Geriforte Stimulates Antioxidant Defense System

Vandana Pathania, Nidhi Syal, Manjinder K Hundal and Khanduja, K.L.*
Department of Biophysics, Postgraduate Institute of Medical Education & Research, Chandigarh, India.
[*Correspondent author]

SUMMARY

Geriforte, a herbomineral preparation, feeding at 1g% dose level for 4 weeks significantly increased catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in liver of mice. In rats, in addition to these enzymes, the levels of reduced glutathione (GSH) were also significantly enhanced. However, only SOD in mice and CAT and SOD activities in rat lung were significantly elevated after Geriforte treatment. A decrease in superoxide (O$_2^-$) and H$_2$O$_2$ release by alveolar macrophages obtained from rats fed on Geriforte containing diet was observed. The results suggest that Geriforte feeding stimulates antioxidant defense system and indicate the future preventive/therapeutic prospects of this preparation against free radical damage under various pathological states.

Ageing is associated with a general decline in physiological functions which leads to morbidity and mortality. Evidences implicate stochastic events as being a fundamental driving force behind this process$^1$. The sustained damage inflicted by endogenously produced oxidants is the likely cause of age related deficits in cellular functions.

In recent years, the issue of chemopreventors in natural products has received considerable attention. Chemopreventers are classified as natural entities of plant origin which can prevent the appearance of long-term age-related diseases like cancer and cardiovascular disease. It has been recommended that chemoprevention should be inexpensive with an easily applicable approach to control ageing and age-related disorders. Chemopreventers can be found in all food categories with plants being the main source. Herbs, spices and food consumed in different parts of the world are being tested for the presence of new chemopreventers$^{2-4}$. Some frequently consumed spices and vegetables in India, Japan and other Asian countries have been reported to have beneficial effects on health, e.g. curcumin in turmeric and d-limonene in citrus oil inhibit chemically induced tumors$^5-7$. Effective agents have been found in herbs like rosemary, sesame seeds, organo, pepper, garlic and ginger. Many independent researchers recommend that people should increase the consumption of fruits, vegetables and herbs.

The role of free radicals has been implicated in various diseases and also in causing ageing. It appears to be more and more obvious that a combination of different chemopreventors could collectively produce a much better beneficial effect than taking a single compound. With this idea the present study was designed to see the effect of Geriforte (The Himalaya Drug Company, Bangalore, India) on the antioxidant defense system of liver and lungs of mouse and rat. This preparation is a combination of several plant ingredients and minerals including amla (*Phyllanthus Emblica* Linn.), Brahmi (*Centella asiatica* Linn.), Asvagandha (*Withania*
somnifera Dunal) Haldi (*Curcuma longa* Linn.), Bhangra (*Eclipta alba* Linn.), Loh bhasma (iron oxide), Jasad bhasma (Zinc oxide), Onion (*Allium cepa* Linn.), Garlic (*Allicin sativum* Linn.), Grape (*Vitis vinifer* Linn.), Carrot (*Docos carota* Linn.) etc. This preparation is very well tolerated by rodents and is reported to have some health benefits including reduction in anxiety disorders, and age-related enzymatic changes in liver and brain. The present study evaluates the effect of oral intake of Geriforte on antioxidant defense system in tissues of two different species i.e. mouse and rat.

Reduced glutathione, oxidised glutathione, glutathione reductase, t-butylhydroperoxide, nicotinamide, adenine dinucleotide phosphate reduced, nitroblue tetrazolium (NBT), 2,2-dithiobis-5-nitrobenzoic acid (DTNB), horseradish peroxidase, phorbol-12-myristate-13-acetate (PMA), RPMI-1640 (without phenol red) culture medium were procured from Sigma Chemical Co., St. Louis, MO. All other chemicals were of analytical grade and purchased locally. Geriforte (powder form) was a generous gift from The Himalaya Drug Company, Bangalore, India.

Male swiss mice (18-20 g) and Wistar rats (180-200 g) were used. They were maintained on a pellet diet supplemented with or without Geriforte and given water *ad libitum*. Animals were divided into 2 groups of 8 animals each while one group served as control the other group was fed the pellet diet supplemented with 1 g% Geriforte for 4 weeks. Animal weights were noted weekly.

