BIOCHEMICAL EFFECTS OF AGING ON SOME ANTIOXIDANT DEFENCE ENZYMES IN LIVER, HEART AND BRAIN OF CAMELS
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ABSTRACT
This study was undertaken to elucidate the possible effect of aging which is an extremely complex multifactorial process with various alterations in cellular components on some antioxidant defence enzymes system in liver, heart and brain of camels for this aim 30 male one-humped camels with age ranged between 1 – 40 years and weighed 450-650 Kg divided according to their ages into 3 groups each of 10 animals: Group (1) below 5 years old representing Young camels - control. Group (11) from 5 – 15 years – Adult, Group (111) over 15 years old - Aged camels. The obtained data showed a high significant increase in liver Superoxide dismutase (SOD),Glutathione peroxidase (GPX) activity of adult, this increase became highly significant in old, a significant increase in liver Catalase (CAT) activity of adult. Moreover a high significant decrease in old. A significant decrease in liver L-Malondialdehyde (MDA) content of adult, whereas a very high significant increase in old. A significant increase of heart SOD and CAT activities of adult, while a very high significant decrease of the heart SOD activity and a high significant decrease of the heart CAT activity in old. Whereas a significant decrease of heart MDA in adult and a significant increase of it in old. A non significant increase in CAT activity in brain of adult. While a significant decrease CAT in old. A significant increase in GPX and MDA in brain of adult, this increase became high significant at old. A high significant increase in brain Monoamine oxidase (MAO) activity in the adult, this increase became very high significant in the old. A high significant decrease in brain Acetylcholinesterase (AchE) activity in adult, this decrease became a very high significant decrease in brain of old camels. It could be concluded that the antioxidant enzymes had vital role in protecting tissues from oxidative damage during aging as they decrease increases the vulnerability of celluarl components to reactive oxygen species.

Key Words: Antioxidant enzymes, liver, heart, brain and Camel.
INTRODUCTION

Aging is the cumulative result of oxidative damage to the cells and tissues of the body that arises primarily as a result of aerobic metabolism also the free radical theory may be used to explain many of the structural features that develop with aging including the lipid peroxidation of membranes, formation of age pigments, cross-linkage of proteins, DNA damage and decline of mitochondrial function (Wickens 2001).

Inal, et al., (2001) mentioned that free oxygen radicals have been proposed as important causative agents of aging and the course of metabolism, reactive oxygen species (ROS) are produced from the respiratory chain of the mitochondria having the capacity to oxidize a variety of cellular constituents including lipids, DNA, and proteins. They include hydroxyl radicals, superoxide anion, hydrogen peroxide and nitric oxide. They are very transient species due to their high chemical reactivity that leads to lipid peroxidation and oxidation of DNA and proteins (Melov 2002). When ROS generation is increased to an extent that overcomes the cellular antioxidants, the result is oxidative stress (Mates 2001) which is defined as damage and an imbalance between pro-oxidants and/or free radicals and antioxidantizing systems (Bonnefoy et al., 2002). Moreover, Reiter, (1995) reported that the major causes of age-related destruction of neuronal tissue is toxic free radicals that are natural results of aerobic metabolism and the brain is susceptible to free radicals attack because it generates more of these toxicants per gram of tissue than does any other organ. Also, the heart faces a high risk of free radicals injury owing to a slow generations of antioxidants (AO) enzymes by its cells (Devi, et al. 2003).

This study was undertaken to elucidate effect of aging on some antioxidant defence enzymes system of liver, heart and brain of male camels which may help to clarify the metabolic changes which may occur. The selected antioxidant enzymes were SOD, CAT, and GPX. In addition to MDA content was also determined. Moreover MAO and AchE activities were also investigated only in brain tissues.

