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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 RBCs count was increased after one and two hours as (1.44 and 1.65) x 10⁶ cells/mm³, haemoglobin amount (Hb) as (8.6 and 9.52) g/dl, haematocrit value (Hct) as (24.6 and 27.88 %), mean cell volume (MCV) as (171.08 and 168.32 um³) and mean corpuscular haemoglobin concentration (MCHC) as (34.99 and 34.53 g/dl). 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 AST as (20 and 38 U/L) after one and two hour cold stress respectively, while the values of plasma ALT (29 M/l) increased significantly only after two hour (p<0.05). In conclusion, the changes in some haematological and biochemical parameters of Yemeni toads were response to cold water stress as adaption to their environment fluctuation.

Keywords: cold stress, Bufo tihamicus, haematology, biochemistry

INTRODUCTION:

Animals have many thermal compensatory mechanisms are known,

which involve changes in the haematological and biochemical parameters

(Prosser and Brown, 1961; Houston and De Wilde, 1969). Cold environment adapted fish and frogs (Houston and De Wilde, 1969) can hardly acclimatize with the change in temperature of their environment. Their acclimatization was accompanied with some changes in their haematological 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.

The increase in the levels of haematological and biochemical parameters followed by environmental changes in amphibians is considered one of physiological mechanisms, which contributes to the animal adaptation with sudden changes in its environment (Roofe, 1961; Leftwich & Burke, 1964; Galten & Brooks 1969). Several species of amphibians are able to survive under conditions of very inappropriate, such as cold temperature (Aarset, 1982; Storey and Storey, 1988,

1992). Haematological parameters are increasingly used as indicators of the physiological stress response to endogenous or exogenous changes in fish (Adams, 1990).

Faxon (1964) reported that the number of RBCs was increased when amphibians adapted in a cold environment, and considered it as one of the compensatory mechanisms of cold adaptation. Also, Krishnamoorthy and Shakunthala (1974) showed that RBCs count and haemoglobin content were increased in cold acclimated frogs. The glycogen level in liver and muscle tissue was decreased and blood glucose was increased when frogs were exposed to cold water for a short period (Steiner *et al.* 2000 and Nelson *et al.* 2007).

The internal mechanisms are important to animal adaptation 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 (Storey and Storey, 1984, 1985, 1986). 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 (Storey and Storey, 1988). 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 (Storey *et al.*, 1996). Other factors are also important to promote cryoprotection like antifreeze proteins and special system of antioxidants proteins (Storey *et al.*, 1996, Steiner *et al.*, 2000).

Some studies demonstrated plasma glucose levels elevated after cold shock in fish (Tanck *et al.* 2000, Chen *et al.*, 2002, and Inoue *et al.* 2008), similar results observed in frogs and toads, when animals exposed to cold shock (Steiner *et al.*, 2000). 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 quantity of glucose (up to 0.5 M), a cryoprotectant that demonstrably reduces freezing injury to cells and tissues (Costanzo and Lee, 1993). 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 Yemeni toad (*Bufo tihamicus*).

MATERIALS AND METHODS:

Treatment of animals:

30 Yemeni toads (*Bufo tihamicus*) weighing approximately 28-30 gm each, were collected from the valley of Tuban. The toads kept in 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 experiment. 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 was 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 sample of about 5 milliliters was collected from each 10 toads for each group with sterile syringe from heart puncture into heparinized tube. RBCs were counted after diluting the blood with saline solution (0.75%) by Neubauer haemocytometer slide. The haematocrite (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 Drabkin (1948). Mean cell volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calcu-

lated respectively using standard formula (Adakole, 2012). The remaining blood was used to obtain the plasma by centrifugation (5000 rpm) for 5 min. Plasma glucose, cholesterol, total protein, activities of aspartate transaminase (AST) and alanine transaminase (ALT) were measured colorimetrically in triplicate using commercial kits 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 in table (1) show that exposure of toad to one and two hour of cold stress led to increases in RBC count, hematocrite (Hct) and Hb levels ($p < 0.05$). However, MCV was decreased after one and two hour of cold stress ($p > 0.05$), whereas MCHC were not significant change. In consistence, Van (1986) reported that when the wa-

ter quality is affected by different temperature, any physiological changes will be reflected in the values of one or more of hematological parameters. These hematological indices are used to assess the functional status of the oxygen carrying capacity of the blood stream (Shah and Altindag 2004), and assess the physiological and biochemical status of fish under the stressed condition (Fernandez and Mazon 2003). Cold temperature increases the oxygen requirement (Julian *et al.*, 1989).

