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The effects of oral ginseng administration on the activities and isoenzyme profiles of murine lactate dehydrogenases

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The rhizome of the perennial herb *Panax ginseng* C. A. Meyer has been of importance in the practice of Chinese medicine for the past 2000 years [1], with a wide variety of curative properties ascribed to its use [2]. The active agents in the root extract are ginsenosides, which on acid hydrolysis yield the tetracyclic dammarene triterpenoids, 20*S*-protopanaxadiol and 20*S*-protopanaxatriol, and the pentacyclictriterpene, oleanolic acid [3, 4]. A number of physiological effects including the prevention of body temperature fluctuation following exposure to heat and cold stress [2], improved exercise rates and prolonged swimming times [5] and a reduction in the effects of X-ray irradiation [6] have been claimed for ginseng in laboratory animals. In biochemical terms, the intraperitoneal injection of ginsenosides into rats and mice has been reported to increase the rates of hepatic RNA and protein synthesis [7, 8] and to accelerate hepatic lipogenesis with accompanying decreases in liver glycogen, blood glucose and serum triacylglycerol levels [9]. Ginseng injection was also reported to increase the activity of the glycolytic enzyme pyruvate kinase (EC 2.7.1.40) [10] but to result in a loss of serine dehydratase activity (EC 4.2.21.13) [11].

Following the addition of ginseng to the culture medium of human diploid fibroblasts, significant increases were observed in the activities of phosphohexose isomerase (EC 5.3.1.9) and lactate dehydrogenase (EC 1.1.1.27), the principal gain being in the lactate dehydrogenase (LDH) isoenzyme fractions 2–5 [12]. The aim of the present study was to determine whether similar effects on LDH activity occurred *in vivo* in mice following the oral administration of ginseng.

Materials and methods. Two groups of 25 male mice, strain LACa, each weighing 26–32 g, were maintained on a 12 hr light, 12 hr dark regime, five to a group. Ginseng saponins in the form of a freeze-dried, aqueous extract supplied by Pharmaton S.A. (Lugano, Switzerland) were dissolved in water and administered to the test group at a rate of 8 mg/kg body wt, equivalent to approximately 40 mg of whole root/kg body wt per day, for a period of 30 days. After 21 days of treatment, the pharmacological activity of the ginseng saponins was verified by open-field stress tests [13]. The mice were killed by cervical dislocation, and the brain, heart, liver, skeletal muscle and testes immediately removed from each animal and stored at −20°. For the enzyme assays, tissues were homogenized in 0.01 M phosphate buffer, pH 7.4, to give an approximate concentration of 10% (w/v). The crude homogenate was centrifuged at 700 *g* for 10 min and the lactate dehydrogenase (LDH; EC 1.1.1.27) activity of each tissue was immediately determined in triplicate on the supernatant by the NADH/NAD-linked method of Wroblewski and LaDu [14], in which 1 unit of LDH activity is defined as a decrease in extinction of 0.001 per min, measured at 340 nm, pH 7.4 and 25°. The total protein of each preparation was assayed by the method of Hartree [15]. Isoenzymes were separated by polyacrylamide gel electrophoresis (PAGE) on rod gels of 7% acrylamide prepared by a modification of the Davis method [16]. Samples equivalent to 25 μg protein were run in a 12 hole rod gel electrophoresis chamber at 18–20° and a constant current of 3 mA per tube for approximately 2 hr, the exact time being determined by the rate of movement of bromophenol blue through the gels. Final visualization was by precipitation of reduced nitro-BT formazans [17]. The extinction of the individual isoenzyme bands was measured on a Cary 210 scanning densitometer (Varian Associates) at 540 nm and the activity of each isoenzyme calculated as a proportion of the relevant total activity. The total LDH activity and the activities of each of the isoenzymes of the five tissues tested from the ginseng-treated and the control animals were statistically compared by the non-parametric two-tailed Mann–Whitney U-test [18].

Results and discussion. Following ginseng administration, LDH specific activity was only increased significantly in the hepatic homogenates (Table 1). There were small but non-significant increases in the activity of LDH from the heart and testis samples of ginseng-treated animals; the LDH activity of the brain samples showed no change while in the skeletal muscle preparations LDH exhibited a small but non-significant decrease in activity (Fig. 1). The administration of the ginseng saponins had no significant effect on the LDH isoenzyme profiles of any of the five tissues tested (Table 2).

In rats 20–25% of a dose of ginsenosides administered via the drinking water was absorbed [19] and was measurable in the heart, lung, liver, kidney and spleen within 1.0 hr, the maximum levels being attained after 1.5 hr [20]. Only the brain failed to show any trace of the ginsenosides, suggesting an inability to cross the blood–brain barrier. Thus with the exception of the brain, the observed tissue-specific differences in LDH activity in the mouse following oral ginseng administration can be considered real rather than representing a failure of the active principle(s) in the extract to reach their particular, potential site of action.