MATERIALS AND METHODS

Thirty male one-humped camels with age ranged between 1–40 years and weighed 450-650 Kg., divided according to their ages into 3 groups each of 10 animals. Group (1) below 5 years old representing Young camels - control. Group (11) from 5 – 15 years – Adult, Group (111) over 15 years old - Aged camels.
Sampling: A total number of ninety fresh (liver, heart and brain tissue specimens) were collected from Toukh abattoir after scarification of camels immediately removed, washed several times with saline. Then minced with a meat chopper and about 0.5 g weighed of representative tissue were homogenized immediately with 4.5 ml (potassium phosphate buffer solution PH 7.4, 0.1 mM EDTA), then briefly centrifuged at 3000 r.p.m. for 13 minutes. The supernatant fraction was re-centrifuged for another 10 minutes at 4000 r.p.m. the clear supernatants were separated and used freshly for the determination of various enzymes activities as in a method described by (Salman et al. 1995).

All of them used directly for determination of: SOD (Nishikimi et al. 1972); CAT (Bergmeyer, 1974); GPX (Beutler et al., 1975); MDA (Ohkawa, et al., 1979); MAO (Mc Eween, 1969) and AChE (Den. Blawen, et al. 1983).

Statistical analysis of the results was carried out using student’s T-test and F-test. according to Kempthorn (1969).

RESULTS

The obtained data showed a high significant increase in liver SOD, GPX activity of adult, this increase became highly significant in old, a significant increase in liver CAT activity of adult. Moreover a high significant decrease in old. A significant decrease in liver MDA content of adult, whereas a very high significant increase in old. A significant increase of heart SOD and CAT activities of adult, while a very high significant decrease of the heart SOD activity and a high significant decrease of the heart CAT activity in old. Whereas a significant decrease of heart MDA in adult and a significant increase of it in old. A non significant increase in CAT activity in brain of adult. While a significant decrease CAT in old. A significant increase in GPX and MDA in brain of adult, this increase became high significant at old. A high significant increase in brain Monoamine oxidase (MAO) activity in the adult, this increase became very high significant in the old. A high significant decrease in brain Acetylcholinesterase (AchE) activity in adult, this decrease became a very high significant decrease in brain of old camels.
DISCUSSION

Aging is usually accompanied with hypometabolic state including alterations in antioxidant enzymes activities as SOD, CAT and GPX which resulted in promoting the free radical damaging effect (Taylor, et al. 1991).

The obtained data (Table 1) showed that liver SOD activity recorded a significant increase of aged male camels this is in harmony with the findings of Kim et al. (2003) who studied the age-dependent changes in the hepatic antioxidant systems in hepatocytes and showed that SOD activity was higher in liver tissues of the older rats than younger.

This increased activity because of the reactive oxygen species when increased in cells may cause overexpression of the enzyme (Portakal et al. 2000) also the enzyme increase reflects the higher efficacy of animal to countered the deleterious effect of superoxide leaving less amount of oxidative damage (Kaikkare, et al. 1996).

The obtained data showed that Heart SOD activity was significant decrease of aged camels came in accordance to the data recorded by, Devi, et al (2003) who investigated the activities SOD activity in heart displayed an age-dependent decline and significantly decreased during aging.

This decrease might be explained as the free radcals resulted in the formation of protein peroxide as well as inactivation of the detoxifying enzyme by splitting of the peptide chain, to the reduction in hepatic protein synthesis and increase in protein degradation or both (Gebicki, et al. 1993).

The obtained data showed that the brain SOD activity is significantly increased of aged camels that is similar to the results reported by Leutner, et al.(2001) who reported that neurodegenerative processes of aging are associated with the generation of ROS during cellular metabolism. These ROS are scavenged by antioxidant enzymes in biological systems. They had found that activity of SOD was increased with age in all regions of the brain except in hippocampus of 2-yr-old mice. This increased activity because of ROS when increased in cells may cause overexpression of the enzyme and enzyme increased reflects the higher efficacy of animal to countered the deleterious effect of superoxide leaving less amount of oxidative damage (Portakal et al., 2000).

