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Original Research Article

Evaluation of Adoptogenic Activity of Wheatgrass Juice in Rodents

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ABSTRACT

The present investigation has been undertaken to evaluate adoptogenic activity of wheatgrass juice in various acute stress experimental animal models. In swimming endurance model, test group was treated for 14 days with wheatgrass juice (5 ml/kg & 10 ml/kg, p.o.) and compared with stress control group. Wheatgrass treated rats showed significant improvement in swimming endurance time. Pretreatment with wheatgrass juice also prevented the alterations in biochemical parameters. Meanwhile, weight of the liver, adrenal glands was significantly decreased and spleen weight was significantly increased by wheatgrass treatment. Similarly, in anoxic stress tolerance, treatment with wheatgrass juice for 14 days showed significant increase in anoxia stress tolerance time. Thus from present investigation, it can be concluded that adaptogenic nature of wheatgrass juice.

Keyword: Adoptogen; Wheatgrass juice; Swim endurance test; Anoxic stress tolerance

INTRODUCTION

In modern days stress becomes the integral part of human life. Stress basically is a reaction of mind and body against change in the homeostasis. High-stress modern living is probably the main factor causing several chronic diseases [1]. Various attempts have been made to counter the aversive effects of stress, ranging from yoga and meditation to antistress drugs, particularly the anxiolytic benzodiazepines (BDZ). However, despite claims to the contrary, these nonpharmacological and pharmacological methods appear to have limited utility [2]. Whereas, adaptogens are herbs that are non-toxic, produce a non-specific defensive response to stress, and have a normalizing influence on the body [3]. Adaptogens help the body adapt to stress, support its normal functions, and restore balance. They increase the body's resistance to physical, biological emotional and environmental stressors. They are unique from other substances in their ability to balance endocrine hormones and the immune system. Adaptogens have the most

broad-spectrum healing properties of any herbal medicines, but their unique value is that they specifically relieve stress. Perhaps the single most important property of an adaptogenic plant is the proven ability to combat stress in all forms [4, 5].

Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. Since the introduction of adaptogens, several plants have been investigated, which were once used as tonics due to their adaptogenic and rejuvenating properties in traditional medicine [6]. Shoot of *Triticum aestivum* Linn. (Hindi Name- Gehun, Kanak, Sanskrit name- Godhuma) is called as a wheatgrass, belonging to family: Gramineae. Wheatgrass, young grass of common wheat plant, is freshly juiced or dried into powder for animal and human consumption- both the forms provide chlorophyll, amino acid, minerals, vitamins and enzymes [7]. Studies have been conducted to evaluate the efficacy of wheatgrass (in form of powder or juice) in the treatment of chronic diseases like cancer [8], rheumatoid arthritis [9], ulcer [10], etc. Wheatgrass was assumed to promote physical and mental health, augment resistance of the body against disease and diverse adverse environmental factors, revitalize the body in debilitated conditions and increase longevity. These attributes are remarkably similar to the properties ascribed to adaptogens. Hence, in the present investigation an attempt has been made to screen the adaptogenic property of wheatgrass juice against immobilization and anoxic stress models.

MATERIALS AND METHODS

Animals

The male albino rats of wistar strain (180 - 200g) and albino mice of either sex (20 - 30 g) were used for the study. The animals were procured from Hindu College of Pharmacy, Guntur, Andhra Pradesh. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry condition; room temperature: 27 ± 3^{0}C, relative humidity: 65% ± 10% and 12 hours of Light/dark cycle. All the animals were fed with rodent pellet diet (Amrut Laboratories Ltd., Hyderabad)) and water was allowed *ad libitum* under strict hygienic condition. Ethical norms were strictly followed during all experiments and the study was approved by institutional animal ethical committee.

Preparation of wheatgrass juice

The seeds of wheat (*Triticum aestivum* L.) were procured from local market and washed with tap water, followed by distilled water. The seeds were soaked in distilled water for 8 h and transferred to the containers and the wheat plants were grown in soil. The wheatgrass was collected on day 10. Only wheatgrass of uniform size and shape, without injuries was selected. Twenty grams of above harvested fresh grass was grounded in a laboratory mortar and the juice was squeezed out through four layers of wet muslin cloth. The residue was twice resuspended in 3 ml of sterile water and similarly squeezed. Each day the fresh juice was prepared prior to administration [11].

