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Circadian Variations of Stomach, Duodenum and Ileum Aldehyde Oxidase in Male and Female Dhubb (*Uromastyx microlepis*)

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Abstract: The activity of the cytosolic enzyme, aldehyde oxidase, was estimated in partially purified preparations from stomach, duodenum and ileum of the male and female Dhubb, *Uromastyx microlepis*,for four times daily at equal intervals. In these tissues of both a male and female Dhubbs a clear circadian variation aldehyde oxidase activity was noticed with two substrates (phthalazine and 3-methylisoquinoline). The maximum activity of male and female stomach, duodenum and ileum aldehyde oxidase appeared at 2100 h, whereas the minimum activity of the same enzyme was observed at 0300 h. The differences between maximum and minimum activity were statistically significant ($P < 0.0005$).

Introduction

Microsomal monooxygeneases or mixed function oxidases (MFO_s) are implicated in the detoxification and biotransformation process of a wide range of drugs and foreign compounds[1-3]. Cytosolic, molybdenum hydroxylases, aldehyde oxidase (E.C. 1.2.3.1) and xanthine oxidase (E.C.1.2.3.2) also contribute to the above mentioned functions[4-7].

MFO_s were found to have temporal variations in several species[8-11]. The early studies reported the presence of daily fluctuations in microsomal enzymes of hepatic male rat[8,9]. Moreover, the activity of microsomal enzymes of mice liver exhibited circadian rhythms in drug metabolism[12]. Lake *et al*.,[13] observed diurnal rhythm in the activity of liver microsomal enzymes of the golden hamster. Furthermore, another group of microsomal enzymes such as a

ethylmorphine N-demethylase, aniline hydroxylase and arylhydrocarbon hydroxylase showed circadian rhythms in male liver of Syrian hamster[14]. In addition, aldehyde oxidase and xanthine oxidase which are involved in drugs and xenobiotics metabolism have demonstrated circadian variations in liver cytosole of guinea pigs and Syrian hamster[15,16]. Accordingly, the aim of the present communication is to study whether such circadian variations of aldehyde oxidase activity could be displayed in tissues of Dhubb such as stomach, duodenum and ileum.

Materials and Methods

Chemicals

Phenanthridine and phthalazine were purchases from Aldrich Chemical Company. 3-Methylisoquinoline was obtained from ICN Pharmaceuticals Inc. Potassium Chloride was supplied by Merck Company, while the rest of the chemicals were analytical grade obtained from BDH.

Animals

Adult male and female Dhubbs, weighing 640-750g at death, were bought from the animal market in Riyadh. All animals were housed in groups of six and allowed food *ad libitum*. They were exposed to a period of natural light ranging from 600 to 1900 h and a dark period exactly from 1900 to 6000 h.

Enzyme Purification

Killing of Dhubbs was achieved by cervical dislocation. Then sampling of stomach, duodenum and ileum was collected . Thereafter, collected tissues were submitted to freezing by liquid nitrogen, and stored at -80°C for one week as a maximum. Partially purified aldehyde oxidase was prepared from tissue homogenate, according to the method explained by Johnson *et al*.,[17] Each tissue was weighed and transferred to a beaker containing 1.15% potassium chloride solution enriched with 10^{-4} M EDTA, two or three times. The tissue was chopped finely by scissors; aliquots were transferred to a glass homogenizing tube and homogenized using Janke & Kunkel homogenizer. The homogenate was heated at 50-55°C for 10 minutes, followed by immediate cooling in ice to 10°C, then centrifuged for 45 minutes at 15,000 g at 4°C using Heraeus Christ 20-3 (LABSCO) centrifuge. The supernatant was filtered through glass wool into a measuring cylinder and the volume of the clear solution was noted. Solid ammonium sulphate was added to 50% saturation (35.4 g/100 ml at 4°C) with stirring using a magnetic stirrer on an ice bath.

When precipitation was complete (15 minutes), the suspension was centrifuged at 6000g for 20 minutes at 4°C. The supernatant was discarded and the precipitate was washed with distilled water and dissolved in approximately 5ml 10^{-4} M EDTA solution. The partially purified enzyme was stored in a deep freezer at –80ºC and used when required.

Enzyme assay

The activity of aldehyde oxidase was evaluated spectrophotometrically by a Varian UV/VIS spectrophotometer, fitted with a thermostatically controlled cell holder maintained at 37°C. The specific activity of the enzyme was determined using two substrates (phthalazine and 3-methylisoquinoline) as described by Johnson *et al.,*[18]. The oxidation rate of either phthalazine (1mM) or 3-methylisoquinoline (1mM) was monitored at 420 nm by following 1mM potassium ferricyanide reduction. Protein concentration was estimated by Biuret method[19] .