The recorded data showed that liver CAT activity high significant decrease of aged camels were similar to the findings of Muradian et al. (2002) who showed The activities of CAT in most tissues displayed an age-dependent decline and significantly decreased during
aging, and the levels of oxidative protein damage (measured as carbonyl content) in the brain and liver were significantly higher in older animals than in young animals.

The recorded liver CAT decreased activity could be attributed to its decreased synthesis or increased its degradation or its inhibition by increased free radicals production as H2O2 and O2 which increased by aging or may be due to the resultant H2O2 can be destroyed by it. The decrease in CAT activity was also attributed to the reduction in hepatic protein synthesis and increase in protein degradation or both (Dillon, et al. 1990).

The reported data showed that the heart and brain CAT activities significant decrease of aged camels that could be attributed to its decreased synthesis or increased its degradation due to the heart faces a high risk of free radical injury owing to a slow generation of antioxidant (AO) enzymes by its cells. Also the decrease in heart CAT activity was attributed to the reduction in protein synthesis and increase in protein degradation or both Kasapoglu and Orben (2001). In addition, brain CAT activity decrease could be explained by (Reiter, et al. 1995) as the major cause of age related destruction in neuronal tissue was toxic free radicals that might resulted in denaturation, aggregation and fragmentation of protein altering physiochemical properties and potentially losing enzyme activity.

The recorded liver GPX activity showed high significant increase of aged camels which might be attributed to the higher level of oxidative stress as its activity has been shown to be raised by oxygen radicals in order to detoxify the toxic metabolites which resulted from lipid peroxidation (Sarkar et al. 1997), also it increased because of ROS when increased in cells may cause overexpression of the enzyme. This increased activity reflects the higher efficacy of animal to countered the deleterious effect of H2O2 leaving less amount of oxidative damage (Portakal et al. 2000).

The obtained data showed that the heart GPX activity was significant decrease of aged camels, that decreased activity might be attributed to the free radical theory of aging which explained that one of the potential major causes of age related destruction in tissues was toxic free radicals, which considered a natural result of aerobic metabolism (Devi, et al. 2003). These free radicals may lead to an inactivation of detoxifying enzymes such as GPX by splitting of peptide chain. Also the decrease of enzyme may be attributed to the reduction in hepatic protein synthesis, increased protein degradation or both (Rikans and Harnbrook, 1997).
The recorded brain GPX activity was high significantly increased of aged male camels; this increase might be attributed to the higher level of oxidative stress which were the potential causes of age-related neuronal damage, the brain is particularly sensitive to oxidative damage as GPX has been shown to be raised by oxygen radicals in order to detoxify the toxic metabolites which resulted from lipid peroxidation (Baek, et al., 1999). Also GPX increased because of ROS when increased in cells may cause overexpression of the enzyme (Portakal et al., 2000) and the increased enzyme reflects the higher efficacy of animal to countered the deleterious effect of H2O2 leaving less amount of oxidative damage.

The recorded data revealed that liver, heart and brain MDA were highly significant increased of aged male camels, which might be due to the ROS generation is increased to an extent that overcomes the cellular antioxidants, the result is oxidative stress (Mates, 2001) and H2O2 produced can produce OH radical which cause lipid peroxidation causing peroxidative damage to the cell and the production of a variety of aldehydic compounds (Gil, et al. 2002). The excessive lipid peroxidation leads to the accumulation and increased of MDA (unsaturated carbonyl products of lipid peroxidation) as a measure and a direct indicator of lipid peroxidation and one of the bi-products in the lipid peroxidation process and considered as a marker of oxidative stress (Lee, et al. 2004).

The recorded brain MAO activity was high significant increase of aged male camels (Table 2). This increase might be due to age-related increase in the rate of mitochondrial O2- and H2O2 generation and huge amounts of oxidative damage leading to several neurodegenerative disorders, due to an imbalance between free radical generation and antioxidant defense system Sandhu and Kaur (2002).

