**EFFECT OF TRICHOPUS ZEYLANICUS LEAF EXTRACT ON THE ENERGY METABOLISM IN MICE DURING EXERCISE AND AT REST**

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Manuscript Received: 6.2.2001           Revised: 22.9.2001         Accepted: 20.10.2001

**Objective:** To investigate the effect of the antifatigue agent, Trichopus zeylanicus leaf (alcohol extract) on energy metabolism in mice during exercise and at rest.

**Methods:** Trichopus zeylanicus leaf (alcohol extract) was orally administered to male adult mice. One hour after the extract administration (25, 50, 100 and 200 mg/kg), plasma glucose level was determined or subjected to swimming performance test. The effects of an optimum dose (100 mg/kg) of the extract on plasma glucose, free fatty acids (FFA), pyruvic acid (PA) and lactic acid (LA) levels were determined at rest (1 hr after drug administration) and after swimming exercise for 45 and 90 min.

**Results:** The alcohol extract of Trichopus zeylanicus leaf (100 mg/kg) decreased plasma glucose levels (1 hr after the administration) and increased the swimming performance of mice which was maximum at 100 mg/kg. At a dose of 100 mg/kg, the extract decreased plasma glucose levels and increased the levels of FFA without significant changes in the levels of PA and LA in the resting mice. In contrast, after exercise for 90 min, glucose level was found to be higher whereas the levels of FFA, LA and PA were found to decrease compared to control.

**Conclusion:** T. zeylanicus leaf (alcohol extract) influences fuel metabolism in mice at rest as well as during exercise. It stimulates utilization of fatty acids during exercise.

**INTRODUCTION**

During the ethnopharmacological investigation on the Agasthiar Hills of Kerala, India, Pushpangadan and co-workers discovered the medicinal value of Trichopus zeylanicus Gaertn., popularly known as Arogyapacha meaning a plant which gives health and vitality. The fruit of this plant is consumed by Kani tribes for getting instant stamina, better health and amelioration of old age related disorders. Earlier studies from this laboratory on T. zeylanicus showed several pharmacological activities such as enhancement in swimming performance, choleretic activity, hepatoprotection, aphrodisiac property and mast cell stabilizing activity.

The leaf powder of T. zeylanicus in the form of an aqueous suspension or its alcoholic extract has been shown to increase swimming performance in normal as well as adrenalectomized mice. However, the mechanism underlying this antifatigue effect of the drug is not known. The present study was undertaken to get some insight on the energy metabolism at rest and during exercise in mice as influenced by the drug.

**MATERIALS AND METHODS**

Plant material and preparation of extract: Trichopus zeylanicus leaves were collected during June and July from the garden of Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram (India) where the plant is cultivated under natural conditions (voucher specimen No. TBGT 31690).
To prepare ethanol extract, the air dried leaf powder was extracted using ethanol (100 ml/10 g) with constant shaking for 4 h. The extract was filtered and filtrate was evaporated to dryness under reduced pressure in a rotary evaporator. Approximately 5.5 ± 0.36% (mean±SD) yield (dried extract) was obtained using absolute ethanol as a solvent. The extract was suspended in 5% Tween 80 and used for studying bioactivity (control animals received 5% Tween 80 of same volume).

Experimental animals: Male Swiss albino mice (10 weeks old, 27-30 g) were used. They were fed with standard rodent pellet (Lipton and Co., Bangalore) and water ad libitum and maintained under standard laboratory conditions (temperature 24-28°C and relative humidity 60-70%).

Swimming exercise and blood collection: Mice were fasted for 12 h (over night) and T. zeylanicus (alcohol extract) was administered orally. To determine the effect of various doses (25, 50, 100 and 200 mg/kg) of the extract on plasma glucose levels, 5 groups of mice with 6 animals in each group were taken. Four different doses (25, 50, 100 and 200 mg/kg) of the extract were administered to 4 groups, respectively and the 5th group served as control which received 5% Tween 80 (vehicle) in an identical manner. Blood samples were collected 1 h after the drug administration by decapitation.

