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ORIGINAL ARTICLE

Combined Effect of Alpha Tocopherol and Ascorbic acid on Body Weight of Rats Exposed to Chronic Restraint Stress

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ABSTRACT

Objective: This study was planned to determine the combined effect of ascorbic acid and alpha tocopherol on body weight of Sprague Dawley rats exposed to chronic restraint stress.

Materials and Methods: The study was conducted in National Institute of Health (NIH), Islamabad. Ninety male Sprague Dawley rats (mean wt 250 ± 50 grams) were used and divided into 3 groups. Each group comprised of 30 rats. Group I was taken as control. Group II was exposed to chronic stress, whereas Group III was given combined doses of ascorbic acid and alpha tocopherol prior to restraint stress.

Results: The comparison of weight gain by the different groups revealed that there was a significant decrease in weight gain of rats exposed to the chronic stress as compared to control group. Combined supplementation with ascorbic acid and alpha tocopherol resulted in significant improvement of their eating habits.

Conclusion: Chronic stress has a detrimental effect on weight. This effect can be minimized by the intake of sufficient amount of antioxidants.

Key words: Ascorbic acid, Alpha tocopherol, Body weight, Chronic stress.

Author`s Contribution
[1] Conception, synthesis, planning of research and manuscript writing Interpretation and discussion
[2] Data analysis, interpretation and manuscript writing, [3] Active participation in data collection.

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Introduction

Stress is a widespread problem of today's society where stressors are increasingly prevalent.[1] Best way to prevent stress in life is to live a healthy lifestyle which includes good nutrition, moderate exercise, adequate sleep, meaningful work.[2] Stress is commonly known to change body weight and food intake in animals. Of the various stress methods available for the study of the effects of stress, the restraint stress model is most commonly used, as it effectively mimics physical and psychological stress.[3] The restraint stress method has also been used as an animal model of depression. Thus, many studies have shown that restraint stress suppresses food intake and body weight gain in rats.[4,5] The central regulation of body weight and food intake occurs in the hypothalamus, which contains 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 sustained activation of this pathway to determine for the maintained suppression of body weight.[8,9] CRF activates hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic nervous system, and catecholamine systems. All of these systems likely to inhibit food intake and reduce body weight, but none of them is activated

significantly during the hours or days after exposure to repeated restraint. Moreover, the preventive role of antioxidant in reducing weight loss is still unclear. Several studies have documented the beneficial 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 combined supplementation of ascorbic acid and alpha tocopherol in preventing the decline in body weight due to chronic restraint stress.

Materials and Methods

Total 90 Sprague Dawley healthy male rats weighing 250±50 grams, 60 days (8weeks) old were purchased from (NIH), Islamabad. Female rats, diseased rats or rats who developed disease during the course were excluded from the study. Rats were divided into 3 groups. Each group comprised of 30 Rats. Group-one was considered as control group. Rats in this group were fed with normal standard diet without any supplementation. They were supplied plain tap water for drinking. Rats in group-two were given standard diet without any supplementations. However, these rats were exposed to daily stress for 15 days. Rats of group 3 were supplemented with ascorbic acid in a dose of 500mg /l added in drinking water & alpha tocopherol 300mg/l supplement with soya bean oil for one month before and during chronic stress. Rats of group 2 & 3 were exposed to chronic stress by keeping immobilized in a meshwire restrainer for 6 hours daily for 15 days.[9] Stress was determined by the serum cortisol level. Samples were taken in the morning between 8.00 and 9.00 am, to avoid bias due to different levels of cortisol owing to the diurnal variations.[11]

Composition of Pelleted Diet for rats

	INGREDIENTS	Weight
1	Whea flour	2.85Kg
2	Wheat Brawn	2.85Kg
3	Dried skimmed milk powder	2.00 Kg
4	Soya bean Oil	0.50Kg
5	Mollasen	0.15Kg
6	Fish meat	0.15Kg
7	Salt (common)	0.05Kg

This diet was prepared at (NIH), Islamabad, according to the standard approved by the Universities Federation for Animals Welfare.

