

Evaluation of Geriforte, an Herbal Geriatric Tonic, On Antioxidant Defense System in Wistar Rats

Balkar Singh, Sharma, S.P. and Ritu Goyal

Laboratory of Nutritional Histopathology and Ageing,
Department of Zoology, Kurukshetra University, Kurukshetra, India.

INTRODUCTION

The free radical theory of ageing proposed by Harman¹ states the involvement of active oxygen species in ageing. Free radical damage to membranes of the nerve cells has been suggested to be the primary cause of ageing of an organism². The monovalent reduction of oxygen that occurs during normal oxidative metabolism results in the production of highly reactive oxygen species including superoxide free radicals (SOR)³.

Some oxygen free radicals are dismutated very actively by superoxide dismutase (SOD)⁴. The product of the dismutation process is hydrogen peroxide (H₂O₂), which is per se less harmful to the biological molecules than the SOR² and is eliminated by catalase (CAT) and glutathione peroxidase (GPx)^{5,6}. Another highly reactive hydroxyl radical (·OH) generated by the action of H₂O₂ and SOR via the iron-catalyzed Haber-Weiss reaction is also a potentially damaging radical. The attack of ·OH on polyunsaturated fatty acids within the cellular membranes results in lipid peroxidation^{7,8} leading ultimately to the formation of the age pigment lipofuscin, which is directly related to the age of animals⁹.

Geriforte, an herbomineral formulation, is an ideal geriatric tonic to solve the problem of ageing. The oral administration of this Ayurvedic drug has been reported to inhibit the formation of lipofuscin in ageing rats^{10,11}. Since SOR is the root cause of lipofuscin formation, alterations in the level of SOD, CAT, and lipid peroxidation could substantially influence the process of ageing. Therefore, in the present study, the Ayurvedic drug Geriforte has been evaluated for SOD, CAT, and thiobarbituric acid reactive substance in terms of malondialdehyde (MDA), a product of lipid peroxidation, in the brain of ageing Wistar male rat.

MATERIALS AND METHODS

Twenty two month old Wistar male rats used in the present experiment were obtained from the Animal House, Kurukshetra University, Kurukshetra, India. The animals were divided into experimental and control groups of 10 animals each. The experimental animals were given Geriforte powder (The Himalaya Drug Co., Bombay, India) orally by mixing it with cane sugar (1:1) at a dosage of 900 mg/kg body weight per day for two months. Another group of animals received an equal amount of sugar only, served as control. Both groups of animals received Gold Mohur rat feed (Hindustan Lever Ltd. Bombay, India) and water *ad libitum*. The animals were housed in the same room maintained at 27 ± 2°C under 12 h light-dark cyclic illumination provided by Laxman Sylvania 40W fluorescent tubes.

The animals were sacrificed after anesthetizing with ether. Before sacrifice, they were observed on starvation for 12 hours. The brain was removed quickly from the skull, and the homogenate was prepared in 10 mM tris-HCl extraction buffer containing 0.32 M sucrose and 1mM EDTA at pH 7.4. The sample was homogenised in a Teflon glass homogeniser. The crude homogenate was centrifuged at 13,600 g for 30 minutes. The supernatant (postmitochondrial) was collected and used for the determination of enzyme activities and lipid peroxidation. The cytosolic Cu-Zn SOD was estimated according to the method of Marklund and Marklund¹² by monitoring the ability of SOD to inhibit the autoxidation of pyrogallol. One unit of SOD activity is defined as the amount of enzyme required to inhibit the rate of pyrogallol autoxidation by 50% at 25°C. The activity of CAT was determined following the method of Aebi¹³. One unit of enzyme activity is defined as the amount of CAT that decomposes 1 µmol of H₂O₂ in 1 min. Lipid peroxidation in terms of MDA was estimated by using the method of Placer *et al.*¹⁴ The data obtained in the present study were analyzed statistically using Student's *t*-test.

