

The Effect of Ascorbic Acid and Selenium intake on serum Cortisol in rats Under Restraint Stress

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Abstract

Over the recent years ,vitamin C and selenium have been a clear line of defense against the effects of stress. The goal of the study is to indicate the effect of restraint stress in rats on the serum levels of cortisol, the role of vitamin C and selenium supplement in stress relief by determining their effect on serum cortisol . forty Wistar rats were divided randomly into four equal groups (n=10), all the animal except negative control exposed to restraint stress for six hours a day . the first group were supplemented with vitamin C in a dose of (50 mg/kg bw/day) orally, the second included given selenium in a dose of (0.02 µg/kg bw/day) orally, the control included ten rats . Blood was collected on 20th days, and serum cortisol were measured by enzyme-linked immunosorbent assay (ELISA). Results showed that supplement of rats with vitamin C had high significant increase in serum cortisol in stressed rat (14.65 ± 5) compared to those with no stress (8.77 ± 4) which constitute the negative control . The study also revealed that supplement of rats with vitamin C , Selenium and (vitamin C + Selenium) have highly significantly increased serum cortisol level to be (10.42 ± 3.78), (10.29 ± 4.54) and (12.77 ± 3.57) respectively compared to stressed rats . It is concluded that chronic stress restriction can result in cumulative initiation of cortisol secretion in rats. Intake of vitamin C can relieve stress by increasing decreasing cortisol .

Keywords: restraint stress, cortisol, vitamin C , selenium.

Introduction

The World Health Organization has considered stress to be the “Health Epidemic of the 21st Century” and is estimated to cost American businesses as much as \$300 billion a year ⁽¹⁾ . The effect of stress on our physical and emotional health can be devastating. More than 50 percent of individuals in the USA study felt that stress had a negative impact on productivity at work. Between 1983 and 2009, stress levels in all demographic groups in the United States increased by 10–30 per cent ⁽²⁾ .

The majority of people experience certain form of stress at any given time. The term stress was used

to describe a number of negative feelings and reactions which follow hazard or challenge cases. But the reactions to stress are not all negative. In fact ,life requires a certain amount of stress. For example, childbirth is one of the most stressful events of life. The high levels of hormones released during birth, which are also involved in the response to stress, are thought to prepare the newborn baby to adapt to the challenges of life outside the womb ⁽³⁾ .

Several studies indicate the effect of stress on the economy; there is clear evidence of economic stress posing a risk to mental health and reduces life satisfaction and behavioral alteration ⁽²⁾ . Stress does not only directly hamper well-being, but also negative effects on other areas of life which lead to economic pressures and economic hardship . vitamin C is also believed to be involved in anxiety, stress, depression, fatigue, and mood ⁽⁴⁾ .

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Oral vitamin C supplementation has been hypothesized to elevate morale as well as decrease depression and anxiety. By its antidepressant role, too, vitamin C can play antioxidant properties ⁽⁵⁾. In older people, vitamin C status has been associated with increased symptoms of depression following acute illness. In addition, the importance of vitamin C in decreasing anxiety caused by stress. Anxiety is an adaptive reaction to unknown danger, but when disproportionate to danger, it is pathological and continues beyond the presence of the stressor. Another research showed that dietary vitamin C supplementation (1000 mg / day) along with vitamins (A and E) resulted in a substantial reduction in depression scores over a 6-week period ⁽⁶⁾.

Another anti-stress element is selenium (Se); it has been shown to have a beneficial effect on mood of the selenium stage, at least if selenium is small. Low selenium status in studies has been linked despite a slightly greater frequency of depression and other adverse mood disorders such as anxiety, frustration, and selenium supplementation, mood tends to be improving ⁽⁷⁾. In the US report, high doses of selenium (226.5 µg per day) and overall mood disruption were significantly improved in clearheaded / confused, confident / unsure, and compound / anxious sub-scores. Similar findings were obtained in a double-blind cross over test conducted in the UK, where a 100 µg selenium supplement significantly reduced anxiety, fatigue and depression ⁽⁸⁾.

Material and Methods

It was experimental study, conducted March 2020 to the end of April 2020 on department of Physiology. Forty fertile adult's albino rats (*Rattus norvegicus*) were used in the present study, their ages average (10-12), the weight range was (150-300) gm, the animals were housed in the animal house in a typical situation, in well ventilated wire-plastic cages with dimensions design cages (50× 35 × 15 cm) with metal covers and containing bedding of wood shaving which was changed once per daily. The animals were maintained under controlled environment about 12- hour light and 12-hour dark with (degree of temperature 22-26 °C) and exact circumstances to the normal laboratory nutrition with profitable diet (pellets) and water provided to animals through the all-time of the experiment, For two weeks before the experiment started, to adapt rats to the new

environment none of the rats had any clinically obvious contagions, the rats were divided into four groups (A, B, C and D) with five rats for each cage.

Forty adult (male and female) lab rats were divided randomly in to four equal groups as following: -

First group: included ten rats (five male and five female) exposed to restraint stress for six hours a day, and supplemented with vitamin C in a dose of (7.2 mg / day) orally by gavage

second group: included ten rats (five male and five female) exposed to restraint stress for six hours a day, and supplemented with selenium in a dose of (0.02 µg / day) orally by gavage.