Eating habits and behavioral changes were also noticed throughout the duration of the study. For statistical analysis statistical package for social sciences (SPSS) version 15 was used. Mean and standard deviation of body weight were calculated. One way anova was applied to calculate p-value

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 was considered 'improved' while less than 15 grams' intake was considered 'reduced' (Table 1). Average weight of all rats at the beginning was 220 grams. The comparison of weight gains by the different groups shown in (table 2) revealed that there was a significant decrease in weight gain of rats exposed to the chronic stress as compared to control group.

Table 2: Group wise comparison of body weight (kg) of rats during different weeks of the study (n=90)

Weeks	Group 1 (n= 30)	Group 2 (n= 30)	Group 3 (n= 30)	p-value
9th	235.17±7.48	234.17±7.32	243.50±7.21	< 0.001
10th	261.00±6.07	261.67±7.11	286.00±7.24	< 0.001
11th	279.33±6.26	279.33±7.63	300.00±6.95	< 0.001
12th	300.00±5.72	299.50±7.70	322.50±6.53	< 0.001
13th	313.83±6.78	306.83±7.13	334.33±7.16	< 0.001
14th	320.17+6.83	309.50±7.58	369.57±6.03	< 0.001

Nevertheless, combined supplementation with ascorbic acid and alpha tocopherol resulted in significant improvement of their eating habits.

Serum cortisol levels were highest in group 2 followed by group 3 and then group 1 (Table 3)

Table 3: Group wise comparison of serum cortisol (n=90)

Variable	Group1 (mean±SD)	Group 2 (mean±SD)	Group 3 (mean±SD)	p-value
Cortisol (ng/ml)	21.4 ± 0.92	34.71± 1.45	24.17± 1.07	< 0.001

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

Table 1: Comparison of eating habits of rats in different group (n=90)						
Groups	**9TH week**	**10th week**	**11th week**	**12th week**	**13th week**	**14th week**
Group 1 (n=30)	Normal	Normal	Normal	Normal	Normal	**Normal**
Group 2 (n=30)	Normal	Normal	Normal	Normal	Reduced	**Reduced**
Group 3 (n=30)	**Normal**	**Improved**	**Improved**	**Improved**	**Improved**	**Normal**

Discussion

In the present study, we investigated the effects of combined supplementation of ascorbic acid and alpha tocopherol 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 changes in body weight and food intake remain to be clarified. Our results have shown that restraint stress rapidly induce a marked decrease in body weight that may be due to a reduction of food intake. The stress-induced decrease in body weight may be due initially 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 activation of the HPA axis. Results of study conducted by Santos, support our results that chronic stress causes reduction in weight gain (2.0 ± 0.65 g/day)[15], while Dallman et has documented contradictory results and postulated that chronic stress resulted in increase in weight gain (2.5 ± .32g / day). It could be due to reduction in growth hormone secretion, reduced linear growth, and sympathetic neural outflow along with reduced fat mobilization, which led to obesity.[16] Moreover, this study also showed that, while exposure to restraint stress significantly decreased food intake, once the stress ended, the food intake of the stressed group returned to the normal level. In our study, serum corticosterone levels were increased by repeated restraint stress. Effect of combined supplementation with ascorbic acid and alpha tocopherol has been documented by different studies as more potent as compared to their individual effects, in improving the immune status. Fuente et al., documented that combined use of ascorbic acid and alpha tocopherol improved not only the immune status by decreasing serum cortisol level but also the body weight.[17]

The data of present study has revealed that stress; whether psychological or physical could lead to lower the immune status of the individual. Stress is one of the major 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

The results of this study showed that chronic restraint stress is responsible for elevated serum cortisol level, which directly or indirectly affect the food intake, and reduces the body weight. However, those rats who were given prior combined supplementation of ascorbic acid and alpha tocopherol did not show that reduction.

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