

**ADAPTOGENIC ACTIVITY OF SEEDS OF TRICHOPUS
ZEYLANICUS GAERTN, THE GINSENG OF KERALA**

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ABSTRACT: The alcoholic extract of seeds of *Trichopus zeylanicus* showed a potent adaptogenic or antistress properties against a variety of stresses in both rats and mice. The extract increased the swimming performance of normal and adrenalectomized mice. Significantly; prevented a variety of stress and chemical induced ulcerations in rats and also prevented milk-induced leucocytosis in mice. The extract further reduced the gastric secretory volume, PH and acid output in pylorusligated rat stomach. No mortality was observed upto a dose of 3 g/kg per oral in mice. The study indicated that *trichopus zeylanicus* seeds induce a state of nonspecific increased resistance against a variety of stress induced biological changes in animals.

Introduction

Trichopus zeylanicus Gaertn. (Family: Dioscoreaceae) is a rare, herbaceous, perennial and rhizomatous wild plant growing in the Agasthyar hill forests of Kerala. The forest dwelling local Kani' tribe call this plant Arogyappacha' meaning greener of health and use it as a health tonic. The fresh kernels of tender fruits are eaten by them to get instant energy, stamina and vitality (Pushpangadan et al¹). It is also claimed by the tribals that a regular intake of the kernels and certain other preparations made of the leaf, stem and rhizomes of *T. zeylanicus* would enable one to enjoy youthful health, vitality, resistance from diseases and longevity. The antifatigue and instant energy giving properties experienced after eating the fresh seed kernels by the scientists who made the expedition to these tribal areas¹ are very significant and therefore, deserve scientific investigation.

Being a very rare plant with absolutely no information on its medicinal value prior to the report of Pushpangadan et al¹, no chemical and pharmacological work has ever been carried out on this plant. It was in this context and against this background that the present authors initiated an in-depth investigation on this plant. The present communication relates to the study on the adaptogenic activity to the study on the adaptogenic activity of the seed of this plant.

Materials and Methods

Animals: Adult male Charles – Foster rats (100-150g) and Swiss albino mice (25-30 g) were used in the present study. They were maintained in air conditioned rooms (Temp. $23\pm 2^{\circ}\text{C}$. humidity 65-70%; 12 h light/dark cycle), fed with standard rodent pellet diet and tap water ad lib.

Seed Extract: Fresh seeds were extracted in cold with 50% ethanol. The solvent was removed by distillation under reduced pressure to obtain the dried extract hereafter referred as TZSE. Fresh seeds were ground into a suspension in water, hereafter referred to as TZS. Aqueous suspension of the extract in 2% gum acacia was used in all experiments and administered intraperitoneally (i.P) unless otherwise indicated.

Anti-Stress Screening

Swimming Endurance Test: The mice were divided into six groups of ten each. Group I served as control and given an equal amount of vehicle alone; Group II and III received TZSE 100 and 200 mg/kg per oral respectively, and Group IV, V and VI received the fresh seed paste suspension TZS at a dose of 100, 200 and 300 mg/kg per oral respectively. After one hour, the animals were put to swimming in plastic buckets filled with water (Temp. 25°C) and were allowed to swim actively till exhausted and drowned, which was taken as the end point. Swimming time' for each animal was recorded. The mean swimming time for each group was then calculated.

Swimming Endurance test in Adrenalectomized Mice: Animals were divided into four groups of 20 animals each group I served as the normal control. Group II animals were sham operated and kept as sham control. Group III & IV mice were bilaterally adrenalectomized through a single mid-line incision under pentobarbitone anaesthesia. Group III animals received an equal amount of vehicle alone, while group IV animals received 100 mg/kg per oral daily of TZS for 7 consecutive days. On 7th day, all surviving mice were subjected to the swimming performance test, one hour after the administration of the drug to the treated group animals (Group IV) along with the other groups. Swimming time for individual animals was recorded and the mean swimming time for each group calculated.

