Antistress Effect of *Fagopyrum esculentum* in Rats subjected to Forced Swimming Endurance Test.

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Summary

The present study was carried out to evaluate the antistress potential of extracts of *Fagopyrum esculentum* on forced swimming endurance test.The effect was assessed by swimming time and estimation of various biochemical parameters like glucose, cholesterol, triglycerides, cortisol and BUN levels. These activities were tested at dose of 100 mg/kg extracts of *Fagopyrum esculentum* using diazepam as standard drug. It was found that extracts significantly ($p<0.001$) increases swimming time in rats. It also showed significant ($p<0.001$) decreased in blood glucose, cholesterol, triglyceride, plasma cortisol and BUN levels as compared to control stress group. The obtained results revealed that *Fagopyrum esculentum* has got significant anti stress activity.

Keywords: Forced swimming endurance test , *Fagopyrum esculentum,* cortisol.

Introduction

The stress is generally considered as the functional adaptation of the organism in order to cope with a changing and challenging environment [1]. Stress defined as the experience of having intrinsic or extrinsic demands that exceed an individual's resources for responding to those demands. Living systems have evolved to reduce these demands and maintain the status through a series of physiological and sometimes behavioral responses [2] that occur when there is a real or perceived threat to homeostasis. While it is generally accepted that these processes are adaptive, designed to re-establish homeostasis and allow coping, it is also apparent that inadequate or excessive and/or prolonged activation of stress systems can disturb normal physiological and behavioral function [3].

Most of the studies using stress models have shown that physiological and psychological stress stimuli activates the sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis, which results in the secretion of catecholamines from rat brain [4] and glucocorticoids from the adrenocortical cells [5]. Excess glucocorticoid concentrations can have harmful effects such as hypertension, ulcers, immunosuppression and reproductive impairment [6]. Furthermore, an elevation of blood glucose, blood pressure, or lipids by stress stimuli results in the onset of lifestyle-related diseases such as diabetes [7].

Numerous models are available for evaluation of antistress activity for example anoxia stress tolerance, heat induced stress, immobilization stress, cold restraint stress, forced swimming endurance test etc. Swimming in small laboratory animals has been widely used for studying the physiological changes and capacity of the organism in response to stress. Swimming has got a number of advantages over trendmill in response to stress.. The amount of work done

during swimming exercise is far greater than that during the treadmill running of identical time duration. Swimming is not simple exercise stress, because emotional factors are difficult to be eliminated.[8] The forced swimming stress developed by Porsolt et al. has now become widely accepted model for studing physical stress in animals [9].

Recently, the use of complementary and alternative medicines is increasing overworldwide [10]. As Allopathic medicine have numerous side effects like benzodiazepine and anxiolytics despite having significant anti-stress activity, limits the clinical utility due to problem of tolerance and physical dependence on their prolonged use. Therefore there is a need for an effective plant anti-stress agent in the therapy of stress induced disorders [11]. The present investigation is aimed at evaluating the antistress potential of one such Himalayan plant, *Fagopyrum esculentum* native to the hills of Garhwal.

Fagopyrum esculentum Moench belonging to family Polygonaceae is also known as common buckwheat. In Hindi it is known as Kotu, Phaphra and in Kumaon as ogal [12]. It is traditional plant of Uttarakhand used in house hold remedies like anaemia and constipation [13]. Different parts of plant reported to have anti-oxidant [14, 15, 16], antidiabetic [17], anti-allergic [18], anticancer [19], antihypercholesterolemia [20], anti-inflammatory activity [21,]. The present study was undertaken to investigate antistress potential of various extracts of *Fagopyrum esculentum.*

Materials and Method

Plant Material

Fagopyrum esculentum (common buckwheat) was collected from the outer areas of Dehradun. The plant was authenticated by the taxonomist Dr. A K Shrivastava of Botanical Survey of India (BSI), Dehradun. The voucher specimen no. 113512. was lodged in the herbarium of the BSI, Dehradun for future reference.

Extraction

The whole plant of *Fagopyrum esculentum* was shade dried and coarsely powdered.The powdered drug was extracted by maceration at room temperature with regular stirring in the order of increasing polarity of solvents (n-hexane, petroleum ether, ethanol and water) separately. After 48 hours the supernatant and the sediment were separated by filtration through double layered muslin cloth. The residue was extracted second time as described above. The filtrate was evaporated and dried. The dried extract was suspended using 0.1% carboxy methyl cellulose. The concentration was adjusted in such a way that it did not exceed 1ml/100 g of rat

Drugs, Chemicals and Reagent

All the chemicals and drugs obtained were of analytical grade. Diazepam (Calmpose®, Ranbaxy, India) was procured locally, n-Hexane and petroleum ether (Rankem, New Delhi), ethanol and methanol were obtained from Merck, Mumbai.

