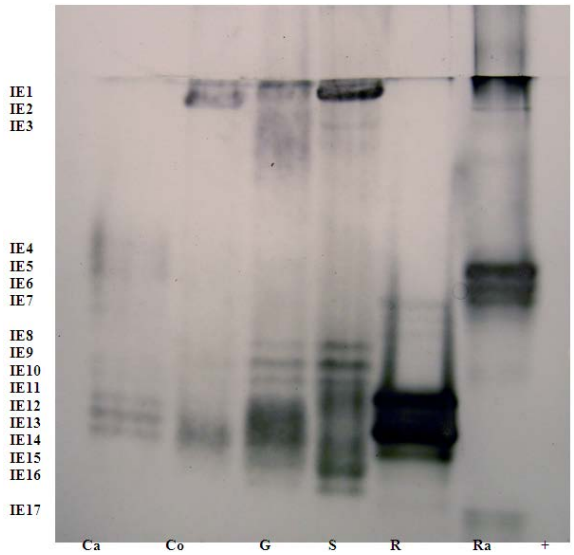

Figure 2- Intestinal esterase (IE) isoenzymes in mammals: (Ca), Camel, 0.3 mg. (Co), Cow, 0.3 mg. (S) Sheep, 0.3 mg. (G), Goat, 0.3 mg. (R), Rabbit, 0.1 mg. (Ra), Rat, 0.1 mg.

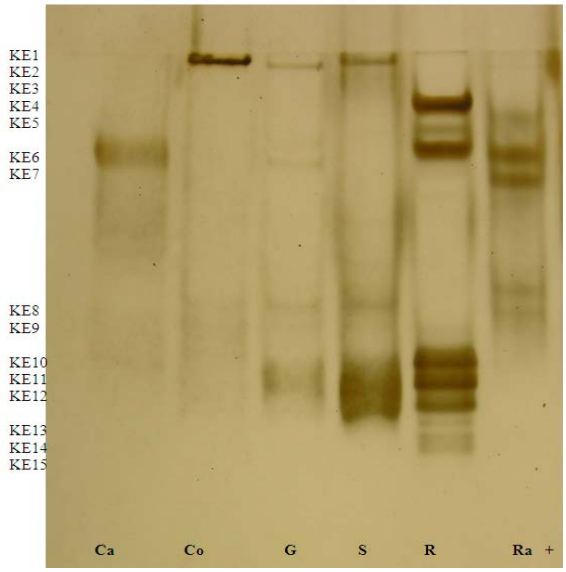

Figure 3- Kidney esterase (KE) isoenzymes profiles of the mammals: Each lane contained 0.4 mg protein.

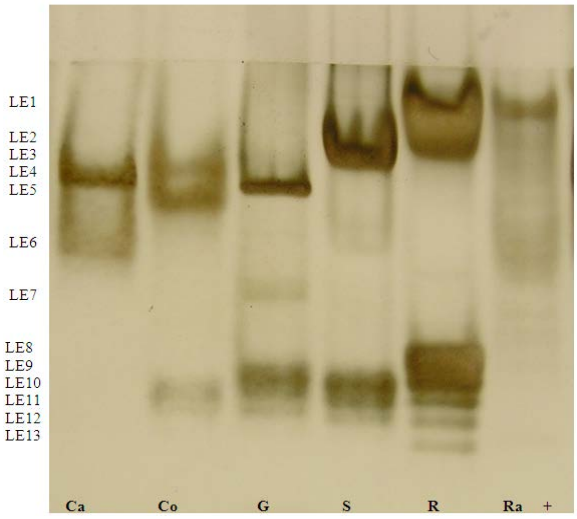

Figure 4- Profiles of liver esterase (LE) isoenzymes of mammalian species: Each lane had 0.1 mg 0.1 mg protein

