



Research Article

Effect hibernation on liver and oviduct of (*Testudo graeca cyrenaica* Linnaeus, 1758)

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Abstract: This study described the effects the hibernation in the structure and functions of liver and oviduct of (*Testudo graeca cyrenaica* Linnaeus, 1758) during hibernation season and activity season, the result revealed that, histological section of oviduct during hibernation season showing atrophy and degeneration of the glandular region, comparing with nearly normal structure after the hibernation, one the other hand, the histological section of liver showing fatty and pigment liver cells were more abundant with damage particularly in female hibernation season while nearly normal structure and almost normal pattern with less abundant fatty after hibernation season.

Keywords: hibernation; liver; oviduct; *Testudo graeca cyrenaica*

Introduction

Hibernation is a state of inactivity and metabolic depression in animals, characterized by lower body temperature, slower breathing, and/or lower metabolic rate. Hibernating animals conserve energy, especially during winter when food supplies are limited, tapping energy reserves, body fat, at a slow rate (Abdalhafid, *et al.*, 2012). Hibernation, ectotherms become dormant in winter simply because they are unable to maintain normal metabolic rates at reduced ambient temperatures occurring at that time of the year Arousal in hibernating mammals leads to restoration of euthermic temperature, metabolic rate, and gas exchange and occurs periodically even as ambient temperatures remain low, whereas body temperature, metabolic rate, and gas exchange of hibernating ectotherms are tightly linked to ambient temperature (Milsom and Jackson, 2011). Temperature plays an important role in various aspects of the life history, ecology, and physiology and histology 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. The present study aimed to illustrate the effects the hibernation on liver and oviduct of *Testudo graeca Cyrenaica* Linnaeus, 1758.

During two seasons, hibernation season (winter), and activity season (summer), (Summer: late June, to mid of July) and (Winter: late November, to mid of January). We collected twenty mature individuals of *Testudo graeca* Cyrenaica Linnaeus, 1758) (ten animals to each season) then brought directly to the laboratory.

Light microscopic investigations

During the each season, we collected specimens and dissected and liver and oviduct were immediately fixed in 10% normal saline. The specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylol, mounted in molten paraplast 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.

Results

The shell of *T. granaica* Cyrenaica contains of dorsal carapace and ventral plastron, and the carapace has thick black lines, males are smaller than females Fig. (1, A & B).

Material and Methods

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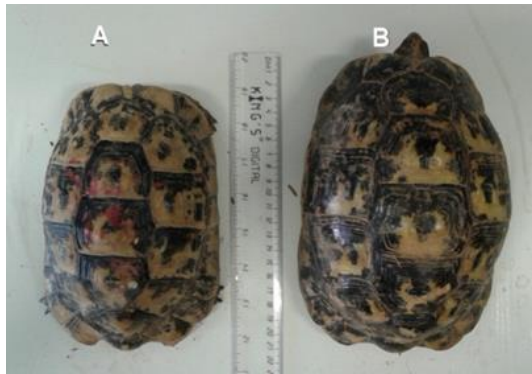


Fig. (1. A male and B female) Photograph showing morphological shape of the adult tortoise (*T. g. Cyrenaica*).

The Viscera of the tortoise (*T. g. Cyrenaica*). Fig. (2) Showing morphological structure of some organs.



Fig. 2. Photograph showing the Viscera of the tortoise (*T. g. Cyrenaica*). 1- Head, 2- Pectorals muscles, 3- The diaphragm, 4- Hart, 5- Right lobe of liver, 6- Mid lob of liver, 7- Left lobe of liver, 8- stomach, 9- Large intestine, 10- ovary, 11- Small intestine, 12- muscles and 13- opening of cloaca.

Effect of hibernation on liver

The chelonian liver acts in the metabolism of lipids, glycogen and protein. It is responsible by the degradation of nucleotides for uric acid excretion, and acts as the main storage of fat in the body. Its functions may change during events such as hibernation and reproduction in females, when vitellogenesis and protein synthesis increase, and the liver may be larger and with changes in color and texture. Such variations are important differentials for primary or secondary liver diseases Figure 3.

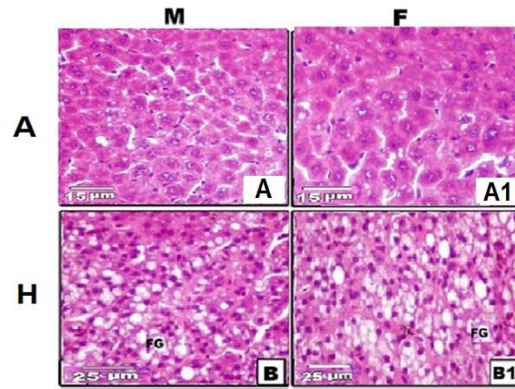


Fig. 3. Photomicrographs of histological sections of adult tortoise (*T. g. Cyrenaica*) liver. male (M) & female (F), during two seasons, Activity season(S), (A&A1) showing nearly normal structure and almost normal pattern with less abundant fatty. Hibernation season (W) (B&B1) showing fatty and pigment liver cells were more abundant with damage particularly in female.