The recorded values revealed that the brain AchE was decreased which was attributed to the direct effect of free radicals OH on the enzyme causing a reduction in protein synthesis and increase in protein degradation or both (Tsakiris, et al., 2000), it might also be explained as those free radicals resulted in the formation of protein peroxide as well as inactivation of the enzyme by splitting of the peptide chain. Also that decrease is the major cause of age-related destruction in neuronal tissue that might resulted in denaturation, aggregation and fragmentation of protein altering physicochemical properties and potentially losing enzyme activity, Moreover the AchE decreased activity attributed to its decreased synthesis or increased its degradation (Kakkar, et al., 1996). Also the decrease of enzyme might be
attributed to the reduction in hepatic protein synthesis, increased protein degradation or both (Rikans and Harnbrook 1997).

From the present investigation it could be concluded that the free radicals can damage the genetic materials causing lipid peroxidation in cell membrane and inactivate membrane bound enzymes and it is known that antioxidant enzymes play a vital role protecting tissues from excessive oxidative damage during aging as depletion of each of antioxidant system increase the vulnerability of various tissues and cellular components to reactive oxygen species.
Table (1): Mean values of SOD (U/mg protein), CAT (U/mg protein), GPX (U/mg protein), and MDA (nmol/mg protein) in liver, heart and brain tissues of homogenates of male camels in different ages

<table>
<thead>
<tr>
<th>Tissue</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GPX (U/mg protein)</th>
<th>MDA (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young Camels</td>
<td>179.92 ±4.00</td>
<td>214.29 ±4.32**</td>
<td>263.60 ±4.33***</td>
<td>194.34 ± 4.10</td>
</tr>
<tr>
<td>Adult Camels</td>
<td>211.43 ± 3.80*</td>
<td>174.42 ± 3.08**</td>
<td>270.49 ± 6.30</td>
<td>308.69 ± 6.94**</td>
</tr>
<tr>
<td>Aged Camels</td>
<td>294.98 ± 3.41*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young Camels</td>
<td>129.08 ±4.30</td>
<td>132.07 ±4.33*</td>
<td>106.76 ±4.26**</td>
<td>126.62 ± 4.08</td>
</tr>
<tr>
<td>Adult Camels</td>
<td>136.09 ± 3.80**</td>
<td>108.42 ± 3.80**</td>
<td>176.30 ± 3.60</td>
<td>162.89 ± 3.80**</td>
</tr>
<tr>
<td>Aged Camels</td>
<td>175.62 ± 3.41*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young Camels</td>
<td>54.51 ±3.06</td>
<td>61.48 ±3.2</td>
<td>86.13 ±3.26**</td>
<td>58.88 ± 1.48</td>
</tr>
<tr>
<td>Adult Camels</td>
<td>66.39 ± 1.80</td>
<td>41.41 ± 1.09*</td>
<td>82.11 ± 3.61</td>
<td>101.27 ± 3.40**</td>
</tr>
<tr>
<td>Aged Camels</td>
<td>89.85 ± 3.41</td>
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</tr>
</tbody>
</table>

S.E.: Standard Error.
*: Significant at (P<0.05)
**: highly significant at (P<0.01)
***: Very highly significant at (P<0.001)

Table (2): Mean values of MAO (U/mg protein) and AChE (U/mg protein) in brain tissues homogenates of male camels in different ages

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MAO (U/mg protein)</th>
<th>AChE (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young Camels</td>
<td>Adult Camels</td>
</tr>
<tr>
<td>Brain</td>
<td>280.65 ± 6.33**</td>
<td>304.41 ± 5.99***</td>
</tr>
</tbody>
</table>

S.E.: Standard Error.
*: Significant at (P<0.05)
**: highly significant at (P<0.01)
***: Very highly significant at (P<0.001)


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