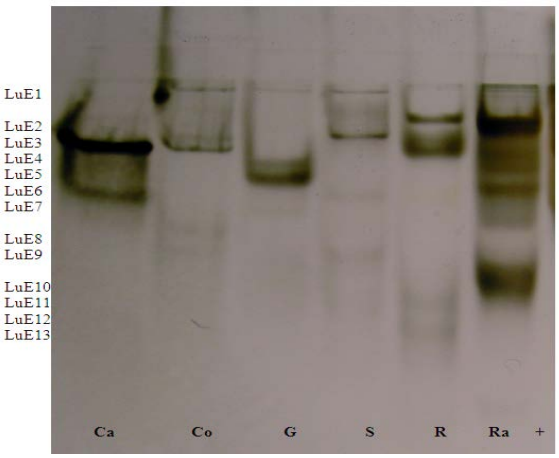

Figure 5- Profile of lung esterase (LuE) isoenzymes of mammalian species: Each lane in the gel contained 0.2 mg protein. Abbreviations of the animal species are the same of those in the previous figures.

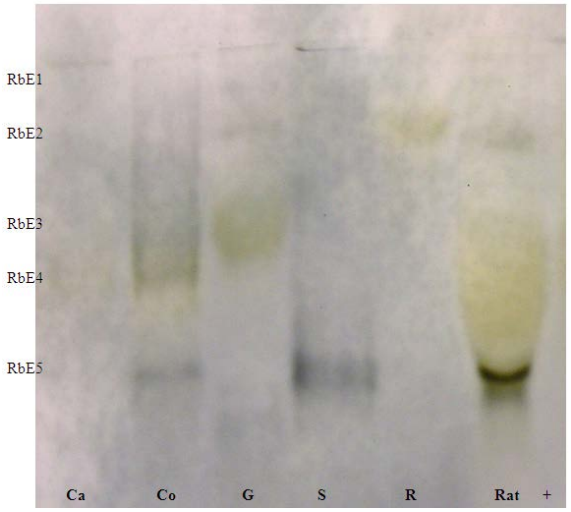

Figure 6- RBC esterase isoenzymes (RbE) in mammalian species: Each lane contained 0.5 mg protein applied to the gel. The abbreviations are the same of those in the previous figures.

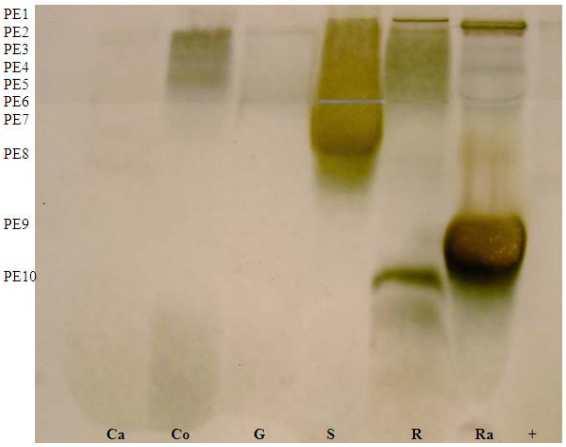

Figure 7- Blood Plasma esterase (PE) isoenzymes of mammalian species: (Ca), Camel, 1.89 mg protein. (Co), Cow, 2.33 mg. protein. (S), Sheep, 1.72 mg protein. (G), Goat, 0.55 mg protein. (R), Rabbit, 0.76 mg protein. (Rat), Ra, 0.82 mg protein.

