

STUDY THE EFFECT OF DIFFERENT TYPES OF STRESS ON SOME BLOOD CONSTITUENTS AND PLASMA BIOCHAMICALS IN MALE RATS

Muna M. Ismail Rabab A. Naser Hanin A. Muhammed
Diyala University, College of Veterinary Medicine
Corresponding author: 73muna@gmail.com

ABSTRACT

The objective of this work was to determine and compare the physiological changes in some: blood components (packed cell volume and hemoglobin) and plasma biochemical parameters (glucose, total protein, albumin, cholesterol and triglycerides) under 3 day of different types of stress: water deprivation, starvation, overcrowding and handling stress. Twenty five male Wistar rats weighted 100-120 gm, were divided randomly into five groups: control, water deprivation, starvation, overcrowding and handling stress. On the third day of stress the animals anesthetized for blood collection; the results of blood component revealed a significant increase in PCV and a significant decrease in Hb of water deprivation group and starvation group respectively. The biochemical changes showed a significant elevation in glucose concentration of starved rats; also there was a significant increase in total proteins of starved rats and overcrowding group, beside there was a significant increase in albumin concentration of water deprivation group, finally the cholesterol and triglycerides showed a significant increase in starved rats. In conclusion the starved rats showed more changes in blood and biochemical parameters followed by water deprivation group, overcrowded and handling stress respectively.

Key words: blood component, handling, rats, starvation, stress, water deprivation.

INTRODUCTION

Physiological or biological stress is an organism's response to a stressor such as an environmental condition or a stimulus (Ulrich-Lai and Herman, 2009). According to the stressful event, the body's ways respond to stress by sympathetic nervous system activation which results in the fight-or-flight response. The body cannot keep this state for long periods of time; afterwards the parasympathetic system returns the body's physiological conditions to normal. In humans, stress typically describes a negative condition or a positive condition that can have an impact on a person's mental and physical well-being (Kloet *et al.*, 2005). Animals have to endure many stressors in their natural environments. For example, they experience food shortages, dwell in areas where predator or parasite densities are high, engage in conflicts with neighbors or group members, and face fluctuations in food and water availability and temperature (McEwen and Wingfield, 2003). Physiologists define stress as how the body reacts to a stressor, real or imagined a stimulus that causes stress. Acute stressors affect an organism in the short term; chronic stressors over the long term. General adaptation syndrome (GAS), developed by Hans Selye, is a profile of how organisms respond to stress; the general adaptation syndrome is characterized by three phases: a nonspecific mobilization phase, which promotes sympathetic nervous system activity; a resistance phase, during which the organism makes efforts to cope with the threat; and an exhaustion phase,

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which occurs if the organism fails to overcome the threat and depletes its physiological resources (Viner, 1999).

Hypothalamus-pituitary-adrenal axis: Basic hypothalamic-pituitary-adrenal axis summary (corticotropin-releasing hormone=CRH, adrenocorticotrophic hormone=ACTH).The HPA axis is a multi-step biochemical pathway where information is transmitted from one area of the body to the next via chemical messengers. Each step in this pathway, as in many biochemical pathways, not only passes information along to stimulate the next region, but also receives feedback from messengers produced later in the pathway to either enhance or suppress earlier steps in the pathway, this is one way a biochemical pathway can regulate itself, via a feedback mechanism (Aldwin, 2007).When the hypothalamus receives signals from one of its many inputs (e.g., cerebral cortex, limbic system, visceral organs) about conditions that deviate from an ideal homeostatic state (e.g., alarming sensory stimulus, emotionally charged event, energy deficiency), this can be interpreted as the initiation step of the stress-response cascade. The hypothalamus is stimulated by its inputs and then proceeds to secrete corticotropin-releasing hormones. This hormone is transported to its target, the pituitary gland, via the hypophyseal portal system (short blood vessels system), to which it binds and causes the pituitary gland to, in turn, secrete its own messenger, adrenocorticotrophic hormone, systemically into the body's blood stream. When adrenocorticotrophic hormone reaches and binds to its target, the adrenal gland, the adrenal gland in turn releases the final key messenger in the cascade, cortisol. Cortisol, once released, has widespread effects in the body. During an alarming situation in which a threat is detected and signaled to the hypothalamus from primary sensory and limbic structures, cortisol is one way the brain instructs the body to attempt to regain homeostasis – by redistributing energy (glucose) to areas of the body that need it most, that is, toward critical organs (the heart, the brain) and away from digestive and reproductive organs, during a potentially harmful situation in an attempt to overcome the challenge at hand (O'Connor *et al.*,2000).

