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CHANGES IN SERUM LEVELS OF TESTOSTERONE, TRIGLYCERIDES AND CHOLESTEROL, BEFORE DURING, AND AFTER HIBERNATION OF THE DESERT TORTOISE, TESTUDO GRAECA CYRENAICA.

Awatif Farag Abdulsyid Abdulkream[1*], Yousef K. A. Abdalhafid[2]
*[*1, 2] Faculty Science, Omar Al-Mukhtar University, Al Bayda, Libya*

***Corresponding Author:-**
***Email**: awatefalmesary@yahoo.com*

Abstract:-
In this study we examined the levels of testosterone, triglycerides and cholesterol, before during, and after hibernation of the Desert Tortoise .The results revealed that testosterone reached high level after hibernation when compared with the level during hibernation. On the other hand, triglycerides and cholesterol reached high levels during hibernation.

Keywords:-*Hibernation; triglycerides and cholesterol,testosterone, T.g. cyrenaica.*

INTRODUCTION

Hibernation is a state of inactivity and metabolic depression inanimals, 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 (Watts *et al.*, 1981).Hibernation in endotherms and ectotherms is characterized by anenergy-conserving metabolic depression due to low body temperaturesand poorly understood temperature-independent mechanisms. Rates ofgas exchange are correspondly reduced. In hibernating mammals, ventilation falls even more than metabolic rate leading to a relative respiratory acidosis that may contribute to metabolic depressionBreathing in some mammals becomes episodic and in some smallmammals significant apneic gas exchange may occur by passive diffusionvia airways or skin. In ectothermic vertebrates, extrapulmonary gas exchange predominates and in reptiles and amphibians hibernating underwater accounts for all gas exchange. In aerated water diffusive exchange permits amphibians and many species of turtles to remain fullyaerobic, but hypoxic conditions can challenge many of these animals (Watts *et al.*, 1981).Oxygen uptake into blood in both endotherms and ectotherms is enhancedby increased affinity of hemoglobin for O2 at low temperature. Regulation of gas exchange in hibernating mammals is predominately linked to CO2/pH, and in episodic breathers, control is principally directed at the duration of the apneic period (Milsomand Jackson, 2011).Control in submerged hibernating ectotherms is poorly understood, although skindiffusing capacity may increase under hypoxic conditions. In aerated water blood pH of frogs and turtles either adheres to alphastat regulation (pH ~8.0) or may even exhibit respiratory alkalosis. Arousal in hibernating mammals leads torestoration 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 (Milsomand Jackson, 2011).

This is study aimed to study Changes in serum levels of testosterone, triglycerides and cholesterol,before during, and after hibernation of the Desert Tortoise ,*Testudograecacyrenaica*.

MATERIAL AND METHODS

Experimental animals:

A total of forty adults , 20 male and 20 female of Desert Tortoise Testudograeca (*T. g. Cyrenaica*) were collected from Al jable Al Kder about 25 km^2, (Al-Blanj in south of Omer Almkhtar University - Al-Bayda city eastern Libya) during winter and summer season between 2016 and 2017 and used in the present study. Once caught, the animals were anesthetized with diethylether and blood was collected and centrifuged at 3000 g for 5 minutes and serum collected and kept in refrigerator at -80ºC.

Determination of serum triglycerides:

Principle:

Concentration of triglycerides was determined according to the method described by Fossati and Prencipe (1982), using biodiagnostic kit according to the following reactions:

Lipase

Triglycerides → Glycerol + fatty acids

Glycerokinase

Glycerol + ATP → Glycerol-3-phosphate + ADP

Glycerol-3-phosphate oxidase

Glycerol-3-phosphate → Dihydroxyacetone phosphate + H_2O_2

Peroxidase

$2H_2O_2$ + 4-chlorophenol + 4-Aminoantipyrine → Quinoneimine

Determination of serum total cholesterol:

Principle:-

Total cholesterol was determined using biodiagnostic kit reagents and according to the method described by **Allain*et al.* (1974)**, and the following reactions.

