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Physiochemical and histological study on the effect of the hibernation on the liver of *Uromastyx acanthinura* (Bell, 1825).

Yousef K.A. Abdel-Hafid[1]

[1]*Department of Zoology, Faculty of Science, Omar Al-Mukhtar University, Libya. Email:* ***Youssef.kh34@yahoo.com***

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Abstract

This study described the changes in the liver of *Uromastyx acanthinura* (Bell, 1825) males and females during hibernation and activity seasons. The results revealed that, hibernation causes increase fatty liver and pigment cells with abundant damage, comparing with nearly normal structure and less fatty liver after the hibernation with almost normal pattern. Genomic DNA showed apparent separation during hibernation. Also, caspase3 and caspase7 activity reached a high level in the liver tissue during hibernation comparing with activity season.

الملخص العربى

وصفت هذه الدراسة التغيرات في كبد الضب الليبي إثناء موسمي السبات والنشاط , وأظهرت النتائج أن السبات يتسبب في زيادة دهون الكبد والخلايا الصبغية مع ضرر كبير في خلايا الكبد مقارنة مع تركيب عادي ودهون اقل وشكل عادي للخلايا في فصل النشاط , وأظهرت الدراسة ايضا تجزؤ الحمض الاميني ال دي ان أي كما وصلت إنزيمات الموت الخلوي إلى اعلي مستوياتها في موسم السبات مقارنة بفصل النشاط.

Keywords: *Hibernation , histology of liver, DNA fragmentation , caspase 3 – caspase 7.*

1. Introduction

Hibernation is a form of hypothermic dormancy that allows animals to escape unfavourable climatic regimes and periods of unavailable food resources. Because the

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metabolic rate of a tissue is reduced when the tissue temperature is reduced hypothermia reduces the thermostatic component of an animal energy budget.

In ectothermic vertebrates, increases in ambient and ground temperatures during spring are thought to play a role in initiating emergence from winter dormancy and subsequent reproductive behavior (Macartney *et al*., 1989; Grobman, 1990; Crawford, 1991). Temperature plays an important role in various aspects of the life history, ecology, and physiology of reptiles and other ectotherms (Angilletta *et al*., 2002). Growth rates (Arnold and Peterson, 1989; Avery, 1994; Litzgus and Brooks, 1998a), reproduction (Schwarzkopf and Shine, 1991; Litzgus and Brooks, 1998b; Rock and Cree, 2003), seasonal activity patterns and habitat use (Webb and Shine, 1998; Whitaker and Shine, 2002), and geographic distribution (Castonguay *et al*., 1999) are all influenced by environmental temperatures. Physiological processes such as metabolic rate generally increase with temperature (Gatten, 1974; Bennett and Dawson, 1976; Beaupre *et al*., 1993; Karasov and Anderson, 1998; McNab, 2002); However, few reptile species are known to have plateaus of temperature-independent metabolism (Waldschmidt *et al.,* 1987). Information on how temperature affects metabolic rates is useful for the development of models that describe the energy budgets of organisms (Lillywhite, 1987; Secor and Nagy, 1994; Beaupre, 1995, 1996), which in turn strongly influence life histories (Congdon *et al*., 1982; Dunham *et al*., 1989). The study aimed to illustrate the effects of seasonal variations on the liver structure and function of *U. acanthinura* (Bell 1825).

2. Material and Methods

A total of 32 adult animals, divided into two groups each group of 16 to each season, 8 Male and 8 female of *U. acanthinura* were caught from south Libya. They were brought directly to the laboratory, from their natural habitats. Specimens were divided into two groups based on the seasons, activity season (Summer: late June to mid of July) and hibernation season (Winter: late November to mid of January).

2.1. Light microscopic investigations

During the two seasons, the collected specimens dissected and liver was immediately fixed in 10% normal saline. The specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylol, mounted in molten parables 58-60 °C . Serial 5µm thick histological sections were cut stained in Mayer's hematoxylin and eosin and processed for investigation under bright field light microscope and photographed.