After 4 weeks the animals were sacrificed by exsanguination under ether anaesthesia. Liver and lungs were perfused immediately with ice cold 0.15M KCL containing 2 mM EDTA, pH 7.4, minced with scissors and homogenized in a Potter-Elvejhem homogenizer at a ratio of 1 g wet weight to 4 ml KCl-Tris buffer (150 mM KCl, 50 mM Tris-HCl, pH 7.4). Homogenates were then centrifuged at 10,000 g for 30 min, and the post-mitochondrial supernatant (PMS) was used for the estimation of antioxidant defense system in liver and lung tissues.

Catalase (CAT) was estimated by a modification Luck’s method. Time taken for decrease in absorbance from 0.450 to 0.400 due to H$_2$O$_2$ breakdown was recorded. One unit of catalase was defined as µmol of substrate converted/min/mg protein at 37°C. Method of Beutler was used for determining the glutathione peroxidase activity (GPx) taking t-butyl hydroperoxide as the substrate. The rate of decrease in extinction was calculated using E-6.22 mM$^{-1}$cm$^{-1}$. Superoxide dismutase (SOD) was estimated by the modified method of Kono *et al*. The rate of NBT reduction by auto-oxidation of hydroxylamine hydrochloride was recorded at 560 nm against a blank. One unit of enzyme activity was defined as the amount of protein required for inhibiting the reduction of NBT by 50%. For measuring the glutathione reductase (GR) activity, method of Carlberg and Mannervick was followed. At 340 nm the rate of change in extinction was recorded for 4 min and E-6.22 mM$^{-1}$ cm$^{-1}$ was used to calculate the enzyme activity. Reduced glutathione (GSH) was measured by the method of Moron *et al.*, using DTNB.

In another experiment alveolar macrophages (Ams) were obtained from male rats of the control and Geriforte treated groups. Broncho-alveolar lavage was performed using 5 ml
warm 0.9% NaCl in 6-7 aliquots. The lavage recovery was 75-80% of the instilled saline. The cells were pelleted by centrifugation at 200 g for 10 min at room temperature. They were washed twice with phosphate buffered saline, pH 7.4. Cell viability was found to be >95% when estimated by exclusion of trypan blue. One million cells were cultured in RPMI-1640 medium without phenol red (supplemented with 10% heat inactivated fetal calf serum, penicillin (200 U/ml), streptomycin (100 µg/ml), 2 mM L-glutamine, 24 mM sodium bicarbonate and 1 mM sodium pyruvate) for 90 min using 1 µg/ml PMA as a stimulant to measure superoxide (O$_2$-) and H$_2$O$_2$ release by macrophage using the method of Pick and Keisari$^{16}$. Briefly, O$_2$- release was measured by the reduction of cytochrome C by O2- radicals at 550 nm using E=2.1 x 10$^4$ M$^{-1}$ cm$^{-1}$. Production of H2O2 by AMs was quantitated on the basis of the H$_2$O$_2$ mediated and horseradish peroxidase-dependent oxidation of phenol red, yielding a reactin product that absorbs maximally at 610 nm. Standard curves were made using 1-30 µM H$_2$O$_2$ solutions. In some instances, 1250 U/ml catalase was used to specifically inhibit H$_2$O$_2$ accumulation.

Lung and liver homogenates as such and the AMs per well after digestion by 1N NaOH were used for protein estimation by the method of Lowry et al.$^{17}$. Statistical analysis was done by impaired Student’s ‘t’ test.

Animals subjected to Geriforte treatment for 4 weeks did not show any change in body weight, organ weight (liver and lungs), and PMS protein content (data not shown). Table 1 depicts the influence of feeding Geriforte on hepatic and pulmonary antioxidant defense system in male mice. Geriforte consumption resulted in an increase in the activities of all the measured antioxidant enzymes except glutathione reductase in liver. The specific activity of catalase, GPx and SOD increased from control values. There was no change in the hepatic level of reduced glutathione. Geriforte feeding did not cause any significant change in the parameters of lung antioxidant defense system except SOD, which showed a significant increase.