Effect hibernation on observation of oviduct

By light microscopy it observed that, during activity season, showing normal structure and less hypertrophied glandular mucosa, on the other hand the oviduct showing atrophy of the glandular mucosa and degeneration of the glandular region in hibernation season (Hx-E). (Fig.4)

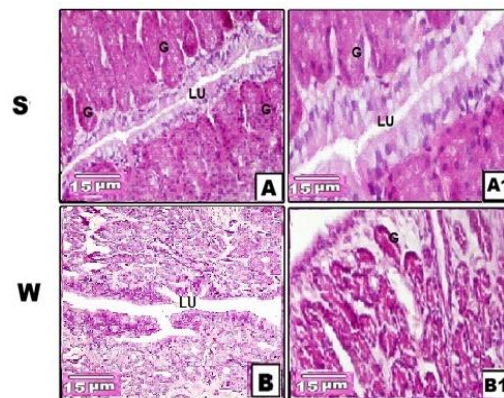


Fig.4. Photomicrographs of histological sections of adult tortoise (*T. g. Cyrenaica*) oviduct during annual cycle. Hx-E, during two seasons, Activity season, (A&A1), showing normal structure and less hypertrophied glandular mucosa. Hibernation season (B&B1) atrophy of the glandular mucosa and degeneration of the glandular region. Abbreviations E, epithelium, G, glandular mucosa, LU, lumen.

Discussion

The ecology and physiology of *T. g. Cyrenaica* (Linnaeus 1758) in North Africa especially 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 environment change in terms of seasonal variation.

The desert Tortoise is a member of the reptile family that is composed of snakes, lizards, crocodiles and other chelonians (turtles). Turtles are obviously different from other reptiles by the shell or box that completely covers the body. The shell is actually a part of the body and hardens about three years after hatching (Muro *et al.*, 1998).

Hibernation in reptiles 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.*, 1995), and glucose utilization (Frerichs *et al.*, 1995) in body organs and in particular the brain. hibernation represents a condition of metabolic depression, where homeostasis is maintained with minimal biological activities. Several regulations need to be made to support hibernation.

Similar observations were previously reported on the *Uromastix acanthinura* (Bell, 1825) by Abdalhafid, (2014). There are strong relationship between hibernation and the levels of TSH, T3, T4, whereby recorded highest levels during spring and summer seasons comparing with winter season (hibernation), during hibernation the reptiles reduce all body process to face up normal circumstances such as low temperature and lack of food, so this is reflected on most body hormones.

Derickson (1976) suggested that, the close correlation between wet fat body mass and total lipid mass in *Agama stellio stellio* may be useful in predicting total lipid mass available at different times of year by measuring wet fat body mass only, thus not destroying the remaining tissues. Tied lipid cycles to life history patterns by considering food availability as a determinant factor. As he stated, low food availability would result in a longer time span for the animal to reach reproductive size and lower quantities of stored lipids. Smaller quantities of lipids can result in lower reproductive effort, which can result in longer life span. *Agama stellio stellio* fits within this category, since it matures in its second year of life (Loumbourdis, unpublished) and stores a low percentage of lipids. Although there are no quantitative data, the environment in which *Agama stellio stellio* lives could not be considered rich in resources, thus resulting in low lipid levels. (Selcer 1987). Reported that, Fat bodies and liver masses in lizards store energy for use during times of high energetic demand such as the breeding season.

Similar findings were reported by (Abdalhafid, 2013) whereby observed that, in the liver of *U. acanthinura*, Genomic DNA showed apparent

separation during hibernation. Also, caspase 3 and caspase 9 activity reached a high level in the liver tissue during hibernation comparing with activity season. In addition, Hibernation in reptiles 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, and glucose utilization, 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.

According to (Abdalhafid *et al.*, 2012), in oviduct of *L. Stellio* found that, during spring and summer, there is a highly vascular and hypertrophy of the glandular portion which are markedly increased in size, there is a marked reduction of the luminal cavity in autumn, there was a considerable atrophy of the mucosa associated with collapsing and non-secretory function of its glandular, massive hyaline degeneration of the glandular mucosa was detected during winter season, and for protein expression, The histological changes of oviduct structures during annual cycle reflected the reduced metabolic activity, in addition, these changes illustrated the drastic damage and degeneration of the glandular region especially epithelium cells in oviduct and comparatively reduction of epithelium cells thickness during hibernation.

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