Evidence for the Role of Succinate Dehydrogenase Enzyme (SDH) in Antistress Activity of Geriforte

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ABSTRACT
Geriforte, an Ayurvedic compound preparation consisting of several plant materials, has been reported to possess antistress activity. As the possible mechanisms of its antistress activity were not evaluated as yet and SDH enzyme plays an important role during stress in the conservation and utilisation of energy, the present study was undertaken for estimating this enzyme in the brain and liver of normal and stressed animals, with and without Geriforte treatment. The results indicate a clear role of the enzyme SDH during stress. Geriforte appears to help adaptive processes during stress by a further increase in this enzyme, which may be a possible mechanism of the antistress activity of this drug.

INTRODUCTION
Geriforte, an Ayurvedic geriatric, restorative tonic, consists of several plant ingredients and has been reported by Singh et al. (1978) as an adaptogenic, "antistress" drug. Energy conservation and release have a vital role to play in the survival of living organisms on the verge of a crisis or under acute stress. To meet this demand there is increased rate of cellular respiration and increased metabolism of carbohydrate and fat to yield energy bundles kin the form of adenosine triphosphate, which ultimately releases biological energy. Succinate dehydrogenase (SDH) enzyme has a key role in Kreb's cycle for energy conservation and utilization. Although the antistress effects of Geriforte have been described in several experimental models, its possible mechanism of action as to how it helps the adaptative process during stress has not yet been evaluated. Therefore, in the present study the effect of Geriforte was assessed on the levels of SDH enzyme. The study was conducted at the molecular level with the cells of the vital organs – the brain and the liver of mice – in relation to stress.

MATERIALS AND METHODS
Geriforte powder provided by The Himalaya Drug Co. was suspended in normal saline and administered orally to animals by a feeding cannula.

Experimental
The study was conducted in male albino mice of the same age and of equal weight groups, each mouse weighing between 20-25 gm. 60 mice were divided into four groups of 15 mice each. The first group served as control and did not receive any treatment or stress. The second group received only Geriforte treatment (100 mg/kg p.o. daily x 14 days). The third group did not receive any treatment but was subjected to stress and served as stress control. The fourth group was given pretreatment with Geriforte (100 mg/kg p.o. daily x 14 days) and was also
subjected to stress and served as the experimental group. All the groups were kept on an identical diet and in exactly the same environmental conditions.

After 14 days, mice of the first and second groups were decapitated and their livers and brains were immediately taken out for enzymatic study. Mice of the third and fourth groups were subjected to swimming stress for five hours, in separate porcelain tanks under identical conditions (temperature of water in the tanks was kept the same as that of the room, 25°C), after which they were also decapitated for SDH enzyme estimation. Five mice of Group III, which drowned during swimming before completion of five hours, were removed alive immediately and decapitated, and their livers and brains were excised for enzyme estimation. These formed the stress-failure group.

**Biochemical**

The activity of SDH enzyme was estimated by the method of Slater and Bonner (1952). SDH activity of the liver homogenate was determined spectrophotometrically using sodium succinate as substrate in mice liver and brain. The reaction mixture in a final volume of 2 ml consisted of 0.1 molar sodium phosphate, buffer brain or liver homogenate equivalent to 20 mg of wet tissue, 0.002 molar K₄Fe(CN)₆, 0.01 molar NaCN and 5 x 10⁻³ sodium succinate. The reaction mixture was incubated for 30 minutes and the reaction was stopped by the addition of 2 ml of 10% TCA. The tubes were centrifuged at 1500 x g for 10 minutes to remove the precipitated proteins and the clear yellow supernatant was read at 400 mm on a Hitachi Perkin Elmer spectrophotometer. After that, protein in respective tissue was estimated and the activity of the enzyme was expressed in terms of optical density change/mg of protein.

**RESULTS AND DISCUSSIONS**

The results are summarized in Fig. 1.

