ORIGINAL ARTICLE

COMPARATIVE EFFECT OF ALPHA TOCOPHEROL AND ASCORBIC ACID ON BODY WEIGHT OF RATS EXPOSED TO CHRONIC RESTRAINT STRESS

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Background: The stress is defined as the constellation of events, which begins with a stimulus, and subsequently results in the activation of certain physiologic systems. This study was planned to determine the comparative effect of ascorbic acid and alpha tocopherol on body weight of Sprague Dawley rats exposed to chronic restraint stress. **Methods:** This comparative experimental study was done in National Institute of Health (NIH), Islamabad. One-hundred-and-twenty male Sprague Dawley rats (mean weight 250±50 grams) were used and divided into 4 equal groups. Group I was taken as control. Group II was exposed to chronic stress, Group III was given only ascorbic acid prior to restraint stress, and Group IV was given only alpha tocopherol prior to restraint stress. Group III & IV were given prior supplementation of ascorbic acid, alpha tocopherol for 1 month. **Results:** There was a significant decrease in weight gain of rats exposed to the chronic stress as compared to control group. Individual supplementation with ascorbic acid and alpha tocopherol resulted in significant improvement of their eating habits with rats supplemented with ascorbic acid. **Conclusion:** Chronic stress has a detrimental effect on weight. This effect can be minimised by the intake of sufficient amount of antioxidants.

Keywords: Ascorbic acid, alpha tocopherol, body weight, chronic stress, cortisol, antioxidant

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INTRODUCTION

Stress is known to change body weight and food intake in animal models. Stress is a widespread problem of today's life where stressors are increasingly prevalent.[1] Best way to prevent stress in life is to adopt a healthy lifestyle which includes good nutrition, moderate exercise, sufficient sleep, meaningful work.[2] Stress is commonly known to change body weight and food intake in animals. Of the various stress models available for the study of the effects of stress, the restraint stress model is most commonly used, as it effectively expresses physical and psychological stress.[3] The restraint stress technique has also been used as an animal model of depression. Many studies have shown that restraint stress decreases food intake and body weight gain in rats.[4,5] The central regulation of body weight and food intake occurs in the hypothalamus, which encompasses multiple neuronal systems, that play important roles in the regulation of energy homeostasis.[6] The weight loss due to stress is dependent on the acute central release of corticotrophin-releasing factor (CRF)[7], but there is no continuous activation of this pathway to determine for the maintained suppression of body weight.[8,9] The CRF activates hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic nervous system, and catecholamine systems. All of these mechanisms likely to inhibit food intake and reduce body weight, but none of them is activated notably during the hours or days after exposure to repeated restraint. Moreover, the defensive role of antioxidant in reducing weight loss is still unclear. Several studies have acknowledged the valuable effects of ascorbic acid supplementation on stress induced changes.

Keeping in view the beneficial role of antioxidants, this study was designed to determine the role of individual supplementation of ascorbic acid and alpha tocopherol in preventing the deterioration in body weight due to chronic restraint stress.

MATERIAL AND METHODS

Total 120 Sprague Dawley healthy male rats weighing 250±50 grams, 60 days (8 weeks) old were obtained from National Institute of Health (NIH), Islamabad. Females, diseased, or those rats developing any disease during the course were excluded from the study. Rats were divided into 4 equal groups. Group-I was considered as control group. Rats in this group were fed with normal diet without any supplementation. They were supplied simple tap water for drinking. Rats in Group-II were given standard diet without any supplementations, and were exposed to daily stress for 15 days. Rats of Group III were supplemented with ascorbic acid in a dose of 500 mg/l added in drinking water and Group IV were supplemented with alpha tocopherol 300 mg/l supplement with Soya bean oil for one month before and during chronic stress. All animals were kept in a separate cage compartments to monitor intake of diet.

Rats of group II, III and IV were exposed to chronic stress by keeping immobilized in a mesh wire restrainer for 6 hours daily for 15 days.[9,10] Stress was determined by serum cortisol level. Samples were taken

early in the morning between 8 and 9 AM, to avoid bias due to different levels of cortisol owing to the diurnal variations.[11]

Composition of pelleted diet for rats was as under:

Wheat flour 2.85 Kg, Wheat brawn 2.85 Kg, Dried skimmed milk powder 2.0 Kg, Soya bean oil 0.5 Kg, Mollasen 0.15 Kg, Fish meat 0.15 Kg, and Table salt 0.05 Kg

This food was prepared at (NIH), Islamabad, according to the standard approved by the Universities Federation for Animals Welfare. Eating habits and behavioural changes were also observed throughout the duration of the study.

For statistical analysis SPSS-15 was used. Mean and standard deviation of body weight were calculated. The statistical significance of difference across the groups was determined by applying ANOVA followed by Post Hoc Test to find difference in various pair of groups. The difference was considered significant if $p<0.005$

RESULTS

The rats remained healthy and active throughout the study. The average intake of feed by each rat was between 15–20 grams. If the rats took more than 20 grams of diet per day, it will be considered 'improved' while less than 15 grams' intake was considered 'reduced' (Table-1). Average weight of all rats at the start was 220 grams.