Results and Discussion

The activity of aldehyde oxidase was assayed each 6- hours intervals utilizing phthalazine and 3- methylisoquinoline. Figures 1 and 2 demonstrate the fluctuation of stomach aldehyde oxidase by using 3-phthalazine methylisoquinoline and potassium ferricyanide as an electronic acceptor. Both substrates appeared to have similar results, the enzymes of male and female, Dhubbs exhibited maximum oxidative activity at 2100 h in the dark period, while the minimum activity was detected at 0300 h for both substrates. The differences between rhythmic extremes was statistically significant ($P<0.05$) as manifested in Figure 3 and 4, duodenum aldehyde oxidase in male and female Dhubbs shows a fluctuation in enzyme activity when phthalazine and 3-methylisoquinoline were used as substrates. Moreover, the enzyme gave similar results for each substrate. In each case, the enzyme activity was maximal at 21 hr, whereas the minimum enzyme activity emerged at 0300 h ($P < 0.005$). Furthermore, similar rhythmic changes were observed when male and female Dhubbs ileum aldehyde oxidase was reacted with phthalazine and 3-methylisoquinoline (Figure 5 and 6).

Molybdenum hydroxylase fractions are including aldehyde oxidase and xanthine oxidase as previously established in many species such as guinea pig, rat, rabbit, baboon and man[6,7,15-17]. However, in the present study one molybdenum hydroxylase, aldehyde oxidase, will be considered, because the other one's xanthine oxidase was detected only in kidney of Dhubb[20]. The aldehyde oxidase with nitrogen containing compounds has response properties to an extensive range from one species to another[21-24]. In present study, male and female Dhubbs aldehyde oxidase prepared from different tissues (stomach ,

duodenum and ileum) and its activity was examined with two substrates (phthalazine and 3-methylisoquinoline). Investigation pointed out that the enzyme failed to react with phenanthridine (data not shown) in comparison with the case of guinea pig or rabbit enzymes mentioned[15,18]. Previous works have reported diurnal rhythms for microsomal monooxygenases in several animal species[8,9,12,14,15,25]. Their findings corroborate the results of the present investigation, which also shows daily variations in aldehyde oxidase activity. Whatever of the species tested, guinea pig and hamster and lizard showed daily variations in aldehyde oxidase and xanthine oxidase[15,16] compatibility, those results are proved again in the present study. It can be observed from Figure 1, 2, 3, and 4 that the activity of male Dhubb aldehyde oxidase with phthalazine and 3-methylisoquinoline was higher than that of female ($P<0.01$). These data are similar to the reported results in Dhubb and guinea pig in which male aldehyde oxidase activity is higher than female enzyme comparatively[20 ,25].This increase in male enzymes has been ascribed to higher testosterone levels[26,27]. Circadian rhythms in drug metabolising enzymes have been attributed to several factors, including light dark–schedule, dietary protein, changes in corticosteroids, feeding times, fluctuations in serum melatonin levels and locomotor activity[8,9,11,14,15]. Consequently, it is concluded that the activity aldehyde oxidase exhibited daily variation in male and female Dhubb.

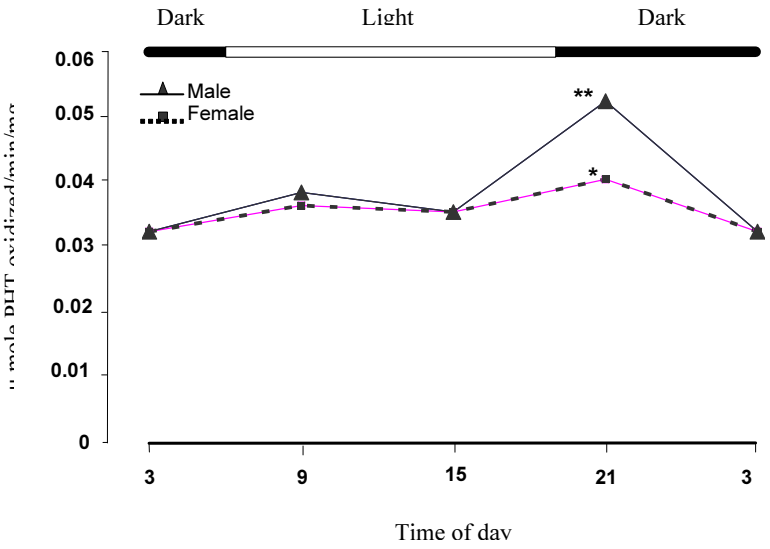

Fig. 1: Diurnal variation in stomach aldehyde oxidase activity. Enzyme activity was measured at 37°C and expressed as µ mole Phthalazine (PHT)oxidized/min/mg protein.Potassium ferricyanide (1mM) was used as an electron acceptor. Each point represents the mean of six animals. Differences between maxima and minima were significant (*P<0.005, **P<0.0005) using a two-tailed students t-test.