Milk-Induced Leucocytosis

Mice were divided into three groups of ten animals each. Blood samples were collected from tail vein at zero hour and leucocyte count was done in each group before milk or extract administration. Group I received an equal volume of gum acacia and served as normal control; Group II and III animals received an injection of sterile milk at a dose of 4 ml/kg sc. One hour prior to milk injection, Group III animals were administered TZES at a dose of 100 mg/kg 24 hours later another leucocyte count was performed on all group.

Anti-Ulcer Activity

The effect of the extract on gastric ulceration induced by Restraint, Cold stress and Aspirin was tested in rats.

Restraint Ulcers:

Groups of rats were deprived of food for 24 hours and restraint ulcers were produced in two groups of 10 animals each by tying all the four legs in the back position and keeping them for 18 hours as described by Selye². Group I served as control, while group II animals received 100 mg/kg TZSE one hour prior to restraining. 18 hours later all animals were sacrificed, stomach removed and examined for the presence of lesions.

Cold Stress-Induced Ulcers: Groups of rats deprived of food 24h prior to the start of experiment were used. Ulcers were produced by restraining the animals in wiremesh and placing supine in a cold (4°C) B.C.D incubator for 2 hours (Hernandez et al. 1988)³. Group I served as control, while group II animals received NSE 100 mg/kg one hour before the cold-stress. Animals were sacrificed and their stomach removed and examined for the presence of lesions.

Aspirin-Induced Ulcers: Rats were divided into two groups and fasted 24 hours prior to the experimentation. Gastric ulceration was produced by Aspirin (200 mg/kg i.p). Group I served as control, while Group II received TZSE (100 mg/kg) one hour prior to aspirin injection. Five hours later the animals were killed and their stomach removed and examined for the presence of lesions.

In all the experiments, ulceration was examined using a hand lens. Ulcer incidence and severity were recorded as described by Hernandez et al.⁴.

Anti-Secretory Activity

Groups of food-deprived rats were anaesthetized with ether and the pylorus ligated as described by Shay et al.⁵. Group I animals served as control, whereas Group II animals received TZSE (100 mg/kg) 30 min. prior to pylorus ligation. Two hours later, the rats were killed and the gastric contents aspirated. The fluid was centrifuged at 3000 rpm for 10 min at 4°C to determine the gastric secretory volume. The pH was determined using a pH meter, while acid output and titratable acid were measured in all gastric samples by titration to pH 7.0 with 0.1 N NaOH solution.

Acute Toxicity and General Behaviour

Groups of 10 mice each received a single dose of TZSE 100, 250, 500, 1000, 2000 and 4000 mg/kg orally. While one group served as the control. They were observed for gross behavioural changes 0.5, 1, 2, 4, 6, 24 & 48 hours after drug administration and further observed till 72 h for any mortality.

Statistical Analysis of the Data

All the grouped data were analyzed statistically using student t' test and p value of 0.05 or less was considered significant.

RESULTS

Effect on Swimming Performance in Normal Mice

Table I shows that the administration of TZSE (100 mg/kg) produced a significant ($p < .02$) increase in the swimming

TABLE 1 Effect of seed extract and fresh seeds of *T. Teylanicus* on Swimming Endurance in Mice.

Group Drug	Dose (Mg/Kg)	Swimming time (Min)
I Normal	---	228±21
II TZSE	100	395 ±36*
III TZSE	200	326±17
IV TZS	100	345±20**
V TZS	200	301±17
VI TZS	300	298±13

Values represent Mean ± SD of ten animals per group.

P values* $< .02$ vs I, ** $< .05$ vs I duration as compared to the control. A dose of 200 mg/kg did not produce significant increase, however, the mean duration of swimming time was high compared to the control group.

