

## Daily Rhythm in Hepatic and Renal Aldehyde Oxidase and Xanthine Oxidase in Male and Female *Uromastyx microlepis*

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**ABSTRACT.** The activities of aldehyde oxidase and xanthine oxidase were determined in partially purified preparations from livers and kidneys of male and female Dhubb, *Uromastyx microlepis*, four times daily at equal intervals. The maximum activity of male and female hepatic aldehyde oxidase appeared at 2100 h, whereas the minimum activity was observed at 0300 h, using phthalazine and 3-methylisoquinoline as substrates. The differences between maximum and minimum activity were statistically significant ( $P < 0.05$ ). No fluctuation was observed in male renal aldehyde oxidase activity with phthalazine, whereas female enzyme showed significant daily variations ( $P < 0.0005$ ). Using 3-methylisoquinoline as a substrate, both male and female renal aldehyde oxidase showed drop in activity at 1500 h, and the differences between minimum and maximum were significant ( $P < 0.0005$ ). Circadian variations in male and female renal xanthine oxidase activities were observed using xanthine as a substrate. The main peak appeared at 0300 h and minimum activity from 1500 to 2100 h, and the difference between rhythmic extremes was statistically significant ( $P < 0.03$ ).

### Introduction

Microsomal monooxygenases and the cytosolic molybdenum hydroxylases, aldehyde oxidase (E.C. 1.2.3.1) and xanthine oxidase (E.C. 1.2.3.2), play very important role in the biotransformation of drugs and xenobiotics<sup>[1-7]</sup>. Many animal species such as mice, rats, hamsters and guinea pigs showed daily rhythmic variations in several enzymes<sup>[8-11]</sup>. These enzymes include: microsomal enzymes, UDP-glucuronyltransferase, sulphotransferase, aldehyde oxidase and xanthine oxidase<sup>[11-13]</sup>. In most situations, greatest enzyme activity has been

observed in the dark period with a corresponding minimum, sometime during the light period<sup>[8-14]</sup>. Therefore, the aim of the present work is to study whether such circadian variations of molybdenum hydroxylases activity are displayed in *Uromastix microlepis*, or Dhubb as it is called in Saudi Arabia.

## Materials and Methods

### Chemicals

Phthalazine was purchased from Aldrich Chemical Company (Gillingham, U.K.). 3-methylisoquinoline was obtained from ICN Pharmaceuticals Inc. (K&K) (Irvine, CA). Xanthine was supplied by Sigma Chemical Company (London, U.K.).

### Animals

Mature male and female Dhubb (644-750 g) were obtained from the animal market in Riyadh. They were housed in groups of seven and allowed food *ad lib*. They were exposed to a natural light period (from 0600 to 1900 h) and a dark period (from 1900 to 0600 h).

### Enzyme preparation

Dhubb were killed by cervical dislocation; livers and kidneys were collected. Collected tissues were frozen by liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for one week. Partially purified aldehyde oxidase was prepared from tissue homogenate, according to the method of Johnson *et al.*<sup>[15]</sup> as follows: each tissue was weighed and transferred to a beaker containing potassium chloride solution (1.15 w/v) containing  $10^{-4}$  M EDTA, two to three times by volume. The tissue was chopped finely by scissors; aliquots were transferred to a glass homogenising tube and homogenised using a Janke & Kunkel homogeniser. The homogenate was heated at  $50-55^{\circ}\text{C}$  for 10 min, followed by immediate cooling in ice to  $10^{\circ}\text{C}$  and then centrifuged for 45 min at 15,000 g at  $40^{\circ}\text{C}$  using a 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  $4^{\circ}\text{C}$ ) with stirring using a magnetic stirrer on an ice bath. When precipitation was complete (15 min), the suspension was centrifuged at 600 g for 20 min at  $4^{\circ}\text{C}$ . The supernatant was discarded and the precipitate was rinsed with distilled water and dissolved in a minimum volume of  $10^{-4}$  M EDTA solution (approximately 3-5 ml). The partially purified enzyme was stored in deep freezer at  $-80^{\circ}\text{C}$  and used when required.

### **Enzyme assays**

The activity of molybdenum hydroxylase fractions was measured spectrophotometrically using a Varian UV/VIS spectrophotometer, fitted with a thermostatically controlled cell holder maintained at 37°C. The specific gravity of aldehyde oxidase was determined using two substrates (Phthalazine and 3-methylisoquinoline) as described by Johnson *et al.*<sup>[16]</sup>. The oxidation rate of either phthalazine (1 mM) or 3-methylisoquinoline (1 mM) was monitored at 420 nm by following potassium ferricyanide reduction. The specific activity of xanthine oxidase was evaluated using xanthine (0.1 mM) at 295 nm as reported previously by Johnson *et al.*<sup>[16]</sup> and Stuble and Stell<sup>[17]</sup>. Protein concentration was determined by the Biuret method.