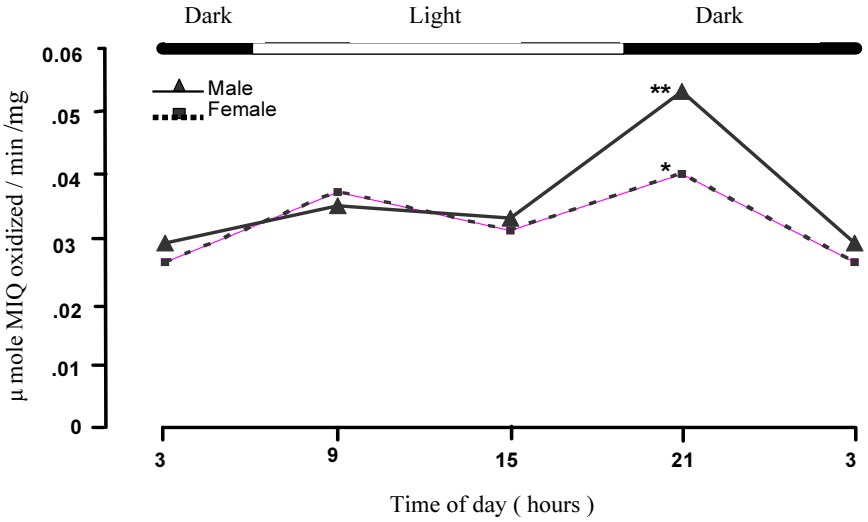

Fig. 2: Diurnal variation in stomach aldehyde oxidase activity. Enzyme activity was measured at 37°C and expressed as µ mole 3-methylisoquinoline (MIQ) oxidized/min/mg protein. Potassium ferricyanide (1mM) was used as an electron acceptor. Each point represents the mean of six animals. Differences between maxima and minima were significant *P<0.005, **P<0.0005 using a two-tailed students t-test .

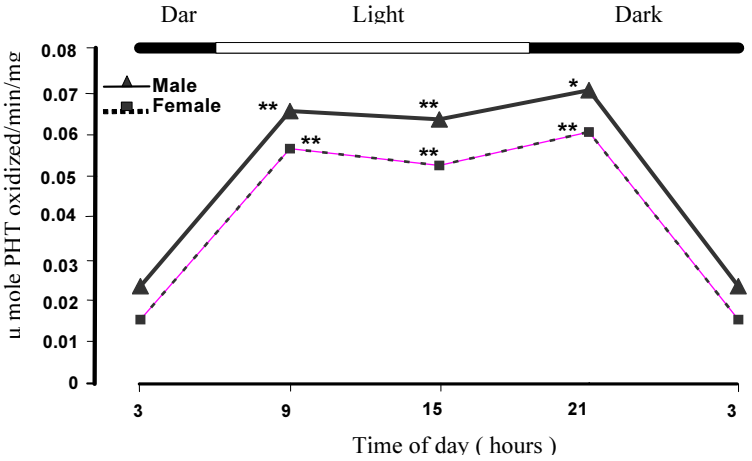

Fig. 3: Diurnal variation in duodenum aldehyde oxidase activity. Enzyme activity was measured at 37°C and expressed as µ mole Phthalazine (PHT) oxidized/min/mg protein.Potassium ferricyanide (1mM) was used as an electron acceptor. Each point represents the mean of six animals. *P<0.005, **P<0.0005 using a two-tailed students t-test .