Third group: included ten rats (five male and five female) exposed to restraint stress for five hours, and supplemented with vitamin C in a dose of (7.2 mg/ day) + Selenium in a dose of (0.02µg /day) orally by gavage.

Fourth group: included five rats (three male and two female) rats exposed to restraint stress for five hours a day and given 2 ml normal saline orally by gavage and served as positive control (PC), and five other rats (three male and two female) without exposed to restraint stress, and given 2 ml normal saline orally by gavage and served as negative control (NC).

Rats were placed in the restraint cage used to produce restraint stress in a glass container (12×5 cm), for six-hour a day ⁽⁹⁾. The rats were exposed to stress between 08:30 AM and 14:30 PM for twenty days of the experiment as seen in figure (3-2) and movement of the rats was highly restricted as they are in the restraint container, negative control was not put in the restraint container all the period of experiment ⁽¹⁰⁾.

Results

All animals in the study were monitored for their feed and well-being and found healthy and active throughout the study period.

The values and statistical difference of serum cortisol at the twentieth day of experiment between rat groups are showed in table (1). According to this table, serum cortisol level has shown a high significant increase ($P < 0.01$) in stressed rat (14.65 ± 5) compared to those with no stress (8.77 ± 4) which constitute the

negative control .

The same table shows that supplement of rats with vitamin C , Selenium and (vitamin C + Selenium) have highly significantly ($P < 0.01$) increased serum cortisol level to be (10.42 ± 3.78), (10.29 ± 4.54) and (12.77 ± 3.57) respectively compared to stressed rats .

Table (1) : Differences in serum Cortisol level among rat groups at the twentieth day of experiment.

Study Groups	Total Number	Serum Cortisol (ng/dl) M \pm SD	F test (P value)
Vitamin C (50 mg/kg)	10	10.42 ± 3.78 B	2.66 (0.04)
Selenium (0.02 mg/kg)	10	10.29 ± 4.54 B	
Vitamin C + Selenium	10	12.77 ± 3.57 AB	
Negative Control (without stress)	5	8.77 ± 4 B	
Positive Control (with stress)	5	14.65 ± 5 A	

A, B : Different letters show high significant difference at $P < 0.01$

Discussion

Stress is a well known health problem that negatively affects physical and emotional status of individuals and the economy of populations and countries ⁽¹⁾. Physiologically, a wide range of hormones can be modulated in response to stress. For example, stress enhances the secretion of glucocorticoids, catecholamines, growth hormone and prolactin. Such physiological changes lead to mobilizing energy sources and tailoring individuals to their current circumstances ⁽¹¹⁾. Importantly, cortisol. Represents the corner stone in psychological stress response, which in turn controls the systemic stress response components hence, cortisol is a main contributor in long-term homeostasis ⁽¹²⁾.

Consistent with the reported data, our findings showed that cortisol level is elevated in response stress as we found a significantly elevated level of cortisol in animals subjected to stress when compared to animals in the negative control group. The mechanism by which stress induces the release of cortisol. For example, blood samples collected from stressed rat showed increased

ACTH/endorphins ratio. Elevated level of ACTH is significant characteristic of response to stress ⁽¹³⁾. Chronic use of glucocorticoid and stress induced release can modulate the activity of a wide range intercellular molecules resulting in altered cell homeostasis. It is suggested that stress can alter the response of the pituitary-adrenal axis to stress resulting in altered peptide processing or ACTH being selectively released ⁽¹²⁾. Our findings showed that vitamin C administration resulted in significantly decreased serum level of cortisol in stressed animals when compared to animals in the negative control group. Although it is studied in various models and study designs, several studies have shown a similar effect of vitamin C. For example, found that ascorbic acid (3000 mg a day) can enhance blood pressure and decrease salivary cortisol in patients exposed to acute psychological stress. Peters and colleagues observed that administration of 1000 mg of the vitamin C during a period of 8 days reduced post-race serum cortisol levels in athletes by 30 % reduction ⁽¹⁴⁾.

In terms of mechanisms by which vitamin C can reduce cortisol, several mechanisms have been proposed. Cortisol reducing effect of vitamin C may be attributed to its ability to inhibit enzymes responsible for steroidogenesis⁽¹⁵⁾. On an alternative way, cortisol released from the adrenals could be associated with oxidative stress-induced release of vitamin. Therefore, it is vitamin C supply with the vitamin may diminish its mobilization from its stores in the body, and consequently, attenuate the cortisol response⁽¹⁶⁾.

Responding to stress involves a wide range of biological processes by direct and or indirect actions of multiple systems. The hypothalamic pituitary-adrenocortical (HPA) axis plays a central biological response that is important to maintain homeostasis during dynamic changes. Both cortisol and DHEA are secreted and released by the HPA and serve as signaling molecules as a part within the hormonal cascade. In addition, they act as pleiotropic molecules centrally and peripherally⁽¹⁷⁾.

Ethical Clearance : Taken from University of Kufa ethical committee

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Conflict of Interest : Nil

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