Experimental Animals

Adult albino rats (200- 250g) of either sex were used for the study.The animals were procured from Indian Veterinary Reasearch Institute, Bareilly. The animals were acclimatized to the environment for a week prior to the studies and maintained under standard 12-hr light / dark cycle throughout the study. The animals were fed with standard pellet diet and given water *ad*

libitum. The study protocol was approved by the Institutional Animal Ethical Committee. (CPCSEA Reg.No. 1156/PO/a/07/CPCSEA)

Forced swimming endurance test:

Treatment groups
The animals were divided into seven groups of six rats in each group.
Group I - received saline given at dose (1ml/100 gm p.o), served as vehicle control
Group II-received saline (1ml/100 gm p.o) and stress, served as negative control.
Group III-received standard drug, diazepam (2 mg/kg, i.p) and stress, served as positive control.
Group IV- treated with aqueous extract (100 mg/ kg, p.o) and Stress.
Group V - treated with alcoholic extract (100 mg/ kg, p.o) and Stress.
Group VI- treated with Petroleum ether extract (100 mg/ kg, p.o) and Stress.
Group VII - treated with n-Hexane extract (100 mg/ kg, p.o) and Stress.

Experimental Procedure
Treatment (extracts/standard/vehicle) was given to rats, once daily for period of 7 days. On 8^{th} day the rats were subjected to swimming stress by keeping them in tank of dimension (37X37X30 cm), filled with water to a height of 25cm. till complete exhaust. The endpoint was taken when the animal started drowning and the mean swimming time for each groups was calculated.

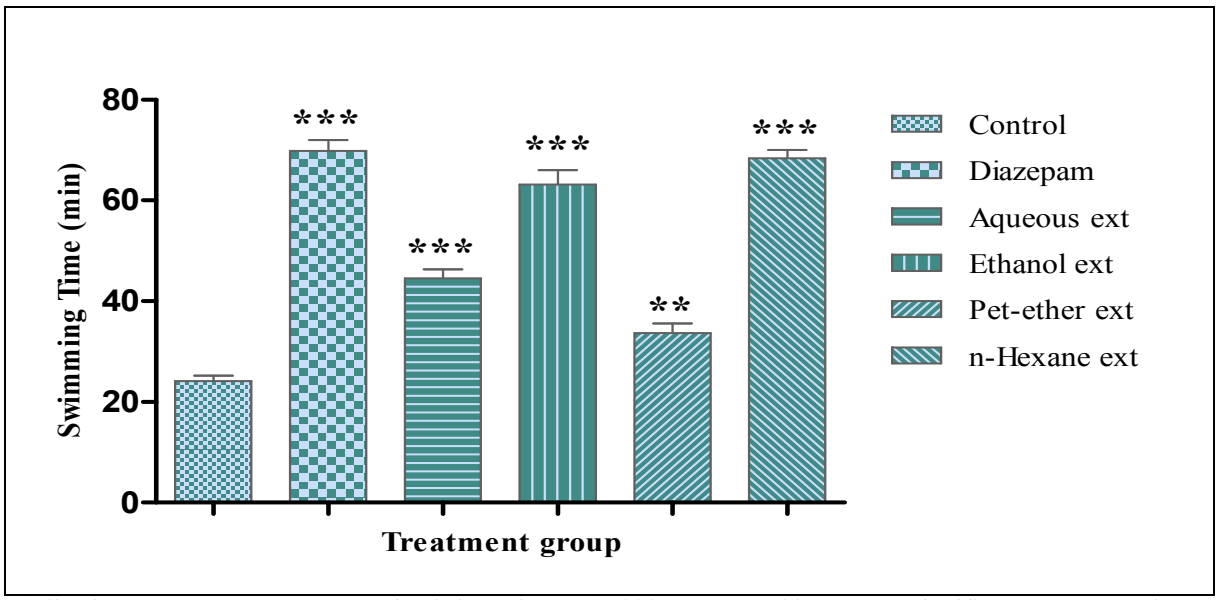
Biochemical estimation
After induction of stress, blood was collected collected by retro-orbital method. Serum was separated and biochemical parameters like serum glucose, triglycerides, cholesterol, BUN, cortisol and blood cell count were estimated. [9]

Statistical Analysis
All values are expressed as mean±SEM. Statical significance was determined using one way ANOVA followed by Dunnett's comparison test.

Results

There are significant differences in the swimming time to exhaustion between the control stress and each group. Thus the swimming times to exhaustion of extracts (aqueous, ethanol, petroleum-ether and n-hexane) groups were significantly longer than that of the control stress group. Ethanol, n-hexane and aqueous extracts were highly significant ($P<0.001$) as compared to control stress. (Table :1)
In forced swimming endurance stress induced elevated plasma corticosterone, glucose, triglyceride, cholesterol and BUN levels were reduced significantly by extracts of fagopyrum esculentum compared to stress control group. (Table 2). It also reduced the white blood cell count compared to stress control group.

Figure 1: Effect of extracts of *Fagopyrum esculentum* in forced swimming endurance test in rats



z(All values are mean ± SEM; n-6 animals in each group). *** P < 0.001, ** P < 0.01: significant as compared to stress control.

Table 1: Effect of extracts of *Fagopyrum esculentum* on biochemical parameters in swimming endurance stress in rats.

