EVALUATION OF ADAPTOGENIC POTENTIAL OF *HIBISCUS CANNABINUS* IN ACUTE STRESS INDUCED MICE

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Summary

To evaluate adaptogenic potential of methanolic extract of *Hibiscus cannabinus* leaves (MEHCL) at different doses against swimming endurance test (SET) and anoxia stress tolerance test (ASTT) in mice. *Withania somnifera* (100 mg/kg, p.o.) used as reference standard and it showed significant effect in both acute stress models. The animals pre-treated with test extract showed significant increase in swimming performance time at higher doses (250 and 500 mg/kg) in dose dependent manner in SET. However, the effect of the test extract at dose of 100 mg/kg was found to be statistically not significant, though there was increase in swimming endurance time seen when compared to control group. In case of ASTT the test extract significantly enhanced the anoxia stress tolerance time in dose and duration dependent fashion only at doses of 100 and 250 mg/kg. Though there was statistically significant antistress activity was observed at the higher dose 500 mg/kg compared to control, but the result was not found to be dose and duration dependent manner when compared to 250 mg/kg dose of the test extract. In conclusion, the results of the present investigation suggest that MEHCL is known to possess significant antistress activity.

Key words: *Hibiscus cannabinus*, adaptogen, anoxia stress and swimming.

Introduction

Adaptability is probably the most distinct characteristic of life. Dr. Hans Seyle[1] defined stress as sum of all non-specific responses of the body to any demands made upon it; fundamentally it was a physiological response; primary object of which was to maintain life and reestablish the normal state. It is evident from the definition that stress cannot be avoided; no matter what one does or what happens to one; there arises a demand to provide the necessary energy to perform the task required to maintain life and to resist and adapt to changing external stimuli. The medicinal substance causing non-specifically increased resistance (SNIR) was variously named as adaptogen or athenkropic[2]. The plant adaptogen is defined as "Smooth pro-stressors which reduce reactivity of host defense systems and decrease damaging effects of various stressors due to increased basal level of mediators involved in the stress response"[3]. A number of plants possess adaptogenic activity due to diverse classes of chemical compounds.

Hibiscus cannabinus Linn. (Malvaceae) also known as Kenaf. The leaves are edible and consumed by South Indians in various forms of food preparation. The plant possesses hepatoprotective[4], haematinic[5], cholesterol lowering[6], and antioxidative[7] activities. Recently, the experimental study has shown that the leaf extract of *Hibiscus cannabinus* exhibited free radical scavenging activity with significant increase in swimming time against cold water swimming stress model[8]. However, there are no reports on adaptogenic (antistress) potential of *Hibiscus cannabinus* leaves available in literature so for. Therefore the present study was taken up.

Materials and Methods

Collection of plant material

The Leaves of *Hibiscus cannabinus* Linn. were collected from the surrounding fields of Chitradurga in the month of November, 2006 after the identification and authentication by Professor K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A voucher specimen has been deposited at the museum of college.

Preparation of extract

The shade dried leaves were coarse powdered and subjected to maceration with methanol for 72 hours at room temperature. The extract was concentrated using rotary flash evaporator. The yield of extract was found to be 10.03 % and stored in airtight container in refrigerator below 10^0C.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out on MEHCL for detection of phytoconstituents present following the literature reported methods [9,10].

Selection and housing of animals

The albino mice of either sex 20 – 30 g were used throughout the experimentation. The animals were procured from Siddaganga College of Pharmacy, Tumkur, Karnataka. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry conditions. All the animals were fed with rodent pellet diet and water *ad-libitum* under strict hygienic condition. Ethical clearance for performing experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC).

Acute toxicity study (LD_{50})

An acute toxicity of MEHCL was carried out in female albino mice (20 – 30 g). The animals were fasted over night prior to the experiment. Fixed dose (OECD Guideline No. 420) method of CPCSEA was adopted for toxicity studies[11]. The MEHCL was administered at dose of 2000 mg/kg i.p.. The extract was found devoid of mortality of the animals. Hence 2500 mg/kg was considered as LD_{50} cut off value. So the doses selected for the screening of adaptogenic activity of the extract as per the OECD (Organisation for Economic Cooperation Development) guidelines No. 420 (Annexure - 2d) fixed dose method were as under

1. 100mg/kg ($1/25^{th}$ of 2500mg/kg)
2. 250 mg/kg ($1/10^{th}$ of 2500 mg/kg)
3. 500 mg/kg ($1/5^{th}$ of 2500 mg/kg)

Evaluation of adaptogenic activity

Acute stress animal models

Swimming endurance test in mice[12-14]

Albino mice of either sex weighing 20 – 30 g divided into five groups of six animals each

Group I – Control (Received only vehicle 1 ml/kg p.o.)
Group II – Standard (*Withania somnifera*, 100 mg/kg p.o.)
Group III – MEHCL (100 mg/kg p.o.)
Group IV – MEHCL (250 mg/kg p.o.)
Group V – MEHCL (500 mg/kg p.o.)