Fresh seeds administered at a dose of 100 mg/kg also produced a significant ($P < .05$) increase in the swimming time versus the controls. Like the alcoholic extract, the fresh seeds at a dose of 200 and 300 mg/kg also did not produce significant increase in the mean swimming time compared to the control. However, the swimming time in 200 mg/kg group was higher than the controls (Table 1).

Effect on Swimming performance of Adrenalectomized Mice

Table 2 shows that in the sham group (group II) and normal control (Group I) animals, the swimming time was not significantly different, whereas adrenalectomy (Group III) produced a

TABLE II Effect of pretreatment with *T. zeylanicus* seed extract on swimming endurance in Adrenalectomized Mice

Group	Drug/Dose	Number of Mice	Swimming Time (Min)
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I Normal Control	Gum (1.0ml)	Acacia	20	296±18
II Sham Control	Gum (1.0ml)	Acacia	20	280±22
III Adrenalectomy	Gum (1.0ml)	Acacia	15	195±36
IV Adrenalectomy	TZSE mg/kg)	(100	18	358±13**

Values represent Mean ± SD

P Values < .01 Vs I & III

<.01 Vs I; <.001 Vs III.

TABLE III Effect of Pretreatment with T. Zeylanicus seed extract on milk induced Leucocytosis in mice.

Group	Dose	Number of Leucocytes/cc (Oh)	Number of Leucocytes/cc (Oh)
I Control	Gum Acacia 1.0ml	9673.50 ± 335.75	10006.25±124.52
II Milk	4.0ml/kg Sc.	9938.87 ± 119.45	17806.37±539.61*
III + TZSE	4.0ml/kg Sc + 100ml/kg ip	10352.65±113.25	10506.87 ± 369.82

Values represent Mean ± SD of Ten animals per group.

P values* <.001 Vs I & IIs,

TABLE IV Effect of pretreatment with T.Zeylanicus seed extract on the development of Gastric Ulcers in Rat.

Stress	Gastric Lesions			
	Incidence a		Severity b	
	Control	TZSE	Control	TZSE
Restraint (18 h)	100	40	16.8 ± 3.2	6.0 ± 2.0*
Cold (2 h)	100	50	11.6 ± 2.4	5.9 ± 1.5**
Aspirin (5 h)	80	40	12.0 ± 1.0	6.7 ± 1.8 **

a= percent of rats wrth one or more gastric lesions (n=10)

b= Mean number of gastric lesion ± SD (n=10)

p values * <.01, ** <.05

TABLE V Effect of pretreatment with T.Teylanicus seed extract on Gastric Acid secretion in Pylorus-Ligated Rats.

Group	Dose	Gastric Volume (ml/rat)	Gastric pH (Units)	Acid Output (meg/2h)
I Saline	1.0 ml ip	4.2 ±0.8	1.6±0.2	856 ± 125
II TZSE	100 mg/kg	2.1±0.3*	3.0%±0.3**	325±98**

Values represent the Mean ± SD of Ten animals per group.

P values: *<.02, **<.01.

Significant (p<.02) reduction in swimming performance of mice. The administration of TZSE (100 mg/kg) produced a statistically significant (p<.02) increase in the swimming time in adrenalectomized (Group IV) animals over the adrenalectomized (non drug treated) as well as normal control group animals.

The mortality rate in the adrenalectomized mice which did not receive the TZSE was higher than that of the mice which received TZSE (25% Vs 10%).

Effect on Milk- Induced Leucocytosis in Mice

Table 3 indicates that the administration of milk (4 ml/kg) resulted in significant (p<.001) increase in the peripheral leucocyte 24 h after administration. The administration of TZSE (100 mg/kg) prior to milk injection, however, significantly (p<.01) inhibited this milk induced increase in leucocytosis. Gum acacia did not produce any alterations in the leucocyte counts.

Effect on Restraint Ulcers in Rats

All the control animals developed varying degrees of gastric ulcers. The pretreatment with TZSW (100 mg/kg) produced a significant reduction in the incidence of ulcer as well as the severity of the ulceration (Table 4).