Groups	BIOCHEMICAL PARAMETERS				
	Cortisol (µg/dl)	**Glucose (mg/dl)**	**Cholesterol (mg/dl)**	**Triglycerides (mg/dl)**	**BUN (mg%)**
Control	3.98±0.55	78±2.43	69.08±1.67	58.83±3.10	12.72±1.49
Control + Stress	16.43 ±2.90###	163 ±1.783###	131.2 ±1.92###	113.3 ±2.60###	46.9 ±2.45###
Diazepam + stress	4.10 ±0.65***	77.67 ±2.275***	69 ±1.32***	60.0 ±3.19***	14.87 ±1.79***
Aqueous extract + Stress	9.20 ±1.65***	109.8 ±1.50***	79.5 ±1.116***	81.0 ±1.89***	20.23 ±2.04***
Ethanol extract + stress	5.0 ±1.04***	78.5 ±1.25***	67.5 ±1.38***	63.34 ±2.89***	19.03 ±2.36***
Pet-ether extract + stress	11.65 ±2.17***	107.8 ±1.47***	103.5 ±1.80***	96.34 ±2.45***	28.50 ±4.98
n-hexane extract + stress	4.03 ±0.56***	76.17 ±2.38***	66 ±1.18***	61.0 ±3.18***	17.18 ±1.71***

(All values are mean ± SEM; n-6 animals in each group) ### P < 0.001: significant as compared to control; *** P < 0.001 : significant as compared to stress control.

Table 2: Effect of extracts of *Fagopyrum esculentum* on white blood cells.

S.No	Group	WBC(white blood cell) /cumm ± SEM
1	Control	8612±7.75
2	Control + Stress	11400±8.21###
3	Diazepam + Stress	8920±4.61***
4	Aqueous + Stress	9888±3.97***
5	Ethanolic + Stress	7316±3.97***
6	Petroleum ether + Stress	8954±3.73***
7	n-hexane + Stress	8114±3.811***

(All values are mean ± SEM; n-6 animals in each group) ### P < 0.001: significant as compare to control; *** P < 0.001 : significant as compared to stress control.

Discussion

The forced swimming is the most widely used method for assessing the antistress property of novel compound. This paradigm is based on the observation that animals 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 rat could not swim further and started drowning. Increased swimming time has been reported in rat pretreated with antistress and adaptogenic activity [9]. *Fagopyrum esculentum* extracts significantly prolonged the swimming time as compared to control stress. Ethanol, n-hexane and aqueous extracts were highly significant (P<0.001) where as pet-ether was less significant ($p < 0.01$) compared to control stress (figure 1). This ability of *Fagopyrum esculentum* extracts to prolong the swimming time in rats suggest antistress activity.

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 [22]. The stress response begins with the generation of cerebral cortical neuronal activity in response to certain environmental stimuli via the sensory system or due to recall of a stressful experience [23]. The major neural pathways activated by stressors are the Sympathetic nervous system and Hypothalamic pituitary adrenal (HPA) axis [24]. Stresses, both physical and emotional, act via neural pathways to hypothalamus and lead to increase in corticotrophin releasing hormone (CRH) secretion [25] and this stimulates the anterior pituitary to secrete adrenocorticotropin hormone (ACTH) into the systemic circulation [26]. In humans, the natural glucocorticoid is cortisol whereas in rodents it is corticosterone [27]. Increased plasma cortisol influences the mobilization of stored fat and carbohydrate reserves, which in turn increases blood glucose, total protein, BUN, cholesterol and triglyceride levels [9].

The increased cortisol levels and increased blood glucose level are reversed by antistress agents [28,29]. During stress, blood glucose and cortisol level increases [9] which is found to be significantly reduced in *Fagopyrum esculentum* extracts treated rats.The glucose and cortsol levels were significantly increased in control stress group ($p < 0.001$) as compared to control group and significantly ($p < 0.001$) decreased in diazepam group and *Fagopyrum esculentum* extracts (n- aqueous, ethanol, pet-ether and n-hexane) groups, compared to control stress group.

Pretreatment with *Fagopyrum esculentum* extracts as well as the standard drug diazepam significantly ($p < 0.001$) reduced the elevated cholesterol, triglyceride, BUN, total protein levels White blood count in diazepam group and *Fagopyrum esculentum* extracts group significantly reduced as compared to control stress

Conclusion

Extracts of *Fagopyrum esculentum* displayed of antistress (adaptogenic) potential against stress model on experimental animals and result suggest that administration of extracts of *Fagopyrum esculentum* is capable of increasing the capacity to tolerate non-specific stress in experimental animals as evident from the restoration of a large number of parameter studied during forced swimming endurance stress. Further studies may be carried out to identify and characterize the active principles responsible for the activity.

Acknowledgement

The authors are thankful to Dr. N.V. Satheesh Madhav (Director, Faculty of Pharmacy, Dehradun Institute of Technology (D.I.T.) Dehradun, for providing the required facilities to carry out the research work.

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