CholesterolEsterase

Cholesterol-ester + H_2O → Cholesterol + Fatty acid

CholesterolOxidase

Cholesterol + O_2 → Cholestene-3-one+H_2O_2

Peroxidase

$2H_2O_2$ +phenol +4-Aminoantipyrine → quinoneimine +$4H_2O$

RESULTS:

Order Chelonia (Testudines) or turtles:

Desert Tortoise divided into two species in Libya *Testudograeca* (*T.g.Cyrenaica*) and Egyptian Tortoise

Testudokleinmanni(marwa,2017)

T. g. Cyrenaica species distributing in Cyrenaica in eastern Libya specially in AlJabal al Akhdar, Tubruk , Al-marj and AL-tmemy, while the range and the status of the Egyptian tortoise *Testudokleinmanni* in Libya are poorly known due to the country's political isolation. Once contiguous with Egyptian populations, now virtually extinct, the species ranges continuously from the eastern border nearly to Tripoli in the west. Contrary to previous indications, *T. kleinmanni* is absent from the tableland of the Jabal al Akhdar and from its northern and western coastal foothills. On the other hand, it is widespread along the coastal area of the Gulf of Sirte, continuing into Tripolitania along the coast to the vicinity of Tripoli. Evidence for the presence of *T. kleinmanni* in the JabalNafusa Range south of there does not exist (Christoph Schneider and Willi Schneider 2008).

The relationship between hibernation and serum triglycerides:

Tables (1) and figure (1) illustrates the levels of serum triglyceride in both male and female of the adult tortoise (*T. g. Cyrenaica*) during activity season (summer season) and hibernation season (winter season).The assayed the levels of serum triglyceride of both sex were markedly decreased during activity season (summer season) .while, the levels of serum triglyceride in both sex were increased in hibernation season (winter season), There was wide variations of the serum triglyceride between males and females during hibernation season.

Table (1). Shows changes of the levels of serum triglycerideof the adult tortoise (*T. g. Cyrenaica*) during hibernation season (winter season) and activity season (summer season) (Mean±SE).

	Male (Mean±SE)	Female(Mean±SE)
Hibernation season	318.0 ±4.16	321.6±4.40
Activity season	230.0±15.23	251.1±6.24
≤0.05p	s	S

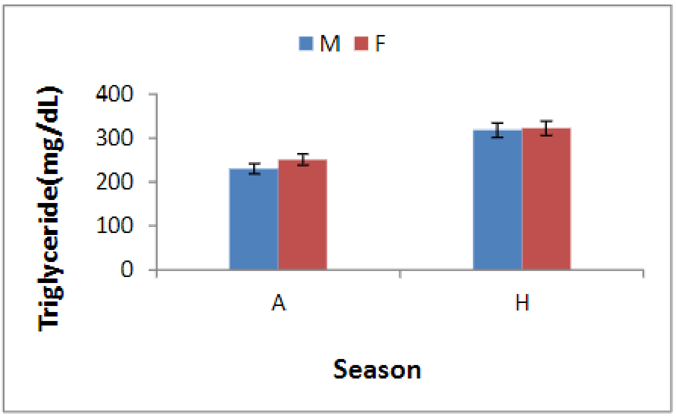

Fig.(1): shows changes of serum triglycerideof the adult tortoise (*T. g. Cyrenaica*) during A. activity season(summer season) and H. hibernation season (winter season) .

The relationship between Hibernation and serum total cholesterol:

Tables (8) and figure (12) illustrates the levels of serum total cholesterol in both males and females of the adult tortoise (*T. g. Cyrenaica*) during activity season (summer season) and hibernation season (winter season).The assayed the levels of serum total cholesterol of both sex were markedly decreased during activity season (summer season) .while, the levels of serum total cholesterol in both sex were increased in hibernation season (winter season), There was wide variations of the serum total cholesterol between males and females during hibernation season.

Table (2). shows changes of the levels of serum total cholesterol of the adult tortoise (*T. g. Cyrenaica*) during hibernation season (winter season) and activity season (summer season) (Mean ± SE).

