Effect of Maharishi Amrit Kalash an ayurvedic herbal mixture on lipid peroxidation and neuronal lipofuscin accumulation in ageing guinea pig brain

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The effects of ayurvedic herbal mixture Maharishi Amrit Kalash(MAK) were studied on brain lipid peroxidation, oxygen consumption, and lipofuscin accumulation in 10 months and 32 months old guinea pigs. Brain regions studied were cerebral cortex, hypothalamus, cerebellum and spinal cord. Parameters assessed were lipid peroxidation, oxygen consumption, and lipofuscin accumulation. The endogenous lipid peroxide was found to be increased significantly (P<0.05) in the 32-month-old animals. Neuronal lipofuscin accumulation in the neurons of cerebral motor cortex, cerebellum and cervical spinal cord was increased (P<0.05) in the older animals. Oxygen consumption was found to be decreased significantly (P<0.05) in the 32-month old guinea pigs. Treatment with MAK at a dose of 500 mg/kg body weight daily for two months reduced the lipid peroxidation and lipofuscin pigment accumulation significantly in brain regions and it also helped in restoring the normal oxygen consumption in the older animals. This indicates antioxidant properties of MAK.

The free radical attack on polyunsaturated fatty acids (PUFA) induces the irreversible and deleterious changes in the cell membrane, and this is believed to contribute to ageing. The lipid peroxidation results in the formation of lipofuscin, which accumulates in cells with ageing. The results on the lipid peroxidation have not been so consistent. Some authors have reported an increase in lipid peroxidation with age, while others have reported decline in lipid peroxidation. Keeping in view such ambiguities we applied two different approaches to measure the lipid peroxidation with age. First, endogenous lipid peroxidation measured in freshly prepared homogenate. Second the tissue homogenate is incubated in the presence of room air and then reacted with thiobarbituric acid. This second approach is felt to provide an assessment of potential substrate available for peroxidation. While, first approach indicates already formed lipid peroxides in the tissue.

Maharishi Amrit Kalash(MAK) 'an herbal mixture' prepared according to the ancient ayurvedic formulation. MAK is available in two forms 'MAK-4 and MAK-5'.

MAK-4 is called ambrosia, it is in the form of tablets. MAK-5 is called nectar it is available in the form of paste. The different component of MAK-4 and MAK-5 are described in various publications. According to Charka Samhita(an ayurvedic medical text) there is no maximum dose of this formulation, it can be taken up to the amount till it does not disturb the normal food consumption. Many of such preparations in ayurveda are called Rasayanas. MAK has shown promises in protection against free radicals attack. Rasayanas are believed to strengthen the body's resistance to infections and diseases and enhances longevity. The mode of action and the cellular effects of MAK are not precisely known. Therefore, in the present study the effects of MAK on lipid peroxidation, lipofuscin accumulation and the oxygen consumption by the animals were studied.

Materials and Methods

Male guinea pigs (Dunkin Harley) of two age groups 8 months and 30 months were used in the present study. Each group was subdivided in two subgroups, each consisting of 40 animals. One subgroup served as control and the other as the experimental group and was given a mixture of MAK-4 and MAK-5 in the ratio of 1:20. The MAK mixture was given intragastrically with the help of a canula at a dosage of 500 mg/kg-body weight daily at 11.00 hrs for two months. Both the groups were fed pelleted food (Hindustan Lever Ltd., New Delhi) ad libitum.

Thiobarbituric acid was purchased from Sigma chemical Co., USA. Other chemicals were purchased.
from SRL or SD-fine chemical Co. Mumbai, India and were of analytical grade. The Ayurvedic preparation MAK was a generous gift from Maharishi Ayurveda Corporation Ltd., Faridabad, India.

Oxygen consumed by each animal from different animal groups was measured using an O2 consumption apparatus29.

After the drug administration, the animals were decapitated and the brains and the spinal cords were dissected immediately and rinsed in chilled normal saline. The following discrete regions: cerebral motor cortex, hypothalamus, cerebellum and brain stem (pons and medulla), cerebral hemisphere without cerebral cortex, hypothalamus and cervical spinal cord were used for measurement25.