2.2. DNA Fragmentation Assay

DNA fragmentation was assayed by a modification of the method of Arends *et al*., (1990) and Bortner *et al*., (1995). Freshly isolated specimens were washed twice with ice-cold PBS and suspended in 100 ml of lyses buffer (10 mM Tris HCl/10 mM EDTA/0.5% Triton X-100, pH 8.0), vortex-mixed, sonicated, and incubated on ice for 20 min. After centrifugation for 20 min at 4°C 14,000 rpm the supernatant containing fragmented (soluble) DNA was transferred to another tube. Lyses buffer (100 ml) was added to the pellet containing insoluble DNA. Both samples were treated with RNase A (0.5 mg/ml) for 1 hr at 37°C and then with proteinase K (Sigma, 0.4 mg/ml) for 1 hr at 37°C. After adding 20 ml of 5M NaCl and 120 ml of isopropanol, the samples were incubated overnight at 220°C, and the DNA concentrations were determined. Fragmented DNA was calculated as 100% X soluble DNA/ (soluble+insoluble DNA). The soluble fraction of DNA was determined by electrophoresis on 1.5% agarose gel and has a ladder-like appearance.

2.3. Caspase 3

Caspase 3 was determine using ELISA kit of Uscn Life Science Inc. Wuhan Cat. No.: E0449Ra. Caspase-3 is a member of the caspase (cysteine aspartate protease) family of proteins, and has been shown to be an executioner protein of apoptosis. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis.

2.4. Caspase 7

It is determined colorimetrically using Stressgen Kit (catalogue No. 907-013). Cells that are suspected to or have been induced to undergo apoptosis are first lysed to collect their intracellular contents. The cell lysate can then be tested for protease activity by the addition of a caspase-specific peptide that is conjugated to the color reporter molecule p-nitroaniline (pNA). The cleavage of the peptide by the caspase releases the chromophore pNA, which can be quantitated spectrophotometrically at a wavelength of 405 nm. The level of caspase enzymatic activity in the cell lysate is directly proportional to the color reaction.

2.5. Statistical analysis

Data were presented as means ± standard error (SE). The statistical analysis was performed with multi-variant analysis of variance (MANOVA) using SPSS (version 13) software package for Windows comparing the multivariations between the groups. *F-test* was calculated and considered statistically significant at $p < 0.05$

3. Results and Discussion

3.1. Histological observation of liver

By light microscopy it observed that, during two hibernation seasons the fatty liver was more abundant with damage, of which restored nearly normal structure in spring and almost normal pattern in summer. Light microscope observations revealed that during hibernation, hepatic cells were less homogeneous in size and more damaged, particularly in female (Fig.1).

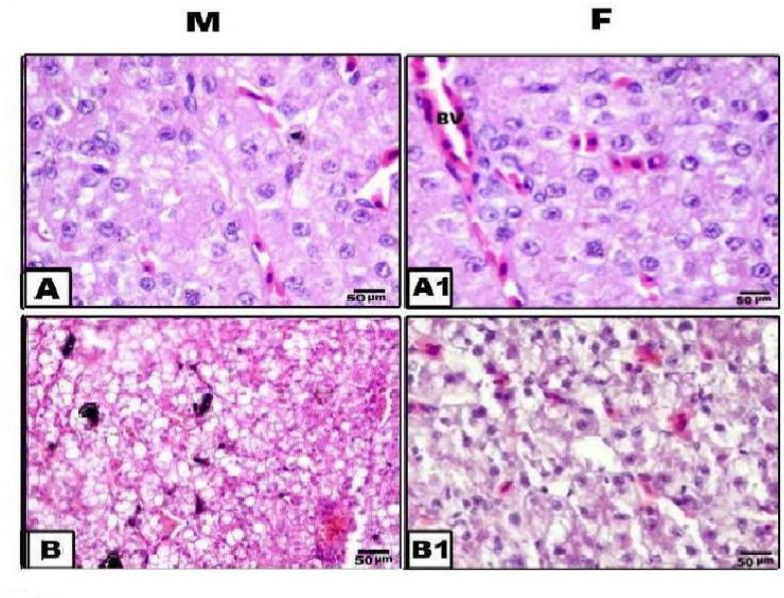

Fig. 1. Photomicrographs of histological sections of *Uromastyx acanthinura* liver, male (M) & female (F), during two seasons, Activity season, (A&A1) showing nearly normal structure and almost normal pattern with less abundant fatty. Hibernation season (B&B1) showing fatty and pigmented liver cells with more abundant damage particularly in female.

3.2. Liver DNA fragmentation

Fig. 2 illustrates the liver DNA fragmentation of males and females *U. acanthinura*. The genomic expression of the degree of laddering (total DNA fragmented) increased and more expressed during hibernation of both male and females. Males showed

highest degree of genomic DNA fragmentation. There was no detected genomic DNA damage during the season of activity.