	Male (Mean ±SE)	Female(Mean ± SE)
Hibernation season	88.0±1.52	83.3±1.76
Activity season	65.00±2.88	68.00±1.52
≥0.05p	s	s

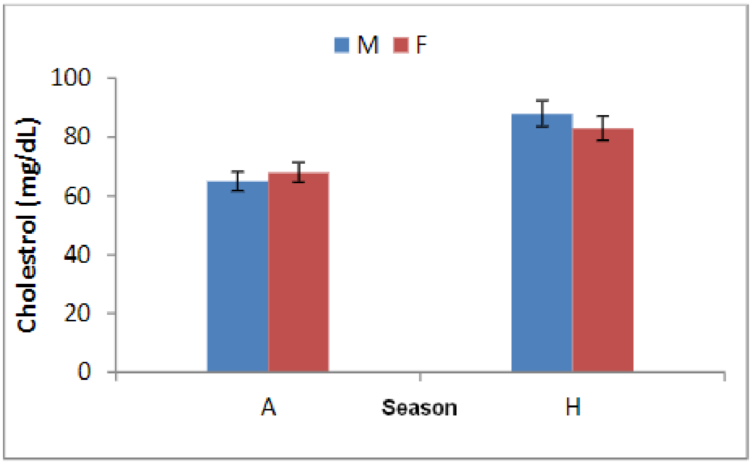

Fig.(2): shows changes of serum totalcholesterolof the adult tortoise (*T. g. Cyrenaica*) during A. activity season(summer season) and H. hibernation season (winter season) .

The relationship between hibernation and testosterone hormone (T) :

Tables (3) and figure (3) illustrates the levels of testosterone hormone (T)in male of the adult tortoise (*T. g. Cyrenaica*) during activity season (summer season) and hibernation season (winter season)The assayed the levels of testosterone hormone (T) in the male were markedly increased during activity season (summer season) . on the other hand, the levels of testosterone hormone (T) in the male were decreased in hibernation season (winter season).

Table (3). Shows changes of the levels of testosterone hormone (T)of the adult tortoise (*T. g. Cyrenaica*) during hibernation season (winter season) and activity season (summer season) (Mean ±SE).

	MALE(Mean ±SE)
Hibernation season	[a]10.0±0.57
Activity season	[b]15.6±0.88
TEST/F	28.900
≥0.05p	S

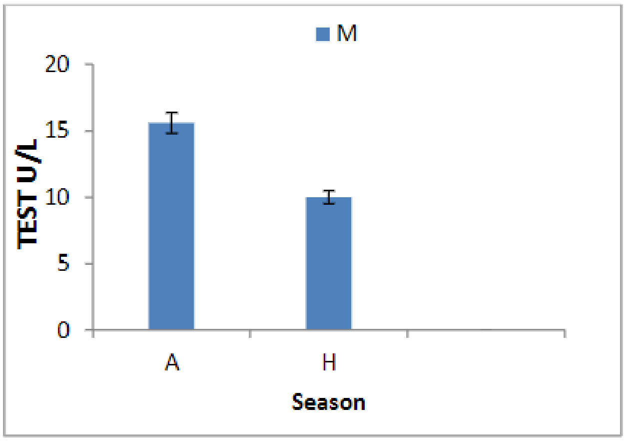

Fig. (3): shows changes of testosterone hormone (T)of the adult tortoise (*T. g. Cyrenaica*) during A. activity season(summer season) and H. hibernation season (winter season) .