RESULTS

The data obtained from the control and Geriforte-treated Wistar male rats on SOD, CAT and lipid peroxidation have been summarized in Table 1. The Geriforte-treated rat brain had significantly ($p<0.01$) a higher level of SOD and CAT contents than that recorded in their respective controls. The contents of SOD and CAT in Geriforte-treated brain increased by 24% and 30% respectively. However, the magnitude of MDA content is reported to be decreased significantly ($p<0.01$) in the Geriforte-treated rat brain. The decrease in MDA content in Geriforte-treated animals was 26%.

Groups	SOD (units/mg protein)		CAT (units/mg protein)		MDA (nmol/g tissue wet weight)	
Control animals	8.00 ± 0.12	$p<0.01$	2.77 ± 0.98	$p<0.01$	164.89 ± 4.98	$p<0.01$
Geriforte-treated animals	9.92 ± 0.27		3.60 ± 0.08		121.87 ± 1.89	

^aEach value is the mean ± standard error of the mean of data from 10 animals.

DISCUSSION

In the present study, the Geriforte administered rats have been found to exhibit and increased level of SOD and CAT with a decreased level of MDA.

It is now well established that the ageing process is associated with an increased susceptibility of tissue to free radical damage as well as elevated level of free radicals, as evidenced by the accumulation of the age pigment lipofuscin¹⁵. Lipid peroxidation, which leads to the formation of the age pigment, has been identified as a primary deteriorative reaction in ageing, caused by free radicals and SOD was shown to inhibit lipid peroxidation *in vitro*¹⁶. A decline in SOD level as a function of age has been studied in a variety of organs of various animals¹⁷⁻²⁰. Thus, it would be of interest to investigate whether the present antiageing Ayurvedic drug, Geriforte, exerts its geriatric effects by inducing the production of SOD and CAT, and inhibiting lipid peroxidation in old animals.

Geriforte, an herbomineral compound, includes a group of Rasayans such as *Centella asiatica* (Brahmi), *Phyllanthus emblica* (Ambla), *Withania somnifera* (Asvagandha) and *Tinospora cordifolia* (Guduchi). Rasayans bring about balance in physiology, enhance immunity, and retard ageing²¹. It seems possible that Geriforte postpones the onset of ageing by inducing the production of SOD and CAT, which scavenge SOR and eliminate H₂O₂ respectively from the biological pathways. It is surmised that the Rasayanic constituents of Geriforte act synergistically to induce the higher production of SOD and CAT *in vivo*. A question to which we have no clear answer yet is, by what mechanism does this antiageing Ayurvedic drug induce the *in vivo* production of SOD and CAT.

As is evident from the present results, Geriforte has a marked potency to induce a large amount of SOD and CAT production against the damaging effects of free radicals. We can safely say that Geriforte is an ideal geriatric tonic for promoting health and longevity. However, further investigations are needed to elucidate the exact mode of action of individual ingredients of Geriforte in the ageing process.

SUMMARY

Geriforte, an herbomineral compound prepared from several herbs and minerals, is being used as a restorative tonic to solve the problems of old age in India. Since active oxygen species have been proposed to be involved in the ageing process of the brain, alterations in the level of enzymes involved in the defense system against free radicals and other active oxygen species could substantially influence the ageing process. Therefore, in the present study, this antistress adaptogenic Ayurvedic preparation has been evaluated for the activities of superoxide dismutase (SOD) and catalase (CAT), key enzymes of the cellular defense system, in the brain of ageing Wistar male rats. The present study was carried out on 22-month old rats. They were given Geriforte powder orally at dosage of 900 mg/kg body weight once a day for two months. The Geriforte-treated rat brain had significantly high levels of SOD and CAT activities than those recorded in their respective controls. The contents of SOD and CAT in Geriforte-administered brain increased by 24% and 30% respectively. In the present investigation, free radical damage was assessed by measuring thiobarbituric acid reactive substance (TBARS) in postmitochondrial fractions of brain homogenate. The magnitude of TBARS has been reported to be decreased by 26% in the Geriforte-treated rat brain. In conclusion, it seems logical to suppose that Geriforte, a combination of several plant ingredients and minerals, protects the body against the damaging effects of free radicals by producing large amounts of antioxidant enzymes.

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