The comparison of weight gains by the different groups (Table-2) showed that there was a significant decrease in weight gain of rats exposed to chronic stress as compared to control group.

Nevertheless, individual supplementation with ascorbic acid resulted in significant improvement of their eating habits as comparted to alpha tocopherol supplementation. (Table-3). Serum cortisol level when compared among different groups was the highest in group II indicating high levels of stress in that group. (Table-4).

Table-1: Comparison of body weight in different study groups of Sprague Dawley rats (gm, Mean±SD)

Weeks	Control (n=30)	Stress (n=30)	Ascorbic (n=30)	Tocopherol (n=30)	*p*
9th	235.17±7.48	234.17±7.32	244.67±7.18	238.33±5.31	<0.001
10th	261.00±6.07	261.67±7.11	282.50±5.37	271.50±6.18	<0.001
11th	279.33±6.26	279.33±7.63	299.00±6.49	291.33±6.94	<0.001
12th	300.00±5.72	299.50±7.70	320.00±6.70	303.33±7.35	<0.001
13th	313.83±6.78	306.83±7.13	330.17±6.09	328.50±11.61	<0.001
14th	320.17±6.83	309.50±7.58	362.50±6.99	305.83±33.43	<0.001

Table-2: Comparison of body weight of the groups during different weeks using Post-Hoc (Tukey) test (*p*=value)

Group Comparison	9th week	10th week	11th week	12th week	13th week	14th week
Group I and II	0.981	0.994	1.000	0.999	0.001	<0.001
Group I and III	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Group I and IV	0.389	<0.001	<0.001	0.328	0.040	<0.001
Group II and III	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Group II and IV	0.144	<0.001	<0.001	0.196	<0.001	<0.001
Group III and IV	0.005	<0.001	<0.001	<0.001	<0.001	<0.001

Table-3: Comparison of eating habits of rats in different groups

Groups (n=30)	9th week	10th week	11th week	12th week	13th week	14th week
Control	Normal	Normal	Normal	Normal	Normal	Normal
Stress	Normal	Normal	Normal	Normal	**Reduced**	**Reduced**
Ascorbic	Normal	**Improved**	**Improved**	**Improved**	Normal	Normal
Tocopherol	Normal	Normal	Normal	Normal	Normal	**Improved**

Normal: daily dietary grams; Reduced= dietary intake less than 15 grams/day; Improved= dietary intake >20 intake of each rat= 15–20 grams/day.

Table-4: Serum cortisol levels in study groups (Mean±SD)

Variable	Group I	Group II	Group III	Group IV	*p*
Cortisol	21.4±0.92	34.71±1.45	30.31±0.81 ng/ml	32.23±1.22 ng/ml	<0.001

Table-5: Statistical differences of serum cortisol between different groups using Post-Hoc (Tukey) test

Comparisons	*p*
Control and Stress	<0.001
Control and ascorbic	<0.001
Control and tocopherol	<0.001
Stress and ascorbic	<0.001
Stress and tocopherol	<0.001
Ascorbic and tocopherol	<0.001

DISCUSSION

In the present study, we examined the effects of separate supplementation of ascorbic acid and alpha tocopherol separately on the body weight and food intake of Sprague Dawley rats exposed to chronic restraint stress. Several studies have tried to establish the fact that chronic exposure to restraint stress reduces the body weight and food intake of rats.[12–14] However, the mechanisms underlying these restraint-induced fluctuations in body weight and food intake remain to be elucidated. Our results have shown that restraint stress speedily induce a marked reduction in body weight that may be due to a decreased food intake. The stress-induced decrease in body weight may be due originally to an early decrease in food intake but then may be subsequently maintained by increases in energy expenditure and body temperature during restraint. Increased serum cortisol levels suggest that physiological responses to repeated stress are associated with the stimulation of the HPA axis. Results of study

conducted by Santos *et al*[14] back our results that chronic stress causes reduction in weight gain (2.0±0.65 g/day), while Dallman *et al*[15] have documented differing results and hypothesized that chronic stress resulted in increase in weight gain (2.5±0.32 g/day). It might be due to reduction in growth hormone secretion, reduced linear growth, and sympathetic neural outflow along with reduced fat mobilization, which led to obesity.[15] This study also showed that, during exposure to restraint stress significantly decreased food intake, once the stress ended, the food intake of the stressed group returned to the normal level. Serum corticosterone levels were increased by repeated restraint stress. Effect of separate supplementation with ascorbic acid and alpha tocopherol has been documented by different studies as more potent as compared to study by Engel *et al*[16] who documented that separate use of ascorbic acid and alpha tocopherol enhanced not only the immune status by decreasing serum cortisol level but also the body weight.

The data of present study has disclosed that stress; whether psychological or physical could lead to lower the immune status of the individual. Stress is one of the important factors, which, in one way or the other, disrupts many physiological functions. Higher the intensity or duration of stress, greater will be the disruption. The use of antioxidants supplements can be one of the means by which we can prophylactically protect our body from the harmful effects of stress.

CONCLUSION

Chronic restraint stress is responsible for elevated serum cortisol level which directly or indirectly affects the food intake, and reduces the body weight. However, those animals on prior supplementation of ascorbic acid showed much improvement in food intake and weight gain as compared to alpha tocopherol supplementation.

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