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).
Desert Tortoise, often known as *Testudograeca* , is a medium–sized Tortoise occurring in desert habitats distributed in the Mediterranean basin, The division of spurthighed tortoises into subspecies is difficult and confusing. Given the huge range over three continents, the various terrains, climates, and biotopes have produced a huge number of varieties, with new subspecies constantly being discovered. Currently, at least 20 subspecies are published, as following: *T. g. graeca* (North Africa and South Spain), *T. g. soussensis* (South Morocco) ,*T. g. marokkensis* (North Morocco) , *T. g. nabeulensis* - Tunisian spur-thighed tortoise (Tunisia) ,*T. g. Cyrenaica* (Libya) , *T. g. ibera* (Turkey) , *T. g. armeniaca* - Armenian tortoise (Armenia), *T. g. buxtoni* (Caspian Sea), *T. g. terrestris* (Israel/Lebanon), *T. g. zarudnyi*(Iran/Azerbaijan) and *T. g. whitei* (Algeria) (Marwa,2017).
Hibernation is a very intricate and sensitive biological event for thereptilian species and entails different physiological mechanisms. Specificbrain regions also remain active during deep torpor (Heller, 1979), whenthe hibernator's brain temperature can drop close to the freezing point ofwater and the brain becomes electrically quiescent to surface electroencephalography (Heller & Ruby, 2004). In fact, as a hibernatorenters into deep torpor, the cortex falls 'asleep', which may be, via c-fosexpression, a result of the activation of the reticular thalamic nucleus thatsuppresses arousal activity (Bratincsak*et al.*,
2007). During hibernation,the 'biological clock' is working; the profound activity of the choroidplexus, the ependymal cells of the lateral ventricles and third ventriclesuggests that the production of the cerebrospinal fluid or
someunrecognized function of these cells might be an important factor inmaintaining torpor (Bratincsak*et al.*, 2007).
Hibernation in reptiles is an evolutionary adaptation to harshenvironmental conditions, such as cold weather and starvation. Thedecrease in body temperature is associated with profound reductions ofblood flow, oxygen delivery (Frerichs et al., 1994), and glucoseutilization (Frerichs et al., 1995) in body organs and in particular thebrain. Hibernation represents a condition of metabolic depression,where homeostasis is maintained with minimal biological activities.Several regulations need to be made to support hibernation.
According to Wade (1997), testosterone can stimulate both masculine courtship and copulatory behaviors, and estrogen and testosterone facilitate feminine receptivity. These results suggest roles for both aromatase (which catalyzes the conversion of testosterone to estradiol) and 5α-reductase (which converts testosterone to dihydrotestosterone). This study documents the presence of both enzymes in the brain of the green anole and indicates that the activity of 5α- reductase is much higher than that of aromatase in whole brainhomogenates. However, differences exist among brain regions, such that aromatase activity is higher in preoptic area/hypothalamic dissections, whereas 5α-reductase is much more active in the brain stem.
Derickson (1974) suggested that, the close correlation between wet fat body mass and total lipid mass in *Agama stelliostellio* 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 stelliostellio fits within this category, sinceit matures in its second year of life (Loumbourdis, unpublished) and stores a low percentage of lipids. Although there are no quantiative data, the environmentin which Agama stelliostellio lives could not be considered rich in resources, thus resulting in low lipid levels.
Unlike to Derickson (1974). In *Agama stelliostellio*, all the lipid depots decline during hibernation, but the most prominent one is that of fat bodies. A calculation based on the absolute values of lipids consumed during hibernation showed that females lost 190 mg from the carcass and 370 mg from the fat bodies. Taking into account that their mean wet weight was 45 g, it was estimated that fe-males consumed lipids equivalent to 1.24% of their total wet weight. Similar calculations for males showed that they consumed 720 mg of lipids which are equiv-alent to 1.26% of their total weight.
(Selcer 1987). Reported that, Fat bodies and liver masses in lizards store energy foruse during times of high energetic demand such as the breeding season.
Ramírez-Bautista (1995). Also mention that, Amphibians and reptileshave within the peritoneal cavity pairs of solid fat bodieseither anchored to the kidneys or near the rectum. Theseare important resource storehouses needed forhibernation and breeding.
DeMarco and Guillette(1992). Suggested that, Although the liver of reptiles serves an immunological role, another major function is glucose metabolism,the liver removessurplus glucose from the blood and stores it as glycogen and reptiles adopt feeding strategies that store energy within these structures in themost efficient manner.
Similar findings were reported by (Abdalhafid , 2013) whereby observed that, in the liver of *U. acanthinura*, Genomic DNA showed apparent separation during hibernation. Also, caspase3 and caspase9 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.

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