Swim endurance test

Rats of either sex (180-200 g) were used for forced swim endurance stress. Group I, administered only distilled water and not subjected to stress, were 'control' animals. Group II, administered only distilled water and subjected to stress, were 'stress control', Group III and Group IV were administered wheatgrass juice (WGJ) 5 ml/kg & 10 ml/kg respectively by oral route. The treatment was given for 14 days. On 14th day one hour after last dose, the rats were subjected to swimming stress by keeping them in propylene tank of dimension (37X37X30 cm), filled with water to a height of 25 cm. Rats were allowed to swim till complete exhaustion and the endpoint was taken when the animal started drowning. The mean

swimming time for each group was calculated. Then animals were killed and blood was collected by cardiac puncture to estimate biochemical parameters like serum glucose, cholesterol, triglycerides and blood urea nitrogen (BUN). The weights of organs such as liver, spleen and adrenal glands were recorded after washing with alcohol [12].

Anoxia stress tolerance

Albino mice of either sex weighing 20 - 30 g were selected and divided into four groups of six each as Group I Negative Control (Unstressed, untreated) , Group II positive control (Stressed, received vehicle) Group III received WGJ (5 ml/kg *p.o.*) and Group IV recieved WGJ (10 ml/kg *p.o.*). Animals were treated for 14 days. At the end of 14th day one hour after the treatment, stress was induced in all mice by placing each animal individually in the hermetic vessel of 1 litre capacity to record anoxia tolerance time. The moment when the animal showed the first convulsions immediately removed from the vessel and resuscitated if needed. The time duration of entry of the animal into the hermetic vessel and the appearance of the first convulsion was taken as time of anoxia tolerance. Appearance of convulsion was very sharp end point, as delay by minute of removal of the animal from the vessel may lead to death of the same [13].

Statistical analysis

Results have been presented as Mean+SEM and the experimental groups were compared with stress control animals. The statistical analysis was done using ANOVA followed by followed by Dunnett's t test. P value of 0.05, 0.01 and 0.001 were considered as statistically significant.

RESULTS

Swim endurance test

Pretreatment with wheatgrass juice (5ml/kg and 10 ml/kg) showed significant improvement in the swimming time as compared to stress control animals (Table 1). Further a rise in blood glucose, cholesterol, triglycerides and blood urea nitrogen (BUN) levels was observed with stress control rats. Treatment with wheatgrass juice, before subjecting animals to stress showed significant decrease in glucose, cholesterol, triglycerides and blood urea nitrogen (BUN) levels (Table 2). Further, stressed rats showed decrease in the weight of the spleen and increase in liver and adrenal glands weight. Animals treated with wheatgrass juice showed significant increase in weight of spleen and decrease in weight of the Liver and adrenal glands (Table 3).

Anoxia stress tolerance in mice

Both the doses of wheatgrass juice offered significant protection against anoxia induces stress changes by increasing anoxia stress tolerance time (Table 4). The Anoxia tolerance test was determined by taking the appearance of convulsion as end point.

Table 1: Effect of wheatgrass juice treatment on swimming endurance time in swim endurance test.

Groups	Swimming endurance time (min)
Normal	---
Stress control	32.94±8.37
WGJ 5 ml/Kg	47.16±5.14*
WGJ 10 ml/Kg	59.57±6.05**

*The values are expressed as Mean±SEM, n = 6 in each group. Significance at *p<0.05, **p<0.01 when compared to stress control as determined by ANOVA followed by Dunnett's t test.*

Table 2: Effect of wheatgrass juice on biochemical parameters in swim endurance test

Group	BIOCHMEICAL ESTIMATIONS(mg/dl)			
	Glucose	Cholesterol	Triglyceride	BUN
Normal control	85.17±3.63	53.14±2.56	68.69±3.54	27.13±2.85
Stress control	128.87± 4.27	101.78±4.33	109.64±5.24	50.92±3.67
WGJ 5 ml/Kg	99.94±3.55*	78.14±1.23*	76.33±3.13*	37.81±3.56*
WGJ 10 ml/Kg	87.13±2.08**	59.78±3.57***	68.37±2.87**	31.13±1.57**