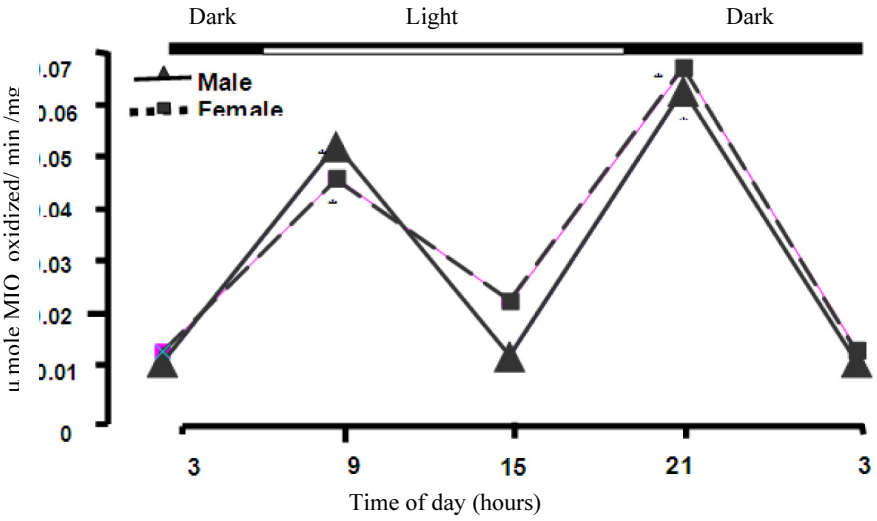

Fig. 4: Diurnal variation in duodenum aldehyde oxidase activity. Enzyme activity was measured at 37°C and expressed as µ mole 3-methylisoquinoline (MIQ) oxidized/min/mg protein. Potassium ferricyanide (1mM) was used as an electron acceptor. Each point represents the mean of six animals. *P<0.005, **P<0.0005 using a two-tailed students t-test .

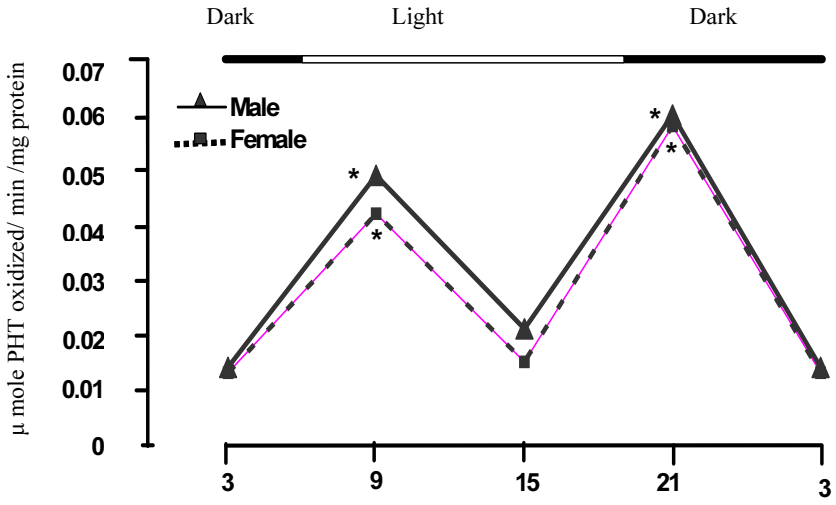

Fig. 5: Diurnal variation in ileum aldehyde oxidase activity. Enzyme activity was measured at 37°C and expressed as µ mole Phthalazine(PHT)oxidized/min/mg protein. Potassium ferricyanide (1mM) was used as an electron acceptor. Each point represents the mean of six animals. *P<0.0005 using a two-tailed students t-test .