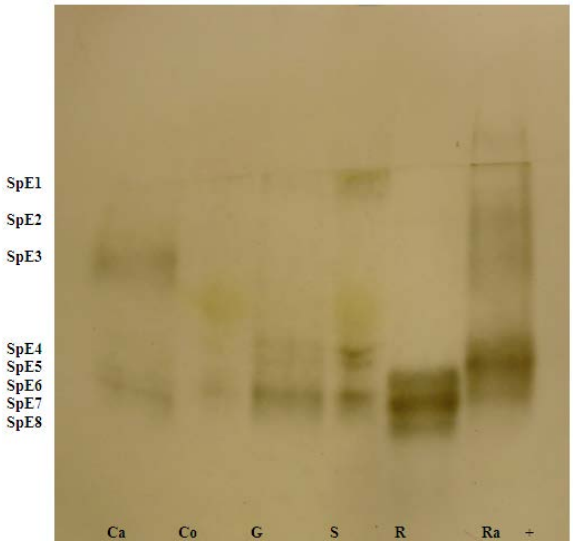

Figure 8- Spleen esterase (SpE) isoenzymes of mammalian species:(Ca), Camel 0.3 mg protein. (Co), Cow 1.04 mg protein. (S), Sheep 0.9 mg protein. (G), Goat 0.45 mg protein. (R), Rabbit 0.46 mg protein. (Ra), Rat 0.4 mg protein.

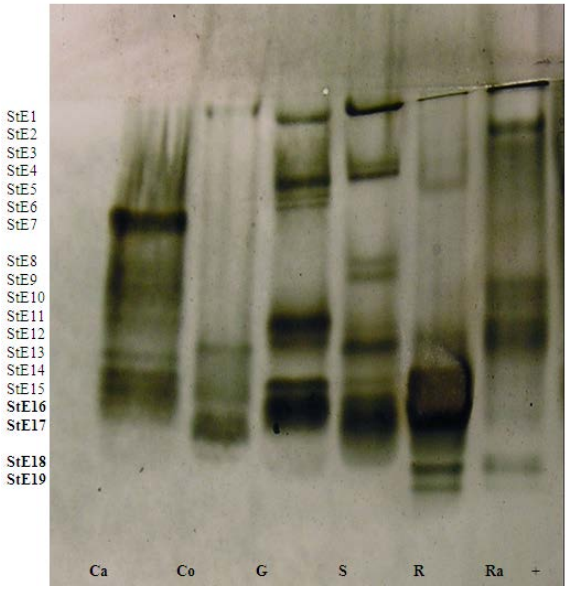

Figure 9- Stomach esterase (StE) isoenzymes of mammalian species:(Ca), Camel 0.32 mg protein. (Co), Cow 0.35 mg protein. (S), Sheep 0.32 mg protein. (G), Goat 0.32 mg protein. (R), Rabbit 0.32 mg protein. (Rat) Ra 0.32 mg protein.

4. Discussion

The results of this study (Table 1) show that liver has the highest esterase activity in camel, cow, goat, rabbit, rat, and sheep. These findings confirm those reported by Hosokawa *et al.* (1990) in rat, mouse, hamster, guinea pig, rabbit, pig, cow, dog, monkey and human and by Holmes and Masters (1968) who reported similar results obtained from sheep and ox tissues. The distribution level of esterase activity in tissues varies among mammals. In this study, camel, cow and rat have the lung in second order after the liver. Goat and rabbit have the kidney in second order. Sheep has the intestine in second order after the liver, which is consistent with Holmes and Masters (1968) results. As observed in previous studies, the liver, the lung, the kidney and the intestine have the highest esterase activity reiterating that esterase has an important role in detoxification and it works as one of the defense mechanism in mammalian bodies. Tsujita *et al.* (1988) studied esterase activity in various rat tissues using methyl butyrate as substrate. For unknown reason(s) their values were lower than the values obtained in this study. However, the present and those of Tsujita *et al.* (1988) gave the same order for esterase distribution in the tested tissues. Various methods were performed to identify the number of esterase isoenzymes.

Paul and Fottrell (1961) used starch gel electrophoresis while Holmes and Masters (1968) used 7.5% PAGE. These procedures gave variable number of esterase isoenzymes. For example, Holmes and Masters (1968) found that sheep liver, kidney, intestine, lung and heart have 7, 11, 8, 6 and 8 esterase isoenzymes, respectively. While the present study showed 6, 4, 9, 5, and 2 esterase isoenzymes for the same mammals and tissues, respectively (Figures 1 to 5). The differences in bands intensities in organ tissues could be due to several reasons of which is rate of gene expression or to the affinity of the