In another set of experiments, 1 h after the administration of different doses of the extract (25, 50, 100 and 200 mg/kg) the animals in all the 5 groups including the control group were subjected to swimming endurance test. The animals were put to swimming in plastic buckets filled with water (25-26°C) and were allowed to swim till exhausted and drowned which was taken as the end point. Swimming time for each animal was recorded.

To determine the effect of the optimum dose (100 mg/kg) on plasma levels of glucose, free fatty acid (FFA), pyruvic acid (PA) and lactic acid (LA), during exercise, mice were fasted for 12 h and T. zeylanicus extract was administered orally (100 mg/kg; single dose) 1 h before swimming exercise. Twelve animals were put in each group and each bucket contained 6 animals.

After swimming for 45 or 90 min each mouse was quickly taken out, blotted using tissue paper and sacrificed under chloroform anaesthesia. The blood was collected by decapitation in heparinized, chilled tubes and quickly placed under ice cold condition. Plasma was separated by centrifugation at 3000 rpm for 10 min in cold centrifuge and kept in deep freezer at -30°C until use. Mice at rest were sacrificed at 60 min after administration of T. zeylanicus extract or 5% Tween 80 (control) and blood was collected; plasma was separated as above.

Biochemical estimations: Plasma glucose was estimated enzymatically using a commercial assay kit (Monozyme, India Ltd.) 10 µL of plasma was used for each assay. FFA was estimated spectrophotometrically and LA was estimated as described by Barker. PA, being an unstable molecule, was estimated by directly applying known volume of the heparinized blood in chilled trichloroacetic acid (TCA, 10%) as described by Theodore and Friendman.

Statistical analysis: The data were analysed and comparisons between control and T. zeylanicus extract treated animals at rest and during exercise were made using a two-way ANOVA followed by Tukey post-hoc test. The data on different doses of the drug (Table 1) were analysed by one-way ANOVA and Dunnett’s multiple comparison test. Statistical significance was accepted at 5% probability level.

RESULTS

The effect of various doses of the extract of T. zeylanicus on plasma glucose level is shown in Table 1. The effect was found to be concentration dependent. At lower doses (25 or 50 mg/kg), the drug did not influence plasma glucose level, but at a higher dose (100 mg/kg) the glucose level was decreased. This decrease was more at 200 mg/kg (Table 1).

The effect of the drug on the swimming endurance of mice was dependent on the dose used. The drug at lower doses (25 or 50 mg/kg) did not enhance swimming performance, while at 100 mg/kg level it markedly stimulated swimming performance. However, further increase in the dose level to 200 mg/kg, slightly decreased the enhancement in swimming performance observed at 100 mg/kg dose.

The plasma glucose concentration at rest (1 h after drug administration) and during exercise, in presence...
Figure 1. *T. zeylanicus* has no significant effect on the concentration of PA in mice at rest and at 45 min. of exercise. At 90 min of exercise in *T. zeylanicus* treated group, PA concentration was significantly lower than that in control group.

The plasma concentration of LA in control mice at rest was $2.35 \pm 0.18 \text{ mM}$ and in *T. zeylanicus* treated group it was $1.87 \pm 0.13 \text{ mM}$. Administration of *T. zeylanicus* caused a slight decline in the concentration of LA at rest. Exercise for 90 min caused a significant increase in the concentration of plasma LA in control animals ($3.21 \pm 0.20 \text{ mM}$) which was very much higher than their respective treated group ($2.15 \pm 0.16 \text{ mM}$). In *T. zeylanicus* treated group, even after 90 min of exercise, the concentration of plasma LA was lower than the resting plasma LA concentration of control animals (Figure 1).

**DISCUSSION**

Earlier study from this laboratory has shown that the antifatigue effect of *T. zeylanicus* (alcohol extract) was maximum at 100 mg/kg dose compared to that at 200 mg/kg. This observation was confirmed in the present study.