The values are expressed as Mean±SEM, n = 6 in each group. Significance at *p<0.05, **p<0.01 and ***p<0.001 when compared to stress control as determined by ANOVA followed by Dunnett's t test

Table 3: Effect of wheatgrass juice on organ weights in swim endurance test

Group	Organ Weight		
	Liver g/100g	Spleen mg/100g	Adrenal gland mg/100g
Normal control	3.18±0.12	269.45± 1.52	16.89±0.55
Stress control	5.54±0.98	194.68±1.84	38.26±1.78
WGJ 5 ml/Kg	4.17±0.72*	219.72 ±11.8*	28.64±1.12*
WGJ 10 ml/Kg	3.52±0.89*	243.66±0.65**	19.58±0.59**

The values are expressed as Mean±SEM, n = 6 in each group. Significance at *p<0.05, **P < 0.01 when compared to stress control as determined by ANOVA followed by Dunnett's t test

Table 4: Effect of wheatgrass juice on anoxia stress tolerance time in mice

Group	Duration of anoxic stress tolerance (min)	% increase in anoxic tolerance
Stressed control	36.57±2.18	---
Wheatgrass juice (5ml /kg, p.o.)	58.66±3.51**	60.40
Wheatgrass juice (10ml /kg, p.o.)	69.13±3.58***	89.03

The values are expressed as Mean±SEM, n = 6 in each group. Significance at **P < 0.01, ***P < 0.001 when compared to stress control as determined by ANOVA followed by Dunnett's t test

DISCUSSION

Stress in optimum quantum acts as stimulator to achieve the best, but when it exceeds, it surely causes imbalance in biochemical parameters as well as leads to suppression in physical endurance [14]. The forced swimming is the most widely used method for assessing the anti-stress property of a novel compound. This paradigm is based on the observation that animals when forced to swim in water eventually assumed a characteristic immobile posture, devoid of any activity. The appearance of immobility therefore, reflects a state of tiredness, fatigue, reduced stamina with the end point being the moment when the rats could not swim further and started drowning [15]. The increased swimming time has been observed in rats, pre-treated with wheatgrass juice with enhanced physical performance significantly longer than untreated (control) group and thus confirming its adaptogenic nature.

The mechanism by which stress rises serum cholesterol is likely to be related to the enhanced activity of hypothalamo-hypophyseal axis (HPA) resulting in liberation of catecholamines and corticosteroids. This could lead to increase in blood cholesterol level since epinephrine is known to mobilise lipids from adipose tissues. The increase in release of catacholamines also leads to elevated levels of glucose and blood urea nitrogen (BUN) [16, 17]. In swimming endurance stress model, treatment with wheatgrass juice inhibited the alteration of serum biochemical parameters in dose dependant manner.

Stress induces adreno-medullary response in man. Adrenaline in turn stimulates β_2 receptors on the pituitary glands causing greater release of ACTH, which can stimulate the adrenal medulla as well as cortex. So adrenal gland weight increases. Cortisol increases mRNA levels in liver cells leading to increase in weight of liver. Spleen constricts to release more blood cells (RBC) during stress. So its weight decreases during stress [18, 19]. In supporting with these conclusions, in our present study we observed increase in liver, adrenal gland weights, and decrease in spleen weight in stressed rats. This stress induced changes of organs weights were significantly reversed by the wheatgrass juice treatment.

Anoxia is a very severe stressor. All the body functions including cellular respiration depend on oxygen supply to them. Any lack of this vital element plays havoc on all body mechanism. Increase in adaptation during this stress by any drug could be considered as its major antistress effect [20]. The results of the study showed that pretreatment with wheatgrass juice significantly prolonged the meantime to develop convulsion, which therefore confirm its antistress property.

This adoptogenic property of wheatgrass could be as a result of its powerful antioxidant and free redical scavenging activities. A variety of biological activities including adaptogenic activity were reported with bioflavanoids [21]. Wheatgrass contains biologically active bioflavanoids like such as apigenin, quercitin, luteoline [7, 22]. The adaptogenic activity may be due to these constituents along with vitamins and minerals.

CONCLUSION

From the present study, we conclude that administration of wheatgrass juice is capable of increasing the capacity to tolerate the non specific stress as evident from the restoration of large number of parameters studied in different stress models suggesting the adoptogenic potential of wheatgrass juice.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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