After enough cortisol has been secreted to best restore homeostasis and the body's stressor is no longer present or the threat is no longer perceived, the heightened levels of cortisol in the body's blood stream eventually circulate to the pituitary gland and hypothalamus to which cortisol can bind and inhibit, essentially turning off the HPA-axis' stress-response cascade via feedback inhibition. This prevents additional cortisol from being released. This is biologically identified as a normal, healthy stress mechanism in response to a situation or stressor – a biological coping mechanism for a threat to homeostasis (O'Connor *et al.*, 2000).

It is when the body's HPA-axis cannot overcome a challenge and/or is chronically exposed to a threat that this system becomes overtaxed and can be harmful to the body and brain. A second major effect of cortisol is to suppress the body's immune system during a stressful situation, again, for the purpose of redistributing metabolic resources primarily to fight-or-flight organs. While not a major risk to the body if only for a short period of time, if under chronic stress, the body becomes exceptionally vulnerable to immune system attacks. This is a biologically negative consequence of an exposure to a severe stressor and can be interpreted as stress in and of itself—a detrimental inability of biological mechanisms to effectively adapt to the changes in homeostasis (O'Connor *et al.*, 2000) .

This study is made to shed the light on the stress effect on following blood parameters in adult male rat: 1.blood packed cell volume 2.Hemoglobin 3.blood glucose 4.plasma total protein 5.plasma albumin 6.plasma cholesterol 7.plasma triglycerides.

MATERIALS AND METHODS

Experimental animals and protocol: Thirty albino mature male rats have been used in this research. They were divided into five groups (5/group with the exception of overcrowding group was 10 animals). The animals weight range from (100-120) gm. They were housed in suitable cages (45× 35× 15) cm at temperature between 22-28c° and dark light cycle (12:12) in the animal house (4m× 5m) at the Collage of Vet. Medicine / Diyala University.

Experimental design: The animals were exposed to different stressful conditions for 72 hours according to their groups as belonging:

1. Control group: This group was fed ordinary pellet diet and water with normal environmental conditions.
2. Water deprivation group: Five male rats were water with held .
3. Starvation group: Ten male rats were food with held.
4. Overcrowding group: Ten male rats were kept in one cage.
5. Handling stress group: Five male rats were exposed to manual physical stress episode twice daily lasts for 5 mints.

Blood sampling: At the end third day of exposed stress and under general anesthesia (use of ketamine xylazin anesthetic) blood samples were obtained via cardiac puncture by use of disposable syringe treated with heparin. The blood samples were used first to measure PCV% and Hb gm/dL, and then centrifuged at 3000 rpm for 15 mints. The plasma used in estimation of biochemical parameters such as plasma cholesterol mg/dL by enzymatic colorimetric method, triglycerides mg/dL by enzymatic colorimetric, glucose mg/dL by a quantitative method, total proteins gm/dL by Biuret method, albumin gm/dL by Bromcresol green colorimetric method.

Statistical analysis: The data were analyzed by F test one way (Steel and Torrie, 1988).

RESULTS AND DISCUSSION

Packed cell volume (PCV): According to table 1, the results shown a significant increase in PCV of water deprivation group as compared to control, besides there was non-significant increase in other groups, since the PCV is the percent of red blood cell to plasma volume, therefore the water deprivation stress caused a reduction in plasma volume and in carcass water, this results agree with Kutscher (1971).

Haemoglobin (Hb): The data pertaining in table 1 explain there was a significant increase in water deprivation group and overcrowding as compare to control in spite of a significant decrease in food deprevated as compared to control, the increasing of Hb concentration may be come as consequences of decrease plasma volume in water deprevated rats in overcrowding group lead insufficient water supply which lead to decrease plasma volume this explanation agree with Khnissi *et al.* (2013).

Glucose concentration: Table (1) illustrated there was a significant increase of glucose in food deprivation rats as compared to control. This elevation may be due to that food

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deprivation correlated with glucose metabolism and decrease in insulin concentration and an increase in other hormones associated with increase processes of glycogenolysis, gluconeogenesis and absorption like glucagon, cortisol and thyroid hormones, this finding agree with Fevold and Petersen (1987).