Lactate dehydrogenase is a tetrameric enzyme, its pattern of activity in specific tissues being determined by varying combinations of the A and B structural sub-units [21]. In the mouse these are coded for respectively by the Ldh-1 locus on chromosome 7 and Ldh-2 locus, as yet

Table 1. LDH specific activity expressed as the mean ± S.E.M., in tissues from ginseng-treated and control mice (U/μg protein)*

	Ginseng-treated	Control	Significance†
Brain	4.07 ± 0.08	4.06 ± 0.09	$P > 0.1$
Heart	6.09 ± 0.11	5.92 ± 0.12	$P > 0.1$
Liver	3.25 ± 0.05	3.00 ± 0.06	$P > 0.05 < 0.055$
Skeletal muscle	18.16 ± 0.29	18.22 ± 0.36	$P > 0.1$
Testis	2.56 ± 0.05	2.41 ± 0.04	$P > 0.1$

* The values are calculated from the means of three determinations on each tissue from the individual treated and control mice; for each group $n = 25$.
† Two-tailed Mann–Whitney U-Test.

Table 2. Mean LDH isoenzyme profiles, expressed as proportions of the total activity (= 1.00), of tissues from ginseng-treated and control mice

	Isoenzyme	1	2	3	4	5	X
Brain							
	Test	0.24	0.21	0.20	0.23	0.12	—
	Control	0.24	0.20	0.21	0.22	0.13	—
Heart							
	Test	0.22	0.34	0.23	0.13	0.07	—
	Control	0.21	0.32	0.25	0.14	0.07	—
Liver							
	Test	<0.01	<0.01	<0.01	0.01	0.99	—
	Control	<0.01	<0.01	<0.01	0.01	0.99	—
Skeletal muscle							
	Test	<0.01	0.01	0.02	0.03	0.94	—
	Control	<0.01	0.01	0.03	0.03	0.93	—
Testis							
	Test	0.26	0.21	0.05	0.02	<0.01	0.46
	Control	0.26	0.20	0.05	0.03	<0.01	0.46

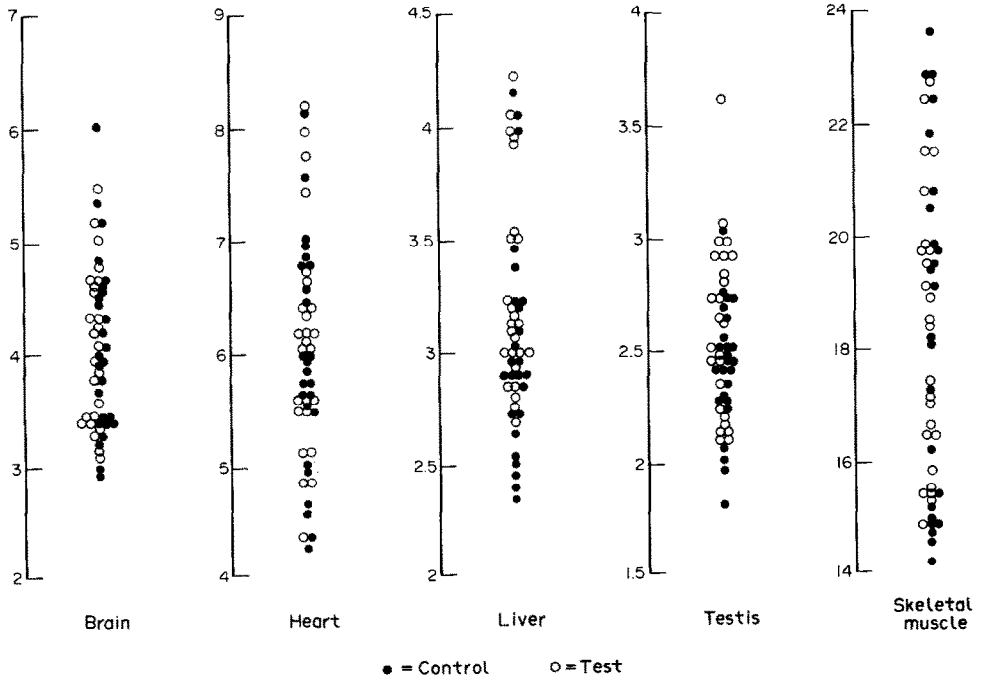

Fig. 1. LDH specific activity of tissues from individual ginseng-treated and control mice (U/μg protein).

unassigned, with additional regulation of the pattern of development of the B sub-unit in the liver provided by the Ldr-2 locus on chromosome 6 [22]. Since the injection of ginseng has been reported to accelerate hepatic protein synthesis [8], the increased specific activity of liver LDH, almost exclusively LDH_5, may be due to protein synthesis *de novo*. However, as the addition of ginseng alkaloids *in vitro* has been claimed to elicit a dose-dependent increase in chicken serum LDH [23], it is also possible that the enhanced hepatic enzyme activity resulted from stabilization of the enzyme molecule by a product(s) present in the ginseng extract.

In mammals the capacity to endure work is inversely correlated with the levels of lactate produced by exercising muscles. The pretreatment of rats with ginseng has been shown to minimize any rise in blood lactate levels following prolonged exercise [24], therefore the increased liver LDH activity observed in the present study may indicate an effective stimulation of the hepatic portion of the Cori cycle responsible for the overall recycling of lactate. Since this would result in a concomitant, enhanced production of pyruvate and glucose via gluconeogenesis, it provides a possible explanation for the anti-fatigue properties that have been attributed to ginseng.

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