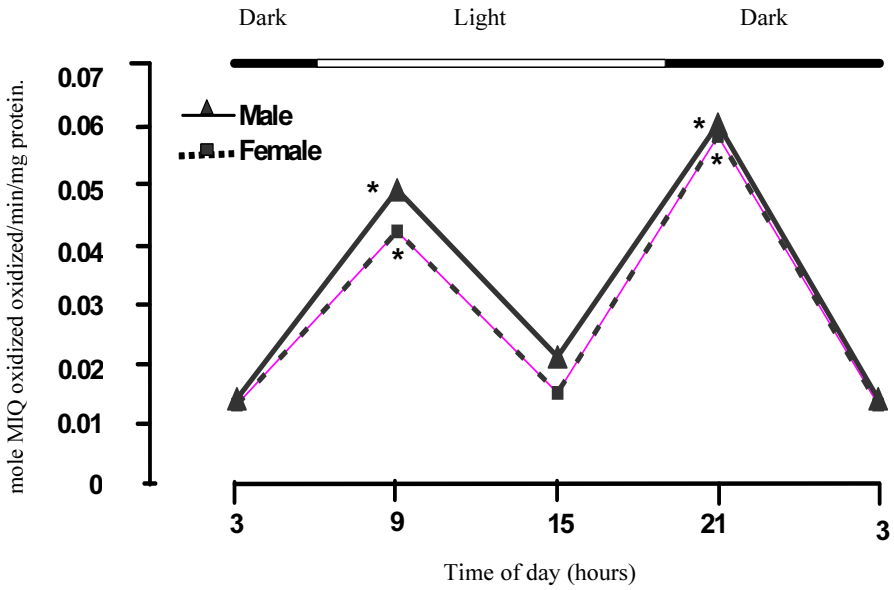

Fig. 6: Diurnal variation in ileum aldehyde oxidase activity. Enzyme activity was measured at 37°C and expressed as µ mole 3-methylisoquinoline (MIQ) oxidized/min/mg protein. Potassium ferricyanide (1mM) was used as an electron acceptor. Each point represents the mean of six animals. *P<0.0005 using a two-tailed students t-test .

References

[1] **Gibson, G.G.** and **Skett, P.** (1986) *Introduction to drug metabolism*, Chapman and Hall, London.

[2] **Nakasa, H., Komiya, M., Ohmori, S., Rikihisa, T., Kiuchi, M.** and **Kitada, M.** (1993) Characterization of human liver microsomal cytochrome P450 involved in the reductive metabolism of zonisamid, *Mol. Pharmacol*., **44:** 216-221.

[3] **Cnubben, N.H., Vervoort, J., Boersma, M.G.** and **Rietjens, I.M.** (1995) The effect of varying halogen substituent patterns on the cytochrome P. 450 catalyzed dehalogenation of 4-halogenated anilines to 4- aminophenol metabolites. *Biochem. Pharmacol*. **49 :** 1235-1248 .

[4] **Beedham, C.** (1985) Molybdenum hydroxylases as drug metabolizing enzymes. *Drug Metab. Rev*. **16**:,119-156.

[5] **Beedham, C., Al-Tayib Y.** and **Smith J.A.** (1992) Role of guinea pig and rabbit hepatic aldehyde oxidase in oxidative *in vitro* metabolism of cinchona antimalarials. *Drug Metab Dispos*. **20:** 889-895.

[6] **Beedham, C., Critchly, D.J.** and **Rance, D.J.** (1995) Substrate specificity of human liver aldehyde oxidase toward substituted quinazolines and phthalazine: A comparison with hepatic enzyme from guinea pig, rabbit and baboon. *Arch. Biochem. Biophys*. **319:** 481-490.

[7] **Rashidi, M.R., Smith, J.A., Clark, S.E.** and **Beedham, C.** (1997) *In Vitro* oxidation of famciclovir and 6-deoxypenciclovir by aldehyde oxidase from human, guinea pig, rabbit and rat liver. *Drug Metab. Dispos*. **25:** 805 -813 .

[8] **Radzialowski, F.M.** and **Bousquet, W.F.** (1968) Daily rhythmic variation in hepatic drug metabolism in the rat and mouse. *J. Pharmacol Exp. Ther*. **163:** 229-238.

[9] **Nair, V.** and **Casper, R.** (1969) The influence of light on daily rhythm in hepatic drug metabolising enzymes in rat life; *Sci*. **23:** 1291-1298.

[10] **Jori, A., Dissalle, E.** and **Santini, V.** (1971) Daily rhythmic variation and liver drug metabolism in rats. *Biochem. Pharmacol*, **20**: 2965-2969.

[11] **Tredger, J.M.** and **Chhabra, R.S.** (1977) Circadian variations in microsomal drug–metabolizing enzyme activities in rat and rabbit tissues. *Xenobiotica*, **7:** 481-489.

[12] **Holcslaw, T.L., Miya, T.S.** and **Bousquet, W.S.** (1975) Circadian rhythms in drug action and drug metabolism in mouse. *J. Pharmacol Exp. Ther*. **195:** 320-322.

[13] **Lake, B.G., Tredger, J.M., Burke, M.D. Chakraborty, J.** and **Bridges, J.W.** (1976) The circadian variation of hepatic microsomal drug and steroid metabolism in the golden hamster. *Chem. Biol. Interact*. **12 :** 81-90 .

[14] **Birt, D.F.** and **Hines, L.A.** (1982) Modifications of circadian rhythms of drug metabolism in Syrian hamster. *Drug – Nutr. Interact*. **1:** 143-151.

