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A STUDY ON THE EFFECT OF HIBERNATION ON ANTIOXIDANT ENZYMES, OF DESERT TORTOISE *TESTUDO GRAECA CYRENAICA*

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ABSTRACT

This study described the effect hibernation on plasma Glutathione-S Transferase (GST) and Catalase (CAT) activities of Desert Tortoise *T. g. Cyrenaica* during hibernation and activity seasons, The results revealed that, The assayed (GST) and (CAT) activities of both sex were markedly increased during activity season(summer season) . However, the levels of (GST) and (CAT) activities of both sex were decreased during hibernation season (winter season) and there were variations of the (CAT) activity between males and females during hibernation season.

KEYWORDS: Hibernation; antioxidant enzymes, *T. g. Cyrenaica.*

INTRODUCTION

Hibernation is characterized by a severe decrease in oxygen consumption and metabolic demand during torpor that is punctuated by periodic re-warming periods, during which oxygen consumption increases dramatically.[1][2] The extreme physiology of torpor or the surge in oxygen consumption during arousal may increase production of reactive oxygen species (ROS), making hibernation an injurious process for lizards.[3] Moreover, fat is the preferred source of energy during hibernation, while the carbohydrates and proteins are spared. Laboratory experiments have revealed that a diet with high levels of polyunsaturated fatty acids (PUFA) is required for hibernation. The PUFA in animal cells undergo peroxidation more rapidly than other fatty acids. Lipid peroxidation is a self-sustaining chain reaction between PUFAs and ROS that produce lipid peroxides and hydrocarbon polymers that are both highly toxic to the cell. Lipid peroxidation has been reported to be increased during torpor in ground squirrels and prairie dogs.[4] However, redox state of lizard during hibernation is largely unknown. Apoptosis of animal cells increases in response to a number of pathological and stressful conditions, including lack of nutrition, ischemia-reperfusion, and oxidative stress.[5]

MATERIALS AND METHODS

Experimental animals

A total of ten animals, 5 males and 5 females mature individuals of Desert Tortoise Testudo graeca (T. g. Cyrenaica) were collected from Al jable Al Kder about 25 km2, (Al-Blanj southern of Omer Almkhtar University - Al-Bayda city eastern Libya) during winter and summer season between 2016 and 2017, 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. and subjected to following experiment.

Catalase activity measurement in plasma

Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase(HRP), remaining H_2O_2 reacts with 3,5-Dichloro-2 hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample.[6]

Glutathione-S-Transferase activity in plasma

Measurement in plasma: Glutathione-S-Transferase assay Kit (biodiagnostic) measures total GST activity (cytosolic and microsomal) by measuring the conjugation of 1-chloro- 2,4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample .[7]

RESULTS

T. g. Cyrenaica species distributing in Cyrenaica in eastern Libya specially in Al-Jabal al Akhdar, Tubruk, Al-marj and AL-tmemy, the shell of *T. g. Cyrenaica* contains of dorsal carapace and ventral plastron, the carapace in *T. g. Cyrenaica* has thick black lines Fig. (1).

Fig. (1) Photograph showing morphological shape 0f Desert Tortoise *T. g. Cyrenaica.*

Fig. (2). Photograph showing the natural habitats of the tortoise (*T. g. Cyrenaica*).

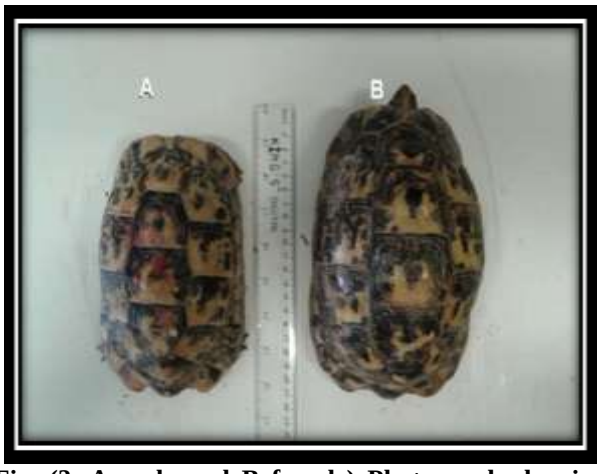

Fig. (3. A male and B female) Photograph showing morphological shape of the adult tortoise (*T. g. Cyrenaica*).