Total protein concentration: Revealed there was a significant increase in the concentration of overcrowding and food deprivation groups as compared to control. These results may be attributed to that starvation lead to exhausted the energy sources (carbohydrate and lipid) and directed to protein catabolism as (proteolysis) when there is huge demand for protein for cell mass recovery especially in the gut, this outcome was agreed with Edward (1991).

Plasma albumin concentration: The present study demonstrates the elevation in albumin concentration in water deprivation group as compare to control, this result may be come from the consequences of decrease plasma volume and impairment of kidney function, this finding was agree with Abdelatif *et al.* (2010).

Plasma cholesterol and triglycerides concentration: The effect of different types of stress on the cholesterol concentration present in table 1 revealed a significant increase in cholesterol and triglycerides in the food deprivation group as compared to control. Metabolic stress like starvation involved with thyroid gland, which lead to severe increase in thyroxine hormone which increase lipolysis of adipose tissues, this result was agree with Wodzicka-Tomaszewska *et al.* (1982) and Marrino *et al.* (1987).

Table (1): Effect of different type of stress: water deprivation, food starvation, overcrowding and handling for 3days on some blood constituents and plasma biochemical parameters in male rats

Groups Parameters	Control	Water deprivation stress	Food Starvation stress	Overcrowdi ng stress	Handling stress
PCV%	32±0.979	37±0.72 A	33±0.82	35±0.34	33±0.39
Hb gm/dl	10.7±1.1	12±0.73 A	9.5±0.919 B	12.03±0.33	11.1±0.894
Glucose mg/dl	96.8±19.7	88.1±7.2	218.2±53.7 A	137.2±11.9	98.1±29.1
Total proteins gm/dl	6.44±0.403	7.1±0.100	10.6±3.409 A	10.7±0.318 A	4.3±1.00
Albumin gm/dL	4.8±0.309	6.5±1.50 A	5.1±1.179	4.9±0.503	3.1±1.19
Cholesterol mg/dL	164±16.9	178±25.5	609.1±134 A	151±11.8	221.8±10.7
Triglycerides mg/dL	327.9±44.9	506.8±53.9	804.5±38.6 A	367.23±60.7	407.4±40.0

Values expressed as mean±SE.

CONCLUSIONS

Starvation stress is more effective stress followed by water deprivation, overcrowding and handling stress respectively.

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دراسة تأثير انواع مختلفة من الاجهاد على بعض مكونات الدم والبلازما في ذكور الجرذان

منى محمد اسماعيل رباب عبد الامير حنين عز اوي محمد
جامعة ديالى / كلية الطب البيطري
الخلاصة

الغرض من هذه الدراسة كان للتحديد ومقارنة التغيرات الفسلجية في بعض مكونات الدم (حجم كريات الدم المرصوص والهيموكلوبين) المعايير الكيميائية الحيوية للبلازما (الكوكوز، البروتين الكلي، الألبومين، الكولسترول والدهون الثلاثية) لمدة 3 أيام تحت عدة أنواع مختلفة من الإجهاد، الحرمان من الماء، التجويع، الازدحام وإجهاد المعاملة. في اليوم الثالث تم تخدير الحيوانات لأجل جمع عينات الدم.

اظهرت نتائج مكونات الدم زيادة ملحوظة في حجم كريات الدم المرصوص ونقصان ملحوظ في الهيموكلوبين في مجموعة الحرمان من الماء و مجموعة التجويع على التوالي، وكانت التغيرات الكيميائية الحيوية مرتفعة بشكل ملحوظ لتركيز الكوكوز والبروتين الكلي في الجرذان المصومة، والمزدحمة وكان هنالك زيادة ملحوظة في تركيز الألبومين في مجموعة الحرمان من الماء أخيرا الكولسترول والدهون الثلاثية أظهرت زيادة واضحة في الجرذان المصومة. يمكن الاستنتاج من هذه الدراسة بأن إجهاد التجويع احدث تغيرات في مكونات الدم وفي القياسات الكيميائية الحيوية يليه إجهاد التعطيش ومن ثم إجهاد الازدحام والمعاملة على التوالي.