In Group III the stress significantly (p<0.001) increased the levels of succinate dehydrogenase enzyme in the brain and liver tissue when compared with the control non-swimmer Group I. Geriforte per se (Group II), has no significant (p<0.05) effect on the enzyme levels both in the brain and liver as compared with the control, non-swimmer Group I. Geriforte per se (Group II), has no significant (p<0.05) effect on the

![Fig. 1: Shows the effect of Geriforte pre-treatment (100 mg/kg p.o. daily x 14 days) on stress-induced activation of cellular mitochondrial succinate dehydrogenase enzyme in the brain and liver of albino mice. There was no significant difference in the levels of the SDH enzyme in the brain and liver of control animals (normal, non-swimming mice) and animals treated for 14 days with Geriforte (non-swimming). However, stress produced a highly significant (p<0.001) increase in the level of this enzyme (stress-control) as compared to the control group.

When Geriforte-treated animals were exposed to the same stress the SDH enzyme levels further increased significantly (p<0.001) as compared to the stress-control group. In those animals of the stress-control group, who failed to continue swimming (stress-failure group), their enzyme levels also increased significantly (p<0.01) as compared to the control (non-swimmers) group, but it was significantly (p<0.001) lower than the stress-control animals.
enzyme levels both in the brain and liver as compared with the control, non-swimmer Group I. Those animals of Group III that drowned and failed to continue swimming much sooner than the scheduled time course of stress (mean 1.75 hr), and coming under the stress-failure group, showed some increase in the levels of the enzyme as compared to the control (non-swimmers). But this was significantly ($p<0.001$) lower when compared with the stress-control Group III animals, who survived the stress (5 hrs of swimming). However, when Geriforte-treated animals (Group IV) were subjected to stress, their SDH enzyme levels both in the brain and liver increased significantly ($p<0.001$) as compared to the stress control (Group III). None of the animals of the Geriforte group (IV) showed stress failure during 5 hrs of swimming.

Stress calls for an increased energy demand through the mobilisation of various physiological processes, to enable the individual to face it. The enzyme SDH is activated following stress and returns to normal after 4 hrs of stress withdrawal (Sharma et al., 1978). The increase in the production of ATP molecules and an increase in turnover of Kreb's cycle. The contention that its activation is essential during stress becomes more obvious from our results, which show that mice of the stress-failure group had significantly ($p<0.001$) lower levels of this enzyme in their brains and livers as compared to those that survived the stress, ("stress-control") (Fig. 1). It appears that animals cope with stressful situations by initiating an increase in this enzyme which catalyses the processes responsible for conservation and liberation of energy.

Geriforte appears to help the adaptive process by enhancing the production of this enzyme during stress. The present study indicates that SDH enzyme level is increased during stress, as also reported earlier by Sharma et al. (1978), and Geriforte helps the adaptive processes as evidenced by the fact that it further induced a significant ($p<0.001$) increase in levels of SDH as compared to the stressed-control Group III (Fig. 1). Apart from this, its antistress, adaptogenic property may also be due to its immunomodulatory effects (Singh et al., 1980, Singh et al., 1981; Kumar et al., 1982).

A decrease in supply of energy, cellular immunity and enzyme synthesis has been described as occurring during ageing (Gianoli, 1975). SDH activity, an important indicator of energy metabolism, decreases in the cerebellum and cerebral cortex in ageing, which results in inadequate ATP production and impairment of the cationic pump mechanism. This causes sluggishness in the response of the neuronal cell activity and, as a result, adaptability to withstand stress in old age is lowered (Meier, 1976).

Furthermore, SDH enzyme may indirectly influence the cholinergic mechanism through the GABA shunt in Kreb's cycle by decreasing the inhibitory influence of GABA on cholinergic neurotransmission. Thus an increase in this enzyme may improve neural cholinergic neurotransmission, which becomes slow and causes reduced memory in ageing subjects (Perry, 1980). Geriforte, an adaptogen, may help the adaptive process by inducing an increase in SDH enzyme levels during stress and also prove useful in aged individuals in whom adaptability to stress is low.
REFERENCES