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Impact of Cold Stress on Haematological and Biochemical parameters of Yemeni toad (*Bufo Tihamicus*)

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ABSTRACT

Changes of some haematological and biochemical parameters of 30 Yemeni toad (*Bufo Tihamicus*) were studied after one and two hours of cold stress. The haematological parameters showed an increase after one and two hours in compare to control. The RBC count increased after one and two hours as (1.44 and 1.65) x 10^6 cells/mm^3, hemoglobin amount (Hb) as (8.6 and 9.52) g/dl, hematocrit value (HCT) as (24.6 and 27.88 %), mean cell volume (MCV) as (171.08 and 168.32 μm^3) and mean corpuscular haemoglobin concentration(MCHC) as (34.99 and 34.53 g/dl) . The studied biochemical parameters changed after one and two hours. The values of serum total protein were significantly ($p<0.05$) increased, as (5.4 and 6.8 g/dl), glucose as (76 and 74 mg/dl), cholesterol as (178 and 149 mg/dl) and GOT as (20 and 38 u/l) after one and two hour cold stress respectively, while the values of serum GPT (29 u/l)only after two hour significantly ($p<0.05$) increased.

Keywords: cold stress, *Bufo Tihamicus*, hematology, biochemistry

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INTRODUCTION

Animals have many thermal compensatory mechanisms are known, which involve changes in the hematological and biochemical parameters. Cold environment adapted fish and frogs can hardly acclimatized with the change in temperature of their environment. Their acclimatization are accompanied with some changes in their hematological and biochemical parameters. The correlation between environmental changes (such as temperature) and these parameters look a bit different in amphibians than higher vertebrates, and this explains the difference in ability of tolerance between amphibians and high vertebrates. [1,2]

The number of red blood cells increased when amphibians adapted in a cold environment, and considered it as one of the compensatory mechanisms of cold adaption. Others like showed that red blood cells count and haemoglobin content increased in cold acclimated frogs. [3,4] The liver and muscle tissue glycogen level decreased and high blood glucose increased when frogs were exposed to cold water for a short period [5]. The increase of he levels of haematological and biochemical parameters followed by environmental changes in amphibians is considered one of physiological mechanisms, which contributes to the animal adapt with sudden changes in its environment [6, 7, 8]. Several species of amphibians are able to survive under conditions of very inappropriate, such as cold temperature [9, 10]. Hematological parameters are increasingly used as indicators of the physiological stress response to endogenous or exogenous changes in fish. [11]

The internal mechanisms are important to animals that these mechanisms help the animals to adapt to their surrounding environment which is called Cryoprotector. This is happened when the low molecular weight molecules, such as glucose or glycerol seem to play an important role in the phenomenon of warranting survival of these animals during freezing [12,13,14]. Cold-adapted ectotherms have achieved this control by evolving a number of physiological and biochemical mechanisms, including production of a high level of cryoprotectants such as glucose and/or glycerol, as well as ice nucleating agents. [15]

This cryoprotector elevates body fluid osmolarity, decreasing the extent of cell volume reduction during extracellular ice formation, preventing cell shrinkage below a critical minimum cell volume and stabilizing membrane proteins [16]. Other factors are also important to promote cryoprotection like antifreeze proteins and an special system of antioxidants proteins [5].

Some studies demonstrated plasma glucose levels elevated after cold shock in fish [17,18,19] similar results observed in frogs and toads, when animals exposed to cold shock [5].

The wood frog (Rana sylvatica) is one of five terrestrially hibernating anurans known to tolerate extensive freezing of their body water. The freeze tolerance of the wood frog depends on the production of large quantities (e.g., up to 0.5 M) of glucose, a cryoprotectant that demonstrably reduces freezing injury to cells and tissues [20].

Very little is known about the freezing tolerance/intolerance of toads, which lives in the Yemeni environment. This research aims to study the effect of cold on some haematological and biochemical parameters in the Tihami toad (*Bufo Tihamicus*).

MATERIALS AND METHODS

Treatment of Animals:

30 Yemeni toad (*Bufo Tihamicus*) weighing approximately 28-30 gm each, were collected from the valley of Tuban. The toads kept in a large glass aquaria with small amounts of water that was changed twice a day. They were fed with earth worms three times a week. The toads acclimatized two weeks in the laboratory at 28°C under good ventilation before testing. Animals were divided into three groups of 10 toads each. The first group served as control group which was maintained at 28°C, while the second and the third groups of toad were transferred directly to tanks in which the water had been decreased in 20°C (cold shock from 28°C to 8°C). The water temperature were decreased by adding ice to the tanks. Then the second and the third groups were kept in cold water for one hour and two hours respectively.