Treatment was given to mice for 7 days. On seventh day 1 hr. after drug administration, all the mice were subjected to swimming endurance test. The mice were allowed to swim individually inside a perplex glass (30 cm height with 20 cm diameter) containing water of 25 cm height maintained at 26 ± 1^0C temperature. The end point was taken till they got exhausted and the moment they drowned. The mean swimming time for each group was calculated.

Anoxia stress tolerance in mice[15]

Albino mice of either sex weighing 20 – 30 g were selected and divided into five groups of six each.

Group I – Control (Received only vehicle 1 ml/kg p.o.)
Group II – Standard (*Withania somnifera*, 100 mg/kg p.o.)
Group III – MEHCL (100 mg/kg p.o.)
Group IV – MEHCL (250 mg/kg p.o.)
Group V – MEHCL (500 mg/kg p.o.)

Animals were treated as shown above for the three weeks. At the end of 1^{st}, 2^{nd} and 3^{rd} week i.e. on 7^{th}, 14^{th} and 21^{st} day one hour after the treatment stress was induced in all mice by placing each animal individually in the hermetic vessel of 300 ml 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 animal 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.

Results

Swimming endurance test in mice

It was observed that MEHCL at doses 250 mg/kg and 500 mg/kg exhibited significant increase in swimming performance time in mice in dose related manner. The effect of the test extract at dose of 100 mg/kg was found to be statistically not significant, though there was increase in swimming endurance time seen when compared to control group. The percentage increase in swimming endurance time was 26.99 – 46.62 %, depending upon the dose of drug. The result of the higher dose (500 mg/kg) of the test extract was found similar to that of standard. The results are given in Table – 1

Anoxia stress tolerance in mice

MEHCL significantly enhanced the anoxia stress tolerance time in mice. The effect was seen dose and duration dependent fashion only at doses of 100 and 250 mg/kg and statistically significant. Though there was statistically significant antistress activity was observed at the higher dose 500 mg/kg, but the result was not found to be dose and duration dependent manner when compared to 250 mg/kg. Pre-treatment with MEHCL (100, 250 and 500 mg/kg b.w.) has significantly increased acute hypoxia time on 7^{th}, 14^{th} and 21^{st} day. This was evident by delaying the latent period for development of clonic convulsion. The antistress activity of higher dose (500 mg/kg) of the test extract was found similar to that of reference standard *Withania somnifera* (100mg/kg). The results are tabulated in Table – 2.

Discussion

In the present investigation MEHCL has been evaluated for the adaptogenic activity against acute stress experimental animal models namely swimming endurance and anoxia stress tolerance test. The well known adaptogen *Withania somnifera* was used as a reference standard in the present study.

Stress alters the normal functioning of the body[16] in special contrivance, when an animal forced to swim becomes immobile after an initial period of vigorous activity. This resembles a state of mental depression[17,18] and causes sever fatigue. The results of the present study showed that pre-treatment with MEHCL increased labor efficiency, as evident by the increase of swimming performance and indicating adaptogenic potential of test extract.

Anoxia is one of the most useful parameter for screening the adaptogenic effect of a drug'[19,20]. In our study with acute anoxia, pre-treatment with MEHCL did prolong the anoxia stress tolerance time in a dose related manner and may be due to its action on pituitary-adrenal gland axis[21]

Literature review indicates that flavonoids and tannins were reported to possess number of pharmacological activities including antistress activity[22,23]. In the present study also preliminary phytochemical screening on MEHCL gave positive tests for flavonoids and tannins, this could be the reason for significant adaptogenic property of test extract.

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Table – 1: Effect of MEHCL on swimming endurance time in mice

Groups	Duration of SET in min	% increase in SET
Control (vehicle)	128.46 ± 9.22	---
Std(*Withania somnifera* 100 mg/kg)	186.44 ± 8.10**	45.13
MEHCL 100mg/kg	163.14 ± 9.87^{ns}	26.99
MEHCL 250mg/kg	182.78 ± 6.202**	42.28
MEHCL 500mg/kg	188.36 ± 10.81***	46.62

Values are mean ± SEM (n = 6). ^{ns}P >0.05, **P<0.01 and *** P < 0.001 as compared to control.

Table – 2 : Effect of MEHCL on anoxia stress tolerance time in mice

Groups	Duration of anoxia stress tolerance (min)		
	7th Day	**14th Day**	**21st Day**
Control (vehicle)	28.72 ± 0.72	29.06 ± 0.27	29.06 ± 0.86
Std. (*Withania somnifera*) 100 mg/kg	36.96 ± 1.67** **(28.69)**	39.10 ± 1.88*** **(34.54)**	40.02 ± 1.87** **(37.71)**
MEHCL100mg/kg	36.49 ± 1.37** **(27.05)**	37.89 ± 1.55** **(30.38)**	38.65 ± 1.60** **(33.00)**
MEHCL 250 mg/kg	38.54 ± 2.05*** **(34.19)**	39.49 ± 1.89*** **(35.89)**	40.37 ± 1.90*** **(38.91)**
MEHCL500 mg/kg	38.72 ± 2.05*** **(34.81)**	39.58 ± 0.75*** **(36.02)**	40.45 ± 0.78*** **(39.19)**

Values are mean ± SEM (n = 6). The values in parenthesis are the % increase in anoxia tolerance. **P<0.01 and *** P < 0.001 as compared to control.