### **Results and Discussion**

Molybdenum hydroxylase fractions containing both aldehyde oxidase and xanthine oxidase were prepared from livers and kidneys of male and female Dhubbs. The activities of both enzymes were assayed at 6-hours intervals using phthalazine, 3-methylisoquinoline and xanthine. Figures 1 and 2 show temporal variations in hepatic aldehyde oxidase activity, using phthalazine and 3-methylisoquinoline as substrates which were monitored indirectly by following the reduction of an artificial acceptor (potassium ferricyanide) at 420 nm. Male and female hepatic aldehyde oxidase gave similar results for each substrate. In each case, enzyme activity was maximal at 2100 h in the dark period, whereas the minimum enzyme activity appeared at 0300 h ( $P < 0.03$ ). As manifested in the Fig. 3, renal aldehyde oxidase in male and female Dhubbs does not show a clear fluctuation in enzyme activity when phthalazine is used as substrate. However, a clear drop in renal aldehyde oxidase activity is observed at 1500 h, when 3-methylisoquinoline is used as substrate (Fig. 4).

The activity of the other renal molybdenum hydroxylase, xanthine oxidase, was assayed using xanthine as substrate and oxygen as the electron acceptor at 295 nm. This enzyme shows less marked variation in its activity. However, one peak is seen at 0300 h (Fig. 5). In addition, female xanthine oxidase shows a significant difference between maximum and minimum activity ( $P < 0.005$ ).

Several workers have reported daily rhythms for microsomal monooxygenases in several animal species<sup>[8-10, 14,18,19]</sup>. Their findings corroborate the results of the present study, which also show daily variations in molybdenum hydroxylases activity. Furthermore, a study carried out by Beedham *et al.*<sup>[11]</sup> found that the activity of aldehyde oxidase and xanthine oxidase exhibited daily variation in guinea pig. Their results resembled those of our study, hence providing further support to the above mentioned daily variations. However, a note

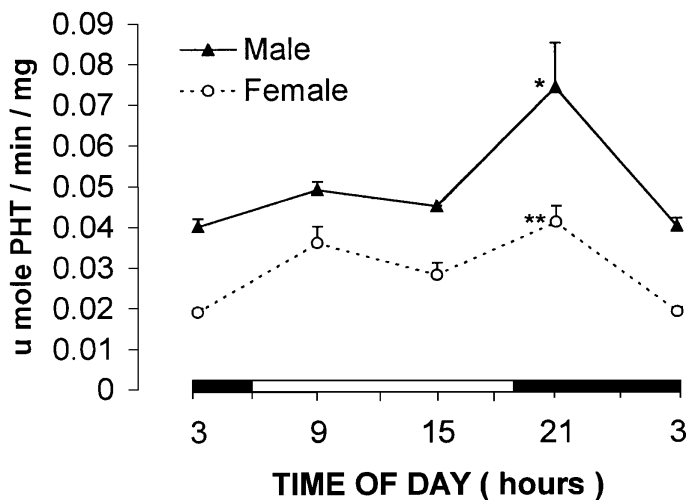


FIG. 1. Circadian variation in hepatic aldehyde oxidase activity. Enzyme activity was measured at 37°C and expressed as *u* mole phthalazine (PHT) oxidised/min/mg protein. Potassium ferricyanide (1 mM) was used as an electron acceptor. Each point represents the mean + SE of five or seven animals. Differences between maxima and minima were significant (\**P* < 0.03, \*\**P* < 0.005) using a two-tailed students t-test.