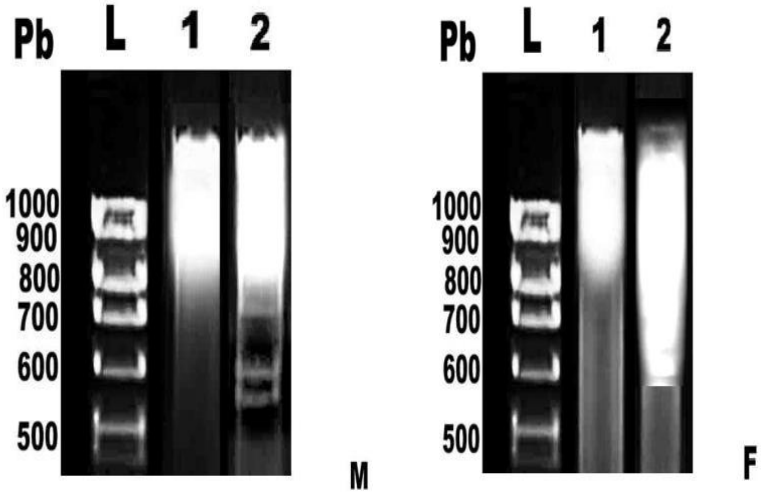

Fig. 2. Male and female of *Uromastyx acanthinura*, DNA fragmentation, Lane (L) Lader, Lane (1) Active season, Lane (2) representing hibernation, Genomic DNA showed apparent separation during hibernation, particularly in female.

3.3. Caspase 3 and Caspase 7

Table 1. and figs (3 and 4) illustrates the changes of liver caspases of both male and female *U. acanthinura*, during activity and hibernation seasons. Caspases 3 and 7 were markedly increased reaching highest level during hebernation season comparing with the activity season.

Table 1. and figs (3 and 4) illustrates the variations of caspases of both male and female of *U. acanthinura* during activity and hibernation seasons. The assayed caspases enzymes were markedly increased reaching highest level during hibernation season. However, during activity season there were a marked declining of the enzymes activities. There were no wide variations of the enzyme activities between both sexes.

Table 1. Changes of liver caspases of both male and female *Uromastyx acanthinura*, during activity and hibernation seasons.

Caspases/Sex	CAS 3		CAS 7	
	M	F	M	F
Activity Season	0.61±0.05	0.60±0.06	0.62±0.04	0.56±0.05
Hibernation Season	0.98±0.04	0.97±0.07	0.92±0.04	0.96±0.04
F-test	55.38	65.40	74.34	65.76
p≤0.05	S.	S.	S.	S.

(Mean±SE), Significancy at p. < 0.05; M:. Male; F: ,.Female .

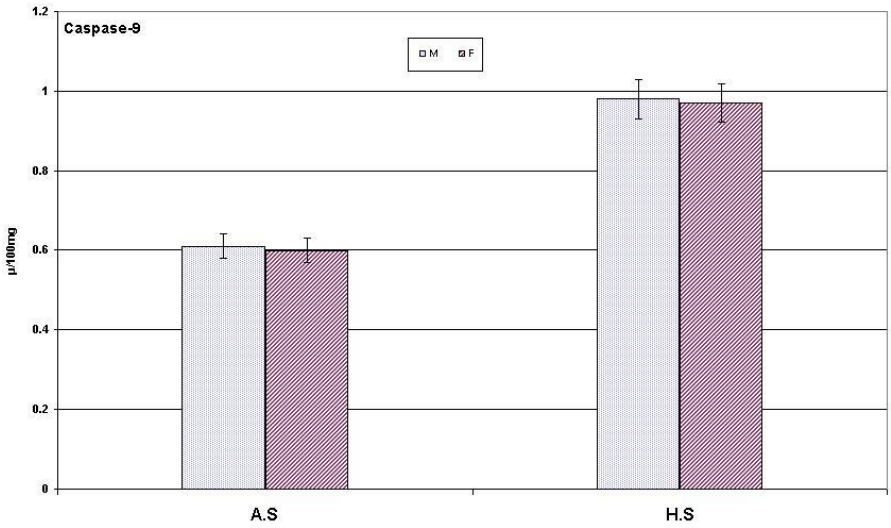

Fig. 3. Changes of Caspase-3 activity in liver of male and female of *Uromastyx acanthinura*, during activity season (A.S) and hibernation season (H.S).