isoenzyme to the substrate used in the given staining procedure. Liver microsomal esterases cannot be identified by the procedure of this study (Hosokawa *et al.,* 1990; Yuet *et al*., 1996). The application of methyl butyrate as substrate is to assay for carboxyl esterase activity (Brüsehaber, *et al.,* 2007). Among the species of this study a number of bands of esterases comigrate on the gel (Table 3). This indicates that there is homologous amino acid sequence of these enzymes on which classification of esterases was based on (Satoh and Hosokawa, 1998 ; Robbi and Beaufay, 1983). An interesting finding is that no esterase activity at least under the conditions of the assay is observed in camel RBC or blood plasma, but it is seen in those of other animals in this study. RBC lysates of cow, goat, rabbit, rat, and sheep yielded 2 bands, 3 bands, 1 band, 2 bands, and 1 band, respectively. Band 5 has the same mobility in that of cow, rat and sheep; and this band of the rat has strongest intensity in all bands (Figure 6). Camel blood plasma has no bands of activity, but the plasma of the cow, goat, rabbit, rat, and sheep showed 3 bands, 2 bands, 3 bands, 6 bands, 1 band, respectively (Fig 7). Li *et al.* (2005) report indicated that human plasma contains four enzymes: Butyrylcholinesterase (EC 3.1.1.8), paraxonase (EC 3.1.8.1), acetylcholine esterase (EC 3.1.1.7), and albumin esterase-like. Albumin was reported to exhibit esterase-like activity towards p-nitrophenyl acetate, α naphthyl acetate, and β naphthyl acetate (Sakurai *et al.*, 2004), and towards nicotinic acid esters (Salvi *et al.*, 1997). The horse (Order Perissodactyla) plasma completely lacks the albumin esterase-like activity (Peter, 1996; Awad-Elkarim and Means, 1988), while dog, rabbit, bovine, rat, and human have this activity. Human serum albumin was shown to have the highest activity among serum albumin preparations from the studied species toward p-nitro-phenyl acetate as substrate (Sakurai *et al.*, 2005). Since our study involves activity-stain on PAGE, using α-naphthyl acetate which is a substrate for serum albumin-like esterase and carboxyl esterase and in the report in which p-nitro-phenyl-phosphate was used (Ahmad Abuerreish, 2011) did not give any band for camel plasma it is concluded that camel plasma albumin has no esterase-like activity; and in this aspect it is similar to the horse. From current studies and Yuet *et al.* (1996), one may conclude that liver does not secrete blood plasma carboxyl esterase. In Yuet *et al.* (1966) study, two carboxyl esterases were retaining C-reactive proteins within the endoplasmic reticulum. This is another example on directed evolution of the promiscuous function of esterase activity of carbonic anhydrase II (Gould and Tawfik, 2005).

References

Abu-Harfeel Nand Abuereish G M. 1984. Isolation and characterization of camel pepsins. *Comp Biochem Physiol*., **77** A: 175-182.

Ahmad M H A and Abuerreish G M. 2011. Purification and biochemical characterization of N-methyl-Histidylesterase from camel (*Camelus dromedarius*) liver. *J Chem Eng*., **5**: 671-683.

Awad-Elkarim A and Means G E. 1988. The reactivity of p nitrophenyl acetate with serum albumin. *Comp Biochem Physiol.,* **91B**:267–272.

Gould M T and Tawfik D S. 2005. Directed evolution of the promiscuous esterase activity of carbonic anhydrase II. *Biochemistry*, **44**, 5444-5452.

Banerjee S, Katz J, Levitz M and Finlay T H. 1991. Purification and properties of an esterase from human breast cyst fluid. *Cancer Res*., **51**: 1092 – 1098.

Bashir N A. 1981. Isolation, characterization, and N-terminal sequence of L-glutamate dehydrogenase from camel liver. M.Sc. Thesis, University of Jordan, Amman, Jordan.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*.,**72**:248-254.