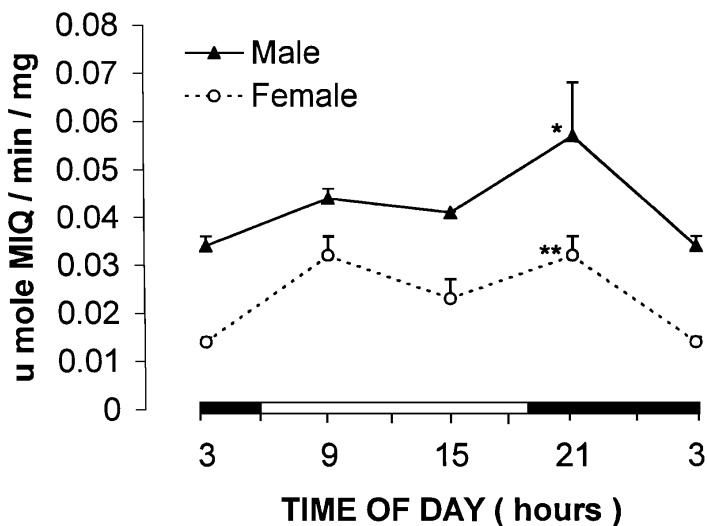


FIG. 2. Circadian variation in hepatic aldehyde oxidase activity. Enzyme activity was measured at 37°C and expressed as *u* mole 3-methylisoquinoline (MIQ) oxidised/min/mg protein. Potassium ferricyanide (1 mM) was used as an electron acceptor. Each point represents the mean + SE of five or seven animals.

\**P* < 0.05

\*\**P* < 0.005

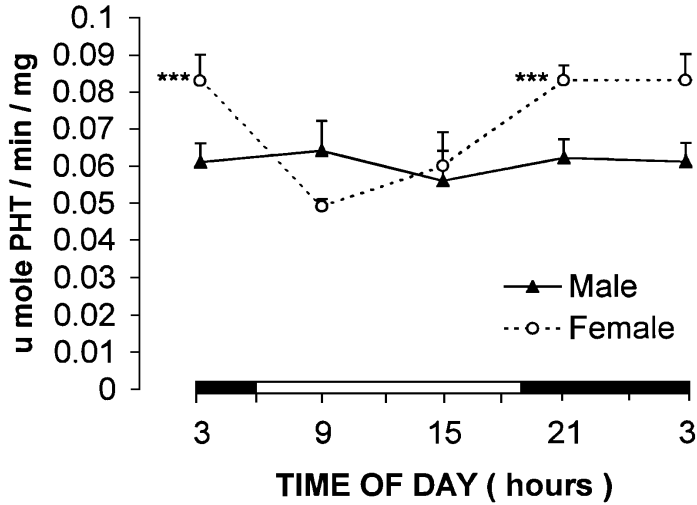


FIG. 3. Circadian variation in renal aldehyde oxidase activity. Enzyme activity was measured at 37°C and expressed as  $\mu$  mole phthalazine (PHT) oxidised/min/mg protein. Potassium ferricyanide (1 mM) was used as an electron acceptor. Each point represents the mean + SE of five or seven animals.

\*\*\*P < 0.0005

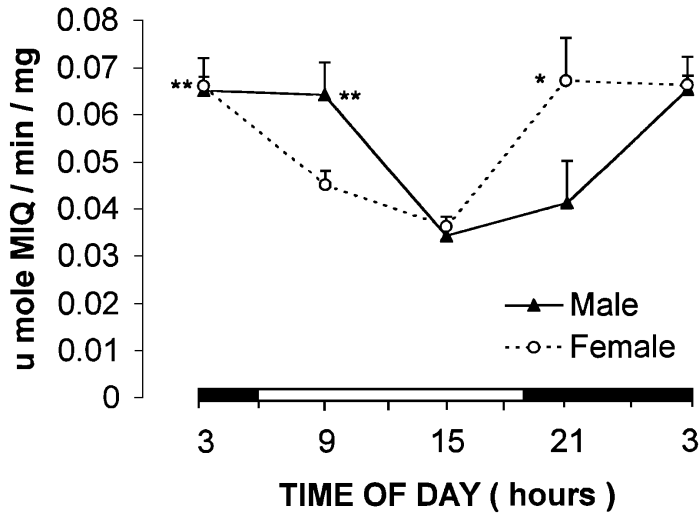


FIG. 4. Circadian variation in renal aldehyde oxidase activity. Enzyme activity was measured at 37°C and expressed as  $\mu$  mole 3-methylisoquinoline (MIQ) oxidised/min/mg protein. Potassium ferricyanide (1 mM) was used as an electron acceptor. Each point represents the mean + SE of five or seven animals.