Homogenates were prepared in a ratio of 1g of wet tissue to 9 ml of 1.15% KCl by using a glass-potter Elvehjen homogeniser. Thiobarbituric acid reactive substance was measured in 1000x supernatant of (1) freshly prepared tissue homogenate and (2) the tissue homogenate was incubated in air at 37°C for one hr. MDA, the end product of superoxide radical induced lipid peroxidation was quantitized by the thiobarbituric acid colored reaction22. Total soluble proteins were measured with method described by Lowry et al23.

For histochemical studies, animals were perfused transcardially with EDTA (3% in physiological saline) for 10 min followed by formaldehyde- saline solution (10% in physiological saline) for 20 min as described by Zeman and Innes24.

Motor cortices, cerebella and cervical spinal cords were dissected out and fixed in formaldehyde-calcium (FCA)25. For lipid histochemistry, animals were dissected out and fixed in formaldehyde-calcium (FCA)25. For lipid histochemistry, animals were decapitated, various tissues were removed and fixed in formaldehyde-calcium (FCA)25. For lipid histochemistry, animals were decapitated and the brain sand the spinal cords were dissected immediately and rinsed in chilled normal saline. The following discrete regions: cerebral motor cortex, hypothalamus, cerebellum and brain stem (pons and medulla), cerebral hemisphere without cerebral cortex, hypothalamus and cervical spinal cord were used for measurement25.

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Results

Oxygen consumption was found to decrease (P<0.05) in the 32 months old animals (Table 1). The endogenous TARS as depicted in the Table 2 ranged from 26.78 nmole/mg protein to 76.89 nmole/mg

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Young animals (10 months)</th>
<th>Old animals (32 months)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Brain-stem</td>
<td>32.37±0.12</td>
<td>26.40**</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>43.81±0.28</td>
<td>32.00*</td>
</tr>
<tr>
<td>Cerebral</td>
<td>41.86±1.37</td>
<td>32.10*</td>
</tr>
<tr>
<td>Cortex</td>
<td>26.42±1.20</td>
<td>29.41*</td>
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<tr>
<td>Hypothalamus</td>
<td>26.40±0.31</td>
<td>24.47*</td>
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Table 1—Effect of MAK on oxygen consumption

| Values, expressed as oxygen consumed ml/min/kg body wt. ±SE of 10 animals |

Table 2—Effect of MAK on the level of endogenous TARS production

| Values, expressed as nmole of TARS/mg protein, are means±SE of 5 animals |

*(P<0.05) compared with controls within the same age group.
***(P<0.05) Compared with 10 months old control animals.
protein in different regions of CNS, the highest being in the rest of the cerebrum and the least in the cerebellum. The amount of endogenous TARS increased in old age \((P<0.05)\). The highest increase was observed in the hypothalamus, i.e. 76.74% and the lowest in the rest of cerebrum, i.e. 59.38%.

The TARS production after incubating the tissue homogenate for one hour in air \((in \ vitro)\) was lower in the tissues collected from the 32 months old animals \((P<0.05)\) than in the 10 months old animals. The maximum difference was observed in spinal cord (60.88%) and brain stem (44.48%) and the minimum in hypothalamus (10.6%) (Table 3). The lipofuscin accumulation with age was highest in cerebral motor

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<tr>
<th>Table 3—Effect of MAK on the level of (in \ vitro) TARS production</th>
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<tr>
<td>[Values expressed as n mole of TARS/mg protein, are means±SE of 5 animals]</td>
</tr>
<tr>
<td>Tissue</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>Hypothalamus</td>
</tr>
<tr>
<td>Rest of the cerebrum</td>
</tr>
<tr>
<td>Cerebrum</td>
</tr>
<tr>
<td>Brain-stem</td>
</tr>
<tr>
<td>Cerebellum</td>
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*\((P<0.05)\) compared with controls within the same age group.
**\((P<0.05)\) compared with 10 months old control animals.

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<th>Table 4—Effect of MAK on the percentage neuronal are occupied by lipofuscin</th>
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<tbody>
<tr>
<td>[Values expressed as percentage neuronal are occupied by lipofuscin ±SE of 10 animals]</td>
</tr>
<tr>
<td>Tissue</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>Cerebellum</td>
</tr>
<tr>
<td>Spinal cord</td>
</tr>
</tbody>
</table>

*\((P<0.05)\) compared with controls within the same age group.
**\((P<0.05)\) compared with 10 months old control animals.