Although the plant, *Trichopus zeylanicus* Gaerton, is experimentally proved as an antifatigue agent in laboratory animals the biochemical mechanism behind the antifatigue effect is not known. Fatigue is defined physiologically as the inability to maintain the expected power output. In exercising mammals, the response to exercise begins with an increase in muscular activity, which switches over to an anaerobic metabolism if the exercise is intense. This leads to an accumulation of LA and depending on the speed at which LA is metabolised in the liver, a metabolic acidosis might be generated. At the same time, respiratory system compensates metabolic acidosis through higher ventilation.

The metabolic effects of *T. zeylanicus* on glucose and LA are intimately connected with the physiological changes which occur during exercise. *P. ginseng*, an adaptogenic and antifatigue plant, did not influence the resting levels of glucose, LA and FFA whereas *T. zeylanicus* in mice at rest produced a significant decrease in glucose and a slight decrease in LA together with sharp increase in FFA (Figure 1). This suggests that the mechanism of action of these

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**Table 1.** Effect of different doses of *T. zeylanicus* leaf (alcohol extract) on plasma glucose level and swimming performance in mice.

<table>
<thead>
<tr>
<th><em>T. zeylanicus</em> extract (mg/kg)</th>
<th>Glucose (mM/L)</th>
<th>Swimming period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>8.0 ± .36</td>
<td>139 ± 15</td>
</tr>
<tr>
<td>25</td>
<td>7.6 ± .34</td>
<td>160 ± 20</td>
</tr>
<tr>
<td>50</td>
<td>7.5 ± .41</td>
<td>148 ± 14</td>
</tr>
<tr>
<td>100</td>
<td>5.4 ± .22*</td>
<td>248 ± 19*</td>
</tr>
<tr>
<td>200</td>
<td>4.6 ± .20*</td>
<td>108 ± 22</td>
</tr>
</tbody>
</table>

One way ANOVA: $F=7.1, df=4, 25, P<.01$; $F=55, df=4, 25, P<.01$.

In one set of experiment, blood samples were collected for glucose determination 1 h after drug administration. In another set of experiments, 1 h after drug administration animals were subjected to swimming exercise (details under materials and methods).

Values are mean±SD; n=6 in each group, *P<0.01 Vs control (Dunnett's test).

(100 mg/kg) and absence of drug is shown in Figure 1. The glucose concentration (mean±SD) at rest was $9.01 \pm 0.6 \text{ mM}$ in control mice and that in *T. zeylanicus* treated group was $6.80 \pm 0.51 \text{ mM}$. After 45 min and 90 min of swimming, plasma glucose concentration of control group halved to that at rest. In contrast, in *T. zeylanicus* treated group, plasma glucose level increased during exercise compared to the glucose level at rest.

Concentration of FFA in control group at rest was $0.40 \pm 0.03 \text{ mM}$ and that in *T. zeylanicus* treated group was almost double ($0.73 \pm 0.05 \text{ mM}$). At 45 or 90 min of exercise, concentration of FFA in control group was elevated whereas in treated group it was declined and the decrease was more marked at 90 min of exercise (Figure 1). At 90 min of exercise the concentration of FFA in control group was $0.69 \pm 0.05 \text{ mM}$ and in *T. zeylanicus* treated group the concentration was half to that of control ($0.37 \pm 0.03 \text{ mM}$). At rest and at 90 min of exercise, but not at 45 min of exercise, *T. zeylanicus* caused significant difference in FFA levels compared to their respective control groups (Figure 1).