\*P < 0.01

\*\*P < 0.03

\*\*\*P < 0.005

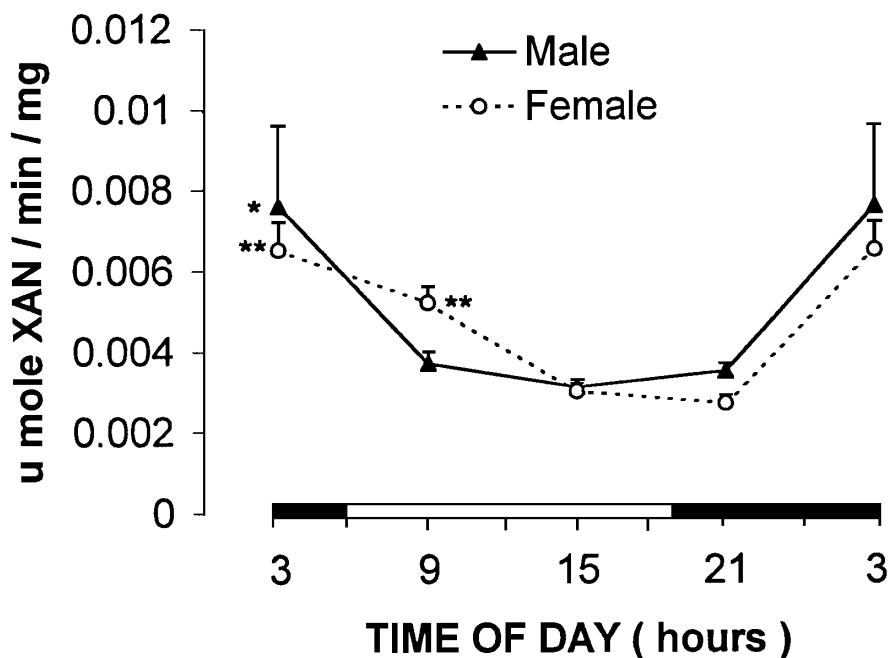


FIG. 5. Circadian variation in renal xanthine oxidase activity. Enzyme activity was measured at 37°C and expressed as  $\mu$  mole xanthine (XAN) oxidised/min/mg protein. Oxygen was used as an electron acceptor. Each point represents the mean + SE of five or seven animals.

\*P < 0.03

\*\*P < 0.005

about their study is worth to be mentioned. That is, the occurrence of maximum enzyme activities was different as for the time. It can be noticed from Fig. 1 and 2 that the activity of male Dhubb hepatic aldehyde oxidase with phthalazine, and 3-methylisoquinoline is significantly higher than that of female ( $P < 0.003$ ). In addition, male Dhubb xanthine oxidase activity is slightly higher than female enzyme activity (Fig. 5). These results are similar to the data reported in mice, rat and guinea pig, in which male aldehyde oxidase and xanthine oxidase activities are higher than female enzymes<sup>[14,18,20]</sup>. This increase in male enzymes has been attributed to higher testosterone levels<sup>[14,18]</sup>.

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## الإيقاع اليومي لإنزيم الألدهايد أو كسيديز وإنزيم الزنثين أو كسيديز في كبد و كلية ذكور وإناث الضب (*Uromastix microlepis*)

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المستخلص . تم في هذا البحث تعيين نشاط كل من إنزيم الألدهايد أو كسيديز في تحضير إنزيمية نصف نقية من أكباد ذكور وإناث الضب ، ثم جمعت هذه الأعضاء في أربع أوقات مختلفة على مدى أربع وعشرين ساعة . كان أقصى نشاط لإنزيم الألدهايد أو كسيديز الكبدي في ذكور وإناث الضب عند الساعة التاسعة مساءً وكان أدنى نشاط عند الساعة الثالثة صباحاً ، وذلك باستخدام الثلاثين و ٣ - ميثايل ايزو كوينولين كمواد يعمل عليها هذا الإنزيم . هذا وكان الفرق بين النشاط الأعلى والأدنى لهذا الإنزيم معنوياً ( $P < 0.05$ ) . ولم يلاحظ أي تموج في نشاط الألدهايد أو كسيديز الكلوي في الذكور ، بينما كان التغير اليومي في نشاطه تغيراً معنوياً في الإناث ( $P < 0.05$ ) باستخدام الثلاثين . وعند استخدام ٣ - ميثايل ايزو كوينولين كمادة يعمل عليها إنزيم الألدهايد أو كسيديز الكلوي لوحظ انخفاض في نشاطية هذا الإنزيم في كل من الذكور والإناث عند الساعة الثالثة ظهراً ، وكان الفرق بين أعلى وأدنى نشاط لهذا الإنزيم في كل من الذكور والإناث معنوياً ( $P < 0.05$ ) . كما لوحظ إيقاع يومي لإنزيم الزنثين أو كسيديز الكلوي باستخدام الزنثين كمادة تفاعل . هذا وكانت قمة نشاط هذا الإنزيم عند الساعة الثالثة صباحاً ، أما أقل نشاط له فكان بين الساعة الثالثة ظهراً والتاسعة مساءً وكان الفرق بين أعلى وأدنى نشاط فرقاً معنوياً ( $P < 0.03$ ) .