[15] **Beedham, C. Padwick, D.J., Al-Tayib Y.** and **Smith, J.A.** (1989) Diurnal variation and melatonin induction of hepatic molybdenum hydroxylase activity in the guinea pig. *Biochem. Pharmacol*. **38:** 1459-1464.

[16] **Al-Tayib, Y.A.** (1999) The circadian variation of the activity of hepatic molybdenum hydroxylases in the female Syrian hamster. *Arab Gulf J. Scientific Res.,* **17(3):** 370-381.

[17] **Johnson, C., Beedham, C.** and **Stell, J.G.P.** (1987) Reaction of 1-amino and 1-chlorophthalazine with mammalian molybdenum hydroxylases *in vitro* Xenobiotica **17:** 17-24.

[18] **Johnson, C., Stubbly–Beedham, C.** and **Stell, J.G.P.** (1983) Elevation of molybdenum hydroxylase levels in rabbit liver after ingestion of phthalazine or its hydroxylated metabolite, *Biochem. Pharmacol*. **33:** 3699-3705.

[19] **Gornal, A.G., Bradwill, C.J.** and **daivd, M.M.** (1948) Determination of serum proteins by means of the biuret reaction. *J.biol.chem* **177**: 751-766.

[20] **Al-Tayib, Y.A.** (2000) Tissue distribution of aldehyde oxidase and xanthine oxidase in the lizard uromatyx microlepis (Dhubb). *J Egypt Ger. Soc. Zool*. **31(A):** 57-65.

[21] **Kaye, B., Offerman, J.L., Reid, J.L., Elliot, H.L.** and **Hillis, W.S.** (1984) A species difference in the presystemic clearance of carbazeran in dog and man. *Xenobiotica*, **14 :** 935-945.

[22] **Taylor, S.M., Stubley-Beedham, C.** and **Stell, J.G.P.** (1984) Simultaneous formation of 2-and 4- quinolones from quinolinium cations by aldehyde oxidase. *Biochem. J*., **220:** 67-74.

[23] **Kay, B., Rance, D.J.** and **Waring, L.** (1985) Oxidative metabolism of carbazeran *in vitro* by liver cytosol of baboon and man. *Xenobiotica*, **15:** 237-242.

[24] **Beedham, C., Bruce, S.E., Critchley, D.C., Al-Tayib, Y.** and **Rance, D.J.** (1987a) Species variation in hepatic aldehyde oxidase activity. *Eur. J. Drug Metab. Pharmacokinet,* **12:** 307-310.

[25] **Beedham, C., Bruce, S.E.** and **Rance, D.J.** (1987b) Tissue distribution of the molybdenum hydroxylases aldehyde oxidase and xanthine oxidase in male and female guinea pigs. *Eur. J. Drug Metab. Pharmacokinet*, **12:** 303-306.

[26] **Huff, S.D.** and **Chaykins, S.** (1967) Genetic and androgenic control of N-methylnicotinamide oxidase activity in mice. *J. Biol. Chem*., **242:** 1262-1270.

[27] **Levinson, D.J.** and **Decker, D.E.** (1984) Biochemical mechanisms for sex specific differences of rat liver xanthine oxidase, *Adv. Exp. Med. Biol*., **165A:** 511-517.

التغيرات اليومية لألدهيد أكسيداز المعدة، والعفج (الإثنا عشري)، واللفائفي في ذكور وإناث الضب

عبدالباسط إبراهيم الصيني

قسم الكيمياء الحيوية – كلية العلوم

جامعة الملك عبد العزيز– جدة– المملكة العربية السعودية

المستخلص: قُدَّر نشاط إنزيم العُصارة الخلوية لألدهيد أكسيداز في التحضيرات النقية جزئيًا, المأخوذة من المعدة، والعفج، واللفائفي لذكور وإناث الضب, لأربع مرات يوميًا، بينها فترات زمنية فاصلة متساوية. لقد لوحظ في هذه الأنسجة المحضرة من إناث وذكور الضب على السواء؛ تغيرًا يوميًا واضحًا في نشاط الدهيد أكسيداز, وذلك باستخدام مادتين وسيطتين اثنتين هما فثالازين و٣– ميثيل ايزو كوينولين. لقد ظهر النشاط الأعظم لألدهيد أكسيداز لمعدة وعفج ولفائي ذكور وإناث الضب في الساعة ٢١:٠٠ في حين لوحظ النشاط الأصغر للإنزيم ذاته في الساعة ٣:٠٠. لقد كانت الفروق بين النشاطين الأعظم والأصغر معوية إحصائيا ($p<0,0005$).