The value of blood PA at rest and during exercise in presence and absence of drug is also shown in
Figure 1. Concentrations of plasma glucose, free fatty acid and lactic acid; and concentration of blood pyruvic acid in control and *Trichopus zeylanicus* (leaf extract) treated mice at rest and after 45 and 90 min of exercise. Values are mean±S.D. n=12 in each group, *significantly different (P<0.01) when compared to respective control.
two drugs are different. The concentrations of FFA in blood have physiological significance, since blood FFA concentration is an important factor in regulating the type of substrate utilization for cellular energy production in non-glucose-obligatory tissues	extsuperscript{14,15}. In this connection, it should be noted that 	extit{T. zeylanicus} treatment resulted in an increase in the concentration of FFA at rest.

A significant increase in the concentration of plasma LA and FFA and blood level of PA after 90 min of exercise in control animals indicated that the exercise was of sufficient intensity to elicit a substantial amount of skeletal muscle anaerobic glycolysis for the energy for muscle contraction. However, pre-treatment with 	extit{T. zeylanicus} extract significantly altered the metabolic responses to the exercise bout.

Interestingly, 	extit{T. zeylanicus} completely inhibited the elevation of plasma LA observed in control animals at 90 min of exercise and also decreased the elevated FFA in mice suggesting that the utilization of FFA was more preferential during exercise (Figure 1). The lower plasma LA and higher plasma glucose in mice after 90 min of exercise in 	extit{T. zeylanicus} treated mice indicate the increased utilization of FFA during exercise.

The potential of 	extit{T. zeylanicus} as a sports medicine, in the form of a single plant preparation or in combination with other adaptogenic plants such as 	extit{Withania somnifera}	extsuperscript{16} is interesting because metabolic adaptation to chronic exercise training results primarily from an enhanced skeletal muscle capacity to oxidize FFA in preference of glucose for cellular energy production during muscular activity	extsuperscript{17}. At rest, the drug may stimulate total glucose utilization and/or decrease glucose mobilisation from liver; and stimulate the efflux of FFA from adipose tissue. This could result in a decrease in blood glucose levels and an increase in FFA. The blood level of FFA is critical for its availability in skeletal muscle; increased utilization of FFA by skeletal muscle during exercise could result in a fall in the production of PA and LA in the muscle and a reduction in fatigue. The mechanism underlying the change in the pathways of intermediary metabolism after administration of 	extit{T. zeylanicus} is not clear. But the present finding provides evidence that this plant extract can have profound effects on the flux of intermediary carbohydrate and lipid metabolism at rest as well as during exercise. During exercise the drug treatment results in an increased efficiency of fuel substrate utilization via a shifting of skeletal muscle energy production towards oxidation of fatty acids.

**ACKNOWLEDGEMENT**

The authors thank Mr. S. Radhakrishna Pilli and Mr. G. Santhoshkumar for their assistance in the laboratory and in the animal house.

**REFERENCES**


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DECLARATION UNDER SECTION 5 OF THE PRESS AND REGISTRATION OF BOOKS ACT, 1867

1. Title of the newspaper ..... Indian Journal of Pharmacology

2. Language(s) in which it is (to be published) ..... English

3. Place of publication ..... Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry-605 006.

4. Periodicity of its publication ..... Bi-monthly published every third week of February, April, June, August, October and December

5. Publisher's Name ..... Dr. R. Raveendran

   Nationality ..... Indian

   Address ..... Department of Pharmacology, JIPMER, Pondicherry-605 006.

6. Printer's Name ..... Mr. Saphal Jhunjhunwala

   Nationality ..... Indian

   Address ..... Kennedy Nagar, Pondicherry-605 001.

7. Editor's Name ..... Dr. R. Raveendran

   Nationality ..... Indian

   Address ..... Department of Pharmacology, JIPMER, Pondicherry-605 006.

8. Names and address of individuals who own the newspaper and partners or shareholders holding more than one percent of the capital ..... Indian Pharmacological Society, Law North West Corner, Patna-800 001.

I, R. Raveendran, hereby declare that the particulars given above are true to the best my knowledge and belief.

Date : 7 January, 2002

R. RAVEENDRAN (Sd)
Signature of Publisher