Fig. (4) Photograph showing Get blood from external carotid artery of the adult tortoise (*T. g. Cyrenaica*).

Effect of hibernation on blood antioxidant enzymes (catalase and glutathione-S-transferase)

Tables (1&2) and figures (5&6) illustrates the levels of blood antioxidant enzymes (GST) and (CAT) activities in both sex males and females of the adult tortoise (*T. g. Cyrenaica*) during activity season (summer season) and hibernation season (winter season). The assayed (GST) and (CAT) activities of both sex were markedly increased during activity season(summer season) . However, the levels of (GST) and (CAT) activities of both sex were decreased in hibernation season (winter season), There were variations of the (CAT) activity between males and females during hibernation season.

Table 1: shows changes of (CAT) activity 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	106.3±3.17	96.00±3.51
Activity season	124.0±3.05	123.0±2.64
p≤0.05	S	S

Values are given as mean ± SE for 5 animals of *T. g. Cyrenaica* in each group.

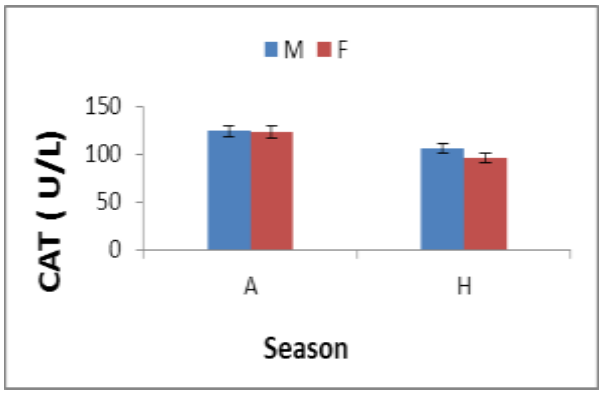

Fig.(5): shows changes of (CAT) activity in blood of the adult tortoise (*T. g. Cyrenaica*) during A. activity season (summer season) and H. hibernation season (winter season).

Table 2: shows changes of (GST) activity 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	132.3±1.20	126.0±2.64
Activity season	162.0±6.11	156.3± 6.33
p≤0.05	S	S

Values are given as mean ± SE for 5 animals of *T. g. Cyrenaica* in each group.

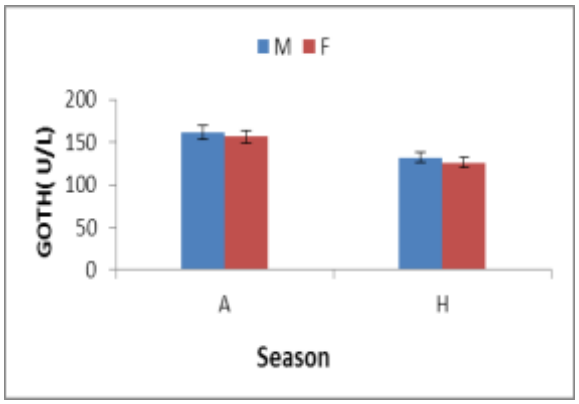

Fig (6): shows changes of (GST) activity in blood of the adult tortoise (*T. g. Cyrenaica*) during A. activity season (summer season) and H. hibernation season (winter season).

DISCUSSION

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.[8] and glucose utilization.[9] 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.

These results agree with the findings recorded[10] which reported that. The assayed catalase and glutathione-S-transferase in both sex of *Laudakia (=Agama) stellio* were markedly increased during activity season(summer season) . However, the levels of catalase and glutathione-S- transferase of both sex were decreased in hibernation season (winter season), under increased oxidative stress. This data is suggestive of an imbalance in production and scavenging of free radicals in the hibernating state. The enzymes superoxide dismutase and catalase are known to inhibit lipid peroxidation in tissues by destroying some of ROS that initiate lipid peroxidation process. It is suggested that levels of these enzymes may be decreased in vital organs during hibernation in reptiles that may lead to increased lipid peroxidation.[10]

Abdalhafid, YKA.[11] showed a decreased 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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