Brüsehaber E, Böttcher D, Musidlowska-Persson A, Albrecht D, Hecker M, Doderer K and Bornscheuer T U. 2007. Identification of pig liver esterase variants by tandem mass spectroscopy analysis and their characterization. *Appl Microbiol Biotechnol* .,**76**:853–859.

Deimling O, Ronai A and Looze S. 1985. Nonspecific esterases of mammalian testis: comparative studies on the mouse (*Mus musculus*) and rat (*Rattus norvegicus*). *Histochemistry*, **82**: 547-555.

Finer Y and Santerre J P. 2004. Salivary esterase activity and its association with the biodegradation of dental composites. *J Dent Res.*, **83**: 22-26.

Göppinger A, Riebschläger M, Ronai A and Deimling O. 1978. Esterase XXVII: purification and characterization of esterase-9A of mouse kidney. *Bioch Biophy Acta*, **525**: 74-86.

Hart N H.and Cook M. 1976. Comparative analysis of tissue esterases of the zebra danio (*Barachydanio rerio*) and the pearl danio (*B. albolineatus*) by disc gel electrophoresis. *Comp Biochem Physiol*., **54B**: 357-364.

Holmes R S and Masters C J. 1968. Acomparative study of the multiplicity of mammatian esterases. *Biochim Biophys Acta*., **151**: 147-158.

Hosokawa M, Maki T and SatohT. 1990. Characterization of molecular species of liver microsomal carboxylesterases of several animal species and humans. *Arch Biochem Biophys.*, **277**: 219-227.

Khanna R, Morton C L, Danks M K and PotterP M. 2000. Proficient metabolism of irinotecan by a human intestinal carboxylesterase. *Cancer Res*., **60**: 4725-4728.

Khullar M, Scicli G, Carretero O A and Scicili A G. 1986. Purification and characterization of a serene protease (esterase B) from rat submandibular glands. *Biochem*., **25**: 1851-1857.

Kouroumalis E, Hopwood D, Ross P E and Bouchier I A D. 1984. Human gallbladder epithelium: non-specific esterases in cholecystitis. *J Pathol*., **142**: 151-159.

Laemmli U K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature***, 277**: 680-685.

Lee W, Wheatley W, Benedict W F, Huang C and Lee E Y P. 1986. Purification, biochemical characterization, and biological function of human esterase D. *Proc Natl Acad Sci USA*, **83**: 6790-6794.

Lexow U, Ronai A and Deimling O. 1980. Purification and characterization of esterase 2B of the house mouse *(Mus musculus*). *Eur J Biochem*., **107**: 123-130.

Li B, Sedlacek M, Manoharan I, Boopathy R, Duysen E G, Masson P and Lockridge O. 2005. Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. *Biochem Pharmacol*., **70**: 1673–1684.

Mentlein R, Rix-Matzen H and Heymann E. 1988. Subcellular localization of non-specific carboxylesterases, acylcarnitine hydrolase, monoacylglycerol lipase and palmitoyl-CoA hydrolase in rat liver. *Biochem Biophys Acta*, **964**: 319-328.

Muyldermans, S. 2001. Single domain camel antibodies: current status. *Rev Mol Biotechnol*., **74**: 277-302.

Oehm H, Looze S D, Ronai A and Deimling O V. 1982. Purification and characterization of esterase 6A, a trimeric esterase of the house mouse (*Musmusculus*). *Eur J Biochem*., **129**: 157-163.

Okada Y and Wakabayashi K. 1988. Purification and characterization of esterases D-1 and D-2 from human erythrocytes. *Arch Biochem Biophys*., **263**: 130-136.

Panda T and Gowrishankar B S. 2005. Production and applications of esterases. *Appl Microbiol Biotechnol*., **67**: 160-169.

Paul J and FottrellP. 1961. Tissue-specific and species-specific esterases. *Biochem J.*, **78**: 418-424.

Peter Jr T. 1996 **All About Albumin, Biochemistry, Genetics, and Medical Applications**, Academic Press, San Diego.