### Table 1: Effect of Geriforte feeding (1g%) on hepatic and pulmonary defense system in male mice

<table>
<thead>
<tr>
<th>Specific activity</th>
<th>Liver</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Geriforte</td>
</tr>
<tr>
<td>CAT</td>
<td>197 ± 49.8</td>
<td>309 ± 84.2$^{a}$</td>
</tr>
<tr>
<td>SOD</td>
<td>26.0 ± 2.81</td>
<td>34.05 ± 2.41$^{c}$</td>
</tr>
<tr>
<td>GPx</td>
<td>0.122 ± 0.02</td>
<td>0.222 ±0.065$^{b}$</td>
</tr>
<tr>
<td>GR</td>
<td>0.035 ±0.005</td>
<td>0.038 ± 0.006</td>
</tr>
<tr>
<td>GSH</td>
<td>5.97 ±1.32</td>
<td>7.51 ± 1.32</td>
</tr>
</tbody>
</table>

$P$ values: $^{a}<0.05; ^{b}<0.01; ^{c}<0.001$

The data related to influence of Geriforte feeding through diet on hepatic and pulmonary antioxidant defense system in rat is presented in Table 2. In rat liver also, except GR, rest of the enzyme activities (CAT, SOD and GPx) and GSH levels increased significantly on
Geriforte treatment. The activities of CAT and SOD were highly significantly increased in rat lung, however GPx, GR and GSH levels remained unaltered.

### Table 2: Effect of Geriforte feeding (1g%) on hepatic and pulmonary antioxidant defense system in male rats (Values are mean ± SD, n=5 to 8)

<table>
<thead>
<tr>
<th>Specific activity</th>
<th>Liver</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Geriforte</td>
</tr>
<tr>
<td>CAT</td>
<td>222 ± 12.0</td>
<td>432 ± 26.6c</td>
</tr>
<tr>
<td>SOD</td>
<td>1.01 ± 0.11</td>
<td>1.50 ± 0.14c</td>
</tr>
<tr>
<td>GPx</td>
<td>397 ± 39.4</td>
<td>577±76.2b</td>
</tr>
<tr>
<td>GR</td>
<td>0.034 ±0.005</td>
<td>0.033 ± 0.004</td>
</tr>
<tr>
<td>GSH</td>
<td>5.42 ± 0.26</td>
<td>6.58 ± 0.37c</td>
</tr>
</tbody>
</table>

P values: a<0.05; b<0.01; c<0.001

There was a significant decrease in O$_2^-$ (47%) and H$_2$O$_2$ (39%) production by AMs obtained from Geriforte fed rats (Table 3).

### Table 3: Effect of Geriforte feeding (1g%) on superoxide O$_2^-$ and H$_2$O$_2$ release by alveolar macrophages in male rat (Values expressed as nmol/90 min/mg protein, are mean ± SD, n=5 to 8)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Geriforte</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2^-$</td>
<td>173 ± 26.8</td>
<td>82.9 ± 14.5a</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>198 ± 37.3</td>
<td>77.22 ± 11.58a</td>
</tr>
</tbody>
</table>

P<0.001

Though extrahepatic tissue and cells also play an important part in the metabolism of compounds and bringing about physiological changes, liver is the principal organ responsible for metabolism of large number of exo-as well as endogenous compound. Therefore, in the present study the effect of Geriforte, an indigenous herbomineral preparation, feeding on antioxidant defense system was studied in liver, lungs and alveolar macrophages (macrophages that reside in aerobic conditions). The latter constitute the first line of defense in lungs and are exposed to higher concentration of O$_2^-$ and other environmental toxins/pathogens$^{18-20}$.

Enhanced activities of CAT, SOD and GPx in liver of both mouse and rat suggest Geriforte’s effectiveness in combating the pro-oxidant state to which the body gets exposed due to various reasons including drug toxicity, diseases and ageing. The increase observed in only some of the antioxidant enzymes of lungs of both mouse and rats presumably is due to the variation in the tissue and species specificity. Additionally, Geriforte, which is a combination of large number of plant extracts and minerals, may be undergoing first pass metabolism by liver due to the oral route of administration. Therefore, comparatively lesser change which Geriforte produced in extrahepatic antioxidant defense in the present study may be due to the fact that lesser constituents of this drug were finding their way into the systemic circulation.

In lungs, more than 95% of the cells in alveolar space are the alveolar macrophages and they are constantly exposed to increased O$_2^-$ tension and exogenous material. The role of AMs in causing chronic inflammation is well established. Geriforte decreased the capacity of AMs to release reactive oxygen species (O$_2^-$ and H$_2$O$_2$) in response to inflammatory agent, PMA, which may be useful in lowering the chronic inflammation associated with various conditions.
This study indicates that Geriforte may be effective in enhancing the antioxidant defense system. Although, additional studies are required to find the effective dose and duration for which Geriforte may be taken orally to bring various effects.

REFERENCES