References

1. Selye H. The evaluation of the stress. American scientist 1973; 61(69): 2695.
2. Breakhman II, Dardymov IV. New substances of plant origin which increase non specific resistance. Ann Rev Pharmacol 1969; 419 - 421.
3. Panossian A, Wikman G, Wagner H. Plant adaptogens, III. Earlier and more rescent aspects and concepts on their mode of action. Phytomedicine 1999; 6(4): 287 - 300.
4. Gabrile A Agbor, Julius E Oben, Blaise Nkegoum, Jean Pierre Takala, Jeanne Y Ngogang. Hepatoprotective activity of *Hibiscus cannabinus* (Linn.) against carbon tetrachloride and paracetamol induced liver damage in rats. Pak J Biological Sci 2005; 13: 397 - 401.
5. Agbor GA, Oben JE, Ngogang JY. Haematinic activity of *Hibiscus cannabinus*. Afr J Biotech 2005 Aug; 4(8): 833 - 337.
6. Tamaki Y, Jinjo K, Uechi S, Hongo F, Sameshima K, Yoga S, Mokuzai Gakkaishi. Cholesterol-lowering effect of water soluble polysaccharides from kenaf (*H.cannabinus*) seeds in rats, Fac.Agric University Ryukyusa Okinawa Japan 2001; 47(2): 159 - 163.
7. Agbor GA, Oben JE, Ngogang JY. Antioxidative activity of *Hibiscus cannabinus* leaf extract. Food Africa 2003; 01 - 05.
8. Ahmed IR, Lakshmi PB, Chithra R, Varghese SS, Shalini K, Chamundeeswari D, Vasanth J. Free Radical Scavenging Activity of *Hibiscus cannabinus* in stress induced rats. Indian drugs 2005 Jun; 42(6): 359 - 363.
9. Kokate CK. Practical pharmacognosy. 4th ed. Delhi : Vallabh Prakashan; 1994.
10. Khandelwal KR. Practical pharmacognosy. 11th ed. Pune: Nirali Prakashan 2004: 149 - 156.
11. Mrs. Prema Veeraraghavan. Expert consultant, CPCSEA, OECD guide line No. 420; Oct 2000.
12. Kannur DM, Hukkeri VI, Akki KS. Adaptogenic activity of *Caesalpinia bondue* seed extracts in rats. J Ethnopharmacol 2006; 327 - 331.
13. Shivakumar H, Talha Javed, Prakash T, Nagendra Rao, Jayakumar Swamy BHM, Veerana Goud A. Adaptogenic activity ethanolic extract of *Tribulus terrestris* Linn. J Nat Rem 2006; 6(1): 87 - 95.
14. Singh B, Gupta DK, Chandan BK. Adaptogenic activity of glycopeptido-lipid fraction from the alcoholic extract of *Trichopus zeylanicus gaertn*. Phytomedicine 2001; 8(4): 283 - 291.
15. Singh B, Gupta DK, Chandan BK. Adaptogenic activity of glycopeptido-lipid fraction from the alcoholic extract of *Trichopus zeylanicus gaertn*. Phytomedicine 2001; 8(4): 283 - 291.
16. Bekhman II. *Eleutherococcus senticosus* A new medicinal herb of the araliaceae family. In proceedings of second international pharmacological meeting 1965; 7: 97 - 102.
17. Porsolt RD, Bertin A, Jalfre M. Behavioural despair in mice. A primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977; 22 (9): 327 - 335.
18. Anisman H, Kokkinidis L, Sklar LS. In stress, psychological and physiological interactions Washington: Hemisphere publication corp 1985; 67-82.
19. Qu, J. B.; Cao, Y.N.; Ma, X. Y. Effect of *Ginseng* leaves and root saponin on animals in acute hypoxia due to negative air pressure.5th Southeast Asia and Western Pacific regional meeting of pharmacologists. Chinese. Pharmacological association Beijing 1988; 14: 6.
20. Kasuga, S.; Ushijima, M.; Morihara, N.; Itakusa, Y.; Nakata, Y. Effect of aged *garlic* extract (AGE) on hyperglycemia induced by immobilization stress in mice. Jpn. J. Pharmacol 1999; 114: 191 - 197.
21. Lu G, Cheng XJ, Yuan WX. Effect of *Ginseng* root saponin on animals in acute hypoxia due to negative air pressure.5th Southeast Asia and Western Pacific regional meeting of pharmacologists. Chinese pharmacological association Beijing 1988; 03: 31.
22. Raj JK, Kapoor S. Flavonoids-review of biological activities. Indian Drugs 1999; 36(11): 668 - 678.
23. Kelly GS. *Rhodiola rosea*. A possible plant adaptogenic. Altern Med Rev 2001; 6(3): 293-302.