Krishnamoorthy and Shakunthala (1974) reported that the RBCs and Hb content increased in cold acclimated frogs. Bozorgnia *et al* (2011) demonstrated that different water temperature effected on hematological parameters in blood of common carp. Weber *et al.* (1967) detected the trout Hb and Hct changed during thermoacclimation. 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) (Inoue *et al.*, 2008).

Lombardi *et al.* (2011) showed that winter swimming, when represented by brief exposure to cold water, induces strong increase of RBC, WBC, PLT counts, Hb and Hct without affecting on MCV, MHC and MCHC. It is reported that such changes in blood cell fraction could be in part due to the reactive spleen contraction, a phenomenon which characterizes physical exercise especially when performed in stressful environmental conditions (Baković *et al.* 2003 and 2005).

The values of plasma 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 than control group. The values of plasma total protein (5.4 and 6.8 g/dl) and AST (20 and 38 U/l) were significantly increased ($p<0.05$) after one and two hour of cold stress respectively, while the values of plasma ALT (29 U/l) significantly increased only after two hours. The increase of blood glucose is the most studied response in this regard. Hyperglycemia during

cold exposure has been reported in many species (Staurnes *et al.*, 1994; Chen *et al.*, 1995). In cold treated fish, increased plasma glucose is used mainly as an osmolyte (Ulfat Jan *et al.*, 2012). Nelson *et al.* (2007) reported that blood glucose increased when frogs were exposure to cold water for a short period. Activation of glycolysis and gluconeogenesis under cortisol control was increased as result of stressful conditions of reduced water temperature (Vijayan *et al.*, 1992 and Pankhurst, 2011).

Cholesterol is very widespread in all body cells, especially nerve tissues and is main compound of plasma lipoprotein and plasma membrane. In the present study plasma cholesterol was increased after cold water shock. It has been shown that cold stress increased the cholesterol level in blood of rats due to increased of the epinephrine (Selamoğlu and Yürekli, 2005).

The increased of ALT and AST enzyme activities in the present study as a result of cold water shock. The liver enzyme AST is sensitive indicators of liver function, since it is

released into the blood under the conditions of stress (de la Torre *et al.*, 2000). The plasma AST activity was significantly increased in response to cold water shock in red sea bream (Hwang *et al.*, 2012). The changes in some hematological and biochemical parameters of Yemeni toads were response to cold water stress as adaptation to their environment fluctuation.

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Table (1): A Summary of cold stress effect on some haematological parameters of Yemeni toad (*Bufo ttihamicus*).

parameters	Control	1 hour	2 hour
Hct	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

Each value represents the mean ± SEM *p<0.05 to 0.01 compared with control value.

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*
AST (U/l)	16 ± 2.64	20 ± 3.6	38 ± 4.35*
ALT (U/l)	8 ± 2	8 ± 1	29 ± 7.21*

Each value represents the mean ± SEM *p<0.05 to 0.01 compared with control value.

تأثير جهد البرودة على المؤشرات الدموية والبيوكيميائية للضفدع اليمني (*Bufo tihamicus*)

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درست بعض المؤشرات الدموية والبيوكيميائية لـ ٣٠ ضفدع يمني (*Bufo tihamicus*) بعد ساعة وساعتين من جهد البرودة. أظهرت المؤشرات الدموية زيادة بعد ساعة وساعتين من جهد البرودة مقارنة بالشاهد حيث ارتفع عدد كريات الدم الحمراء بعد ساعة وساعتين إلى $(1.44 \text{ and } 1.65) \times 10^6$ خلية/ملم^٣، الهيموجلوبين إلى $(8.6 \text{ and } 9.52 \text{ g/dl})$ ، مستوى الهيموكريت إلى $24.6 \text{ and } 27.88$ (%، وحجم متوسط الخلية (MCV) إلى $(171.08 \text{ and } 168.32 \mu\text{m}^3)$ و الـ MCHC إلى $(34.99 \text{ and } 34.53 \text{ g/dl})$. كذلك تغير مستوى المؤشرات البيوكيميائية المدروسة بعد ساعة وساعتين من جهد البرودة، حيث ارتفع مستوى البروتين الكلي $(5.4 \text{ and } 6.8 \text{ g/dl})$ ، وسكر الدم $(76 \text{ and } 74 \text{ mg/dl})$ ، كوليسترول الدم $(178 \text{ and } 149 \text{ mg/dl})$ ونشاط AST $(20 \text{ and } 38 \text{ U/l})$ بعد ساعة وساعتين من جهد البرودة على التوالي، بينما نشاط الـ ALT اظهر ارتفاع بعد ساعتين من جهد البرودة (29 U/l) . وخلصت الدراسة إلي أن التغيرات في بعض المؤشرات الدموية والبيوكيميائية في دم الضفدع نتيجة جهد البرودة هو تكيف للتغيرات البيئية المحيطة.