Prusakiewicz J J, Ackermann C and Voorman R. 2006. Comparison of skin esterase ctivities from different species. *Pharmac Res*., **23**: 1517-1524.

Redinbo M R and Potter P M. 2005. Mammalian carboxylesterases: from drug targets to protein therapeutics. *DDT*, **10**: 313-325.

Robbi M and Beaufay H.1983. Purification and characterization of various esterases from rat liver. *Eur J Biochem.*, **137**: 293-301.

Rudd E A, Mizuno N K and Brockman H L. 1987. Isolation of two forms of carboxylester lipase (cholesterol esterase) from porcine pancreas. *Biochim Biophys Acta*., **918**: 106-114.

Sakurai Y, Ma S-F, Watanabe H, Yamaotsu N, Hirono S, Kurono Y, Kragh-Hansen U and Otagiri M. 2004. Esterase-like activity of serum albumin: Characterization of its structural chemistry using p-nitrophenyl esters as substrates. *Pharmaceutical Research*, **2 (2):** 285-292.

Salvi L, Carrupt P-A, Mayer M and Testa B. 1997. Esterase-like activity of human serum albumin toward prodrug esters of nicotinic acid. *Drug Metabolism and Disposition* **25 (4):** 395-398.

Sanghani S P, Quinney S K, Fredenburg T B, Sun Z, Davis W I, Murry D J,Cummings O W, Seitz D E and Bosron W F. 2003. Carboxylesterase expressed in human colon tumor tissue and their role in CPT-11 hydrolysis. *Clin Cancer Res.*, **9**: 4983-4991.

Satoh T and Hosokawa M. 1998. The mammalian carboxylesterases: from molecules to functions. *Annu Rev Pharmacol Toxicol*., **38**: 257-288.

Satoh T, Taylor P, Bosron W F,Sanghani S P, Hosokawa M and Du B N L. 2002. Current progress on esterases: from molecular structure to function. *Drug Metab Dispos*., **30**: 488-493.

Soni K G, Lehner R L, Metalnikov P, O'Donnell P, Semache M, Gao W, Ashman K, Pshezhetsky A V and Mitchell G A. 2004. Carboxylesterase 3 (EC 3.1.1.1) is a major adipocyte lipase. *J Biol Chem*., **279**: 40683-40689.

Swell L, Byron J E and Treadwell C R. 1950. Cholesterol esterases IV: cholesterol esterase of rat intestinal mucosa. *J Biol Chem*., **186**: 543 - 548.

Tanaka M, IIo T and Tabata T. 1987. Purification and characterization of a carboxylesterase from rabbit liver lysosomes. *J Biochem*., **101**: 619-624.

Tsujita T, Nagai K and Okuda H. 1979. Purification and properties of human serum esterase. *Biochim Biophys Acta*., **570**: 88-95.

Tsujita T and Okuda H. 1983. Carboxylesterases in rat and human sera and their relationship to serum aryl acylamidases and cholinesterases. *Eur J Biochem*., **133**: 215- 220.

TsujitaT. Miyada T and Okuda H. 1988. Purification of rat kidney carboxylesterase and its comparison with other tissue esterases. *J Biochem.*, **103**: 327-331.

Weber K and Osborn M. 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel elctrophoresis. *J Biol Chem*., **244**: 4406-4412.

Welner AM, Platt T and Weber K. 1972. Amino-terminal sequence analysis of proteins purified on a nanomole scale by gel electrophoresis. *J Boil Chem*., **247**:3242-3251.

Yang F, Bian C, Zhu L, Zhao G, Huang Z and Huang M. 2007. Effect of human serum albumin on drug metabolism: structural evidence of esterase activity of human serum albumin. *J Struct Biol*., **157**: 348-355.

Yuet CC, Muller-Greven J, Dailey P, Lozanski G, Anderson V and Macintyre G. 1996. Identification of a C-reactive protein binding site in two hepatic carboxylesterases capable of retaining C-reactive protein within the endoplasmic reticulum. *J Biol Chem*., **271**: 22245-22250.