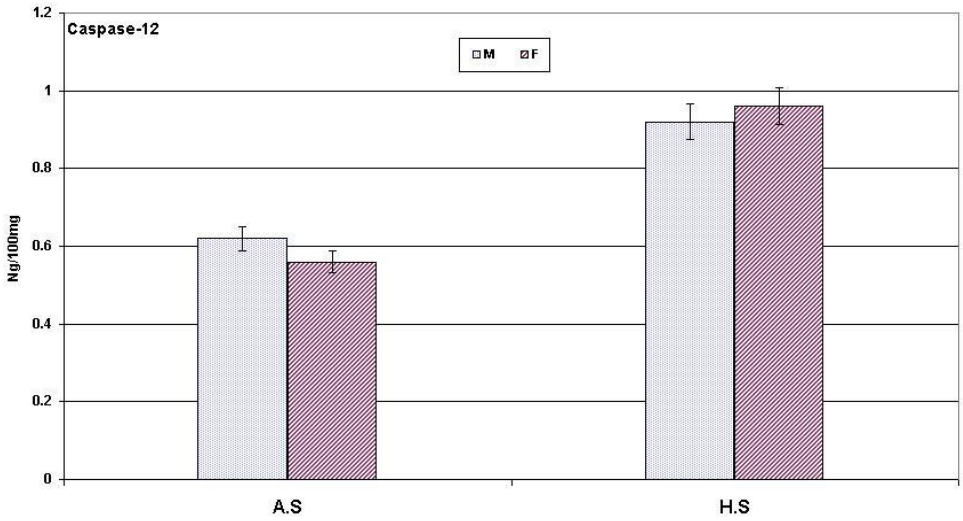

Fig. 4. Changes of Caspase-7 activity in liver of male and female of *Uromastyx acanthinura*, during activity season (A.S) and hibernation season (H.S).

4. Discussion

U. acanthinura often known as the North African Spiny-tailed Lizard, is a medium sized lizard occurring in desert habitats of north-western Africa, and the northern part of western Libyan desert .

The ecology and physiology of *U. acanthinura* in Libya is still little studied, although the amount of information on the subject has increased considerably within the last ten years. This lack of knowledge hampers understanding of how ecological and physiological differences may arise as a result of the environmental changes in terms of seasonal variation.

Hibernation in lizards is an evolutionary adaptation to harsh environmental conditions, such as cold weather and starvation. The decrease in body temperature is associated with profound reductions of blood flow, oxygen delivery (Frerichs *et al.*, 1994), and glucose utilization (Frerichs *et al.*, 1995) in body organs and in particular the brain and liver.

Hepatic cells and structures during hibernation reflected the reduced metabolic activity of *U. acanthinura*. In addition, these changes illustrated the drastic edematous lesions and damage of the natural cells especially hepatic cells in liver.

The observed hepatic cells damage was confirmed by assayed hepatic cells segregation of double helical DNA fragmentation during hibernation as well as of increased caspase 3 and 7 activities, similar observations were previously reported on the snake *Eryx colubrinus* and the lizard *Eumeces schneideri* by Abdel-Raheem *et al.,* (1989 a) and on the ground squirrel *spermophilus tridecemlineatus* by Squire and Andrews (2003). The maintenance of a minimal DNA content in liver and kidney during the hibernating cycle might be responsible for retardation of protein biosynthesis is such organs.

These results run in agreement with the previous studies of Abdel-Raheem *et al.,* (1989c). Also it might be responsible for declining mitotic index of cells. Studies performed by Kruman *et al.,* (1986) indicated that hibernation induced a decline in DNA synthesis in intestinal crypt cells ground squirrels. Also Abdel- Raheem *et al.,* (1989 a) reported that, the decreases in DNA content was observed in different tissues of two reptilian species, *E. colubrinus* and *E. schneideri* during the hibernating season.

According to Cicero *et al*., (1989) and Zuasti *et al*., (1990)., in heterothermic vertebrates, Extra-cutaneous melanin containing cells may be found in various tissues and organs such as the kidney, liver, spleen and lungs, during hibernation.

5. Conclusions

It can be concluded that the biological change of body temperature during seasons of *U. acanthinura* affected the biological structure and function of liver and leading to all changes through all assayed hormonal secretion, hepatic cells structures, and DNA biosynthesis.

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