عنوان المشروع باللغة العربية	تنقية انزيم مختزل الألدوز المستنسخ من كبد الأبل
عنوان المشروع باللغة الإنجليزية	Purification and characterization of recombinant Arabian camel liver aldo-keto reductase
المشرف الرئيس	ا.د عبد الرحمن بن محمد السنيدي
التخصص الدقيق للمشرف الرئيس	كيمياء البروتينات
المشرف المساعد	
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	ثمانية اشهر
Abstract or synopsis of the proposal (200 words or less):	Mammalian tissues produce enzymatic systems belonging to the family or members of the aldo-keto reductase (AKR) such as aldose reductase, aldehyde reductase, dihydrodiol dehydrogenases, and several other reductases. Aldo-keto reductases (AKRs) metabolize a diverse range of compounds by ca talyzing NAD(P)(H)-depe ndent reduction or oxidation reactions. In general, an aldehyde or ketone moiety is converted to the corresponding alcohol. AKR substrates include sugars, steroids, amino acids, pesticides, neurotransmitters, substituted benzenes, polycyclic aromatic hydrocarbons, chemotherapeutic agents, and lipid aldehydes. Metabolic processes in Arabian camels may lead to the accumulation of harmful endogenous carbonyl compounds and these compounds can in turn damage healthy cells by forming adduct with proteins and nucleotides. In a previous study, we reported a novel, thermostable AKR from Arabian camel liver. Based on this study, we plan to purify the camel liver recombinant AKR to characterize enzymatic properties and assess kinetic parameters.
Hypothesis or scientific justification of the proposal	he Arabian camel has many interesting physiological adaptations that enable it to survive the harsh desert environment. This fact has promoted several investigations to determine the "normal" values of several biochemical parameters in the camel, including blood enzymes, hematological parameters, blood metabolites, and others. Previous studies reveal that the normal blood glucose concentration in the camel is significantly higher than in human blood. Camel displayed high blood glucose concentration (9.7 ± 2.8 mM) as compared to other

	mammals. Glucose is reduced in the polyol pathway by aldose reductase enzyme to sorbitol in an NADPH consuming reaction. This enzyme has assumed considerable interest because of its possible involvement in diabetic complications such as cataract, retinopathy, nephropathy, neuropathy and cornea1 epitheliopathy. In the evaluation of aldose reductase inhibitors, the diabetic and galactosemic rat models have been extensively employed. Significant differences, however, have been reported between the susceptibility to inhibition of aldose reductase from rat lens versus human placenta or lens. Moreover, antibodies raised against human placental aldose reductase fail to cross-react with the rat lens enzyme.
Specific objectives	1. To study the similarities and differences between aldose reductase from camel and other sources, the camel liver aldose reductase (AKR) will be expressed and purified from Escherichia coli cultures and its enzymatic properties will be compared with that of purified rat aldose reductase.
Methodology & Major Techniques to be used< /td>	amel liver aldo-keto reductase (AKR) will be expressed and purified from Escherichia coli cultures. Briefly, AKR construct will be transformed into BL21 (DE3) pLysS and grown in 500 ml of Luria broth media supplemented with 50 µg/ml kanamycin sulfate and 25 µg/ml chloramphenicol at 37°C. Expression will be induced by addition of IPTG admin istration at a concentration of 1 mM, and the cultures grown for a further 3 h. Bacterial pellets then collected by centrifugation and suspended in 50 mM NaH2PO4, pH 8.0. Lysis of the cell suspensions and purification of the AKR protein will be carried out using nickel nitrilotriacetic acid agarose and protein purity assessed by SDS-PAGE
Availability of Samples	YES
If the answer is no, kindly justify	
Availability of Chemicals	YES
If the answer is no, kindly justify	
Availability of Instruments	YES

Availability of Ethical Approval (if needed)	YES
Recent References	1. Bains OS, Grigliatti TA, Reid RE, and Riggs KW (2010) Naturally occurring variants of human aldo-keto reductases with reduced in vitro metabolism of daunorubicin and doxorubicin. J Pharmacol Exp Ther 335:533–545. 2. Barski OA, Tipparaju SM, and Bhatnagar A (2008) the aldo-keto reductase superfamily and its role in drug metabolism and detoxification. Drug Metab Rev40:553–624. 3. Byrns MC, Jin Y, and Penning TM (2011) Inhibitors of type 5 17β-hydroxysteroid dehydrogenase (AKR1C3): overview and structural insights. J Steroid Biochem Mol Biol 125:95–104. 4. oorn JA, Srivastava SK, and Petersen DR (2003) Aldose reductase catalyzes reduction of the lipid peroxidation product 4-oxonon-2-enal. Chem Res Toxicol16:1418–1423. 5. Endo S, Matsunaga T, Kumada S, Fujimoto A, Ohno S, El-Kabbani O, Hu D, Toyooka N, Mano J, 6. Tajima K, et al. (2012a) Characterization of rabbit aldose reductase-like protein with 3β-hydroxysteroid dehydrogenase activity. Arch Biochem Biophys 527:23–30. 7. Jin Y and Penning TM (2007) Aldo-keto reductases and bioactivation/detoxication. Annu Rev Pharmacol Toxicol 47:263–292 8. Matsunaga T, El-Kabbani O, and Hara A (2013) Aldo-keto reductases as new therapeutic targets for colon cancer chemoresistance, in Molecular Mechanisms of Tumor Cell Resistance to Chemotherapy (Bonavida B, ed) pp 109–134, Springer,New York.