Determination of Haematological and Biochemical Parameters:

The blood samples of about 5 milliliters was collected with heparinized syringe from heart puncture. RBCs were counted after diluting the blood with saline solution (0.75%) by Neubauer haemocytometer slide. The hematocrite (Hct) values were determined by microhaematocrit reader after centrifugation (4000 rpm) of microhaematocrit capillary for 5 min. The haemoglobin (Hb) content was measured according to [21]. Mean cell volume (MCV) and Mean corpuscular haemoglobin concentration (MCHC) were calculated respectively using standard formula [22]. The remaining blood was used to obtain the plasma by centrifugation (5000 rpm) for 5 min.

Plasma glucose, cholesterol, total protein, activity of enzymes (Glutamic-Pyruvic Transaminase : GPT and Glutamic -oxlate Transaminase: GOT) and blood urea was measured by UV Spectrophotometer. All estimates Biochemical solutions has been using in kit from (Spinreact, SA, Spain))

Statistical analysis of data was performed by SPSS 10.0 version for Windows. One-way analysis of variance (ANOVA) was used for the differences between groups. Differences were considered as significant when *P* value was less than 0.05. All data were expressed as means ± standard error of the mean (SEM).

RESULT AND DISCUSSION

The results were indicated in (Table 1.). These results show that the one and two hour of cold stress led to a number of RBC (red blood cell), hematocrite (Hct) and Hb (hemoglobin) indices increased significantly ($p<0.05$).The MCV (mean corpuscular volume) decreased after

one and two hour of cold stress, whereas the results of the changes in MCHC (mean corpuscular hemoglobin concentration) were not significant ($p>0.05$).

Table 1: A Summary of Cold Stress Effect on Some Haematological Parameters of Yemeni Toad (Bufottihamicus).

Parameters	Control	One Hour	Two Hour
PCV	21.8 ± 1.9	24.6 ± 1.8	27.88 ± 1.6
Hb	7.46 ± 0.52	8.6 ± 0.43	9.52 ± 0.48
RBC	1.16 ± 0.13	1.44 ± 0.1	1.65 ± 0.065
MCV	189.54 ± 22.6	171.08 ± 11.66	168.32 ± 10.5
MCHC	34.3 ± 1.9	34.99 ± 1.03	34.53 ± 1.78

Significant changes were observed in the RBC, Hb, Hct, and MCV after one and two hour of cold stress. [4] reported that the red blood cells count and haemoglobin content increased in cold acclimated frogs. [23] demonstrated that different water temperature effected on hematological parameters in blood of common carp. [24] detected the trout haemoglobin and haemocrite changed during thermo acclimation. Hematocrit and hemoglobin levels did not change either during or after the experimental cold shock, when *Brycon amazonicus* was exposed to cold shock (from 28 °C to 18 °C) [19].

Table 2: Summary of cold stress effect on some biochemical parameters of Yemeni toad (Bufo Tihamicus)

Parameters	Control	One Hour	Two Hour
Glucose (mg/dl)	45 ± 4.58	76 ± 8.88	74 ± 12.1
Cholesterol (mg/dl)	126 ± 4	178 ± 14.5	149 ±7.93
Total protein (g/dl)	4 ± 1	5.4 ± 0.72	6.8 ± 0.91
GOT (u/l)	16 ± 2.64	20 ± 3.6	38 ± 4.35
GPT (u/l)	8 ± 2	8 ± 1	29 ± 7.21

Table (2) shows that the biochemical parameters, the values of serum glucose (76 mg/dl) and cholesterol(178 mg/dl) significantly increased ($p<0.05$) after one hour of cold stress and then decreased after two hours of cold stress but they still maintained higher levels than control group. The glucose was as (74 mg/dl) and cholesterol as (149 mg/dl). The values of serum total protein (5.4 and 6.8 g/dl) and GOT (20 and 38 u/l) were significantly increased ($p<0.05$) after one and two hour cold stress respectively, while the values of serum GPT (29 u/l) significantly increased only after two hours.

The increase of blood glucose is the most studied response in this regard. It is the result of the activation of glycogenolysis that is under cortisol control [25]. Hyperglycemia during cold exposure has been reported in many species [26, 27]. In cold treated fish, increased plasma glucose is used mainly as an osmolyte [28].

Nelson et al (2007) reported that blood glucose increased when frogs were exposure to cold water for a short period.

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