Fig. 1—Fluorescent micrograph from unstained motor cortex section of 32 months old guinea pig. Showing autofluorescent pigment aggregations (p) in pyramidal neurons at Juxtanuclear position. x1520
Fig. 2—Fluorescent micrograph from unstained motor cortex section of 32 months old guinea pig treated with MAK. Note very few fluorescent pigment granules. x1050
Fig. 3—Photomicrograph from unstained cerebellum section of 32 months old guinea pig showing Purkinje neurons. Note heavy deposits of lipofuscin. x1120
Fig. 4—Photomicrograph from cerebellum section of 32 months old guinea pig treated with MAK showing decreased amount of lipofuscin. x1050
Fig. 5—Photomicrograph of cervical spinal cord section showing anterior horn cells of 32 months old guinea pig. Note heavy accumulation of lipofuscin x 320
Fig. 6—Fluorescent micrograph of 32 months old treated guinea pigs showing anterior horn cells with decreased lipofuscin. x 320
The treatment with MAK increased the oxygen consumption only in the 32 months old animals \((P<0.05)\). The drug treatment decreased the level of TARS significantly in all the regions of CNS of both the age groups. The reduction was highest in the brain stem \((70.20\%)\) followed by the hypothalamus \((57.02\%)\). The reduction in the TARS was lower in 10 months old animals as compared to the 32 months old animals in each region of CNS. The drug treatment resulted in a reduction in the in vitro TARS production in both the age groups of animals \((P<0.05)\).

The lipofuscin accumulation was found to be decreased to a significant level in the MAK treated groups \((P<0.05)\) (Figs 2, 4 & 6).

**Discussion**

The endogenous TARS was found to be increased with age in all the regions of CNS studied. The present study reveals lower in vitro TARS production in 32 months old animals. It appears that as the level of endogenous peroxides increases with age, the amount of substrate available for peroxidation, as measured by incubating tissue homogenate prior to reacting them with TBA, actually decreases with advancing age\(^9\). This view is also supported by the observations that the ratio of unsaturated to saturated fatty acids decreases in membrane with age\(^9,10\). Thus, the endogenous lipid peroxidation is quite different from TARS produced by incubating the homogenates in the air. Treatment with MAK may be effective in preventing the free radical induced damage as it contains a large number of compounds namely, tannic acid, flavanoids, catecholamines, tocopherol, polyphenols, ascorbates, riboflavin, carotenes, mucilage, octacosanol, saponins, sphaeranthine, asparagine, glycyrrhizin, camphene, limonene, pinene, etc\(^{13,17}\). Some of the above mentioned chemical components act as potent antioxidants\(^{15-16}\). The lipofuscin accumulation was found to decrease significantly in MAK-treated groups \((P<0.05)\). This may be due inhibition of lipid peroxidation because lipofuscin formation results from lipid peroxidation. Hence it is concluded that MAK can be effective in checking the lipid peroxidation and decreasing the percentage neuronal area occupied by lipofuscin. Since there are many other anti-ageing compounds like deprenyl and acetyl carnitine which has also shown their positive effects\(^{32-36}\), MAK increases the activity of various enzymes only in the old animals in a very region specific way\(^{19}\). Deprenyl increases the activity of superoxide dismutase \((SOD)\) and catalase \((CAT)\) but its effect on glutathione peroxidase \((GPx)\) is still a matter of controversy\(^{32}\). Similarly acetyl carnitine also does not show any effect on the activity of GPx\(^{30}\). However the treatment with MAK increases the activity of SOD, CAT and GPx\(^{19}\).

Since GPx has lower Km value than CAT hence can be considered more effective in protection against \(H_2O_2\)\(^{37}\). The reduction in the total oxygen consumption may have certain adverse effects on the Brain as the brain itself consumes 25% of the total oxygen consumed by the body\(^{3}\). Thus the normalization of oxygen consumption after MAK treatment in older animals may be helpful to normalize the physiological functions of the brain, although more studies are required to establish this claim. Thus MAK appears to provide a good protection against free radicals like centrophoxine deprenyl and acetyl carnitine\(^{37}\).

**References**