Effect on cold Stress Ulcers in Rats

The incidence of cold-stress produced gastric lesions was 100% in control group, whereas the prior treatment with 100 mg/kg TZSE significantly lowered the incidence and severity of ulceration (Table 4).

Effect on Aspirin Induced Ulcers in Rats

Aspirin was able to produce gastric ulceration in 80% of animals after 5 hours of its administration. The group of animals pretreated with TZSE (100 mg/kg) showed significantly reduced rate of incidence and severity of gastric ulceration (Table 4).

Effect of Pylorus-Ligated Rats

Table 5 shows the effect of TZSE pretreatment on gastric acid secretion in pylorus-ligated rats. TZSE significantly reduced the gastric secretory volume ($p < .02$), increased the gastric pH ($p < .01$) and decreased acid output ($p < .01$).

Effect on General Behaviour and Acute Toxicity

All the animals received different doses of TZSE showed no sign of any change in their gross behavioural patterns throughout the period of observation. There was no mortality upto a dose of 3 g/kg during the 72 h. period of observation. However, a dose of 4 g/kg produced 10% mortality after 48 h of its administration.

DISCUSSION

The antifatigue, stimulant and antistress claims of the seeds of *T. zeylanicus*¹ appears to be similar to those of the South American Coca leaves (*Erythrozyllon coca*) and the Korean Ginseng. The stimulant action of Coca leaves is explained as due to the presence of the alkaloid /cocaine (Eiswirth et al⁶) or Cocaine and other metabolites (Burczynski et al⁷). Preliminary chemical screening of *T. zeylanicus* seeds so far did not indicate the presence of any Cocaine or Cocaine like alkaloids. The physical antifatigue action experienced after eating the seeds of *T. zeylanicus* by humans¹, is akin to the nonspecific ginseng like effect. Our present study provides experimental evidence to the antifatigue and antistress effects of the seeds of *T. zeylanicus*.

The administration of the alcoholic extracts of the seeds of *T. zeylanicus* in mice produced antifatigue effect as judged from the significant increase in their swimming time/performance. The swimming time of the mice group administered fresh seed paste suspension has also prolonged significantly as compared to these of the control group. This may explain the antifatigue properties of the seeds of this plant. Since the administration of the seed extract produced a similar increase in the swimming performance of the adrenalectomized group of mice, the antistress effects of the seeds does not appear to be mediated via hypothalamo-pituitary-adrenal axis.

The administration of seed extract also inhibited the milk-induced leucocytosis. The seed extract possesses potent anti-ulcerogenic property against a variety of stresses as is evident by the significant decrease in the rate of incidence and severity of gastric ulceration in the rat stomach induced by restraint stress, cold stress and aspirin. Increase in gastric acid and pepsin production and decrease in gastric mucus has been implicated in the development of ulceration³. The treatment of animals with TZSE resulted in decrease of the gastric output. Further it has also produced an increase in the pH of the luminal fluid and a decrease in the total acid output. This possibly explains the anti-ulcerogenic actions of the seeds of *T. zeylanicus*.

The higher safety doses coupled with the antagonistic effects during a variety of stressful situations indicates that the seeds of *T. zeylanicus* produce a state of non-specific increased resistance, which proves its capability to qualify as an adaptogen' (Brekhman & Dardymov⁸) and thus hold great promise as a therapeutic antistress agent.

Detailed pharmacological investigations including the immunostimulant /modulating actions (if any) of the seed and other parts (stem, leaves and rhizome) of *T. zeylanicus* are in progress.

Acknowledgement

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NOTE:

Due to unavoidable circumstances the release of this quarterly journal 'Ancient Science of Life' is a bit delayed and we regret for the delay. However, in order to make up this and to be up-to date we are compelled to combine the January and April 1989 issues (Vol. VIII, Nos 3&4) to-gether and release now. July 1989 issue (Vol. IX, No 1) onwards the journal will be in its regular frequency. – Editor.