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Molecular correlates of ageing: Anti-oxidant and protective effects of feeding honey in aging albino rats

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ABSTRACT : The effects of feeding milk and honey once a day in a restricted diet regime on lipid per oxidation and catalase activity in discrete regions of the brain of aged albino rats were studied. Feeding milk and honey decreased lipid per oxidation ($P<0.01$) and elevated enzyme catalase activity in the cerebral cortex, cerebellum and medulla oblongata of ageing 18 months old rats.The correlation between the lipid peroxidation and kinetics of enzyme catalase in the cerebral cortex, cerebellum and medulla oblongata of aging albino rat brain has been examined. The results indicated that lipid peroxidation was increased with age while the activity of enzyme catalase decreased. The inverse correlation between lipid peroxidation and catalase activity was also evident when the aging animals were fed honey with milk for 15 days. The Km value was decreased and energy activation (Eac) increased for catalase (CAT) during aging. Feeding with honey and milk (15 days) reversed these effects indicating the enhanced efficiency of enzyme catalase in aging rats due to protection offered by honey. The inverse relationship between lipid peroxidation and catalase activity is consistent with the oxidative-stress induced impairments of neuronal activity as indicated by the decline in the efficiency acetyl cholinesterase (AChE) Feeding milk and honey on restricted diet regime has ameliorated the age induced oxidative stress and has elevated the catalytic potential of enzyme catalase and acetyl cholinesterase. This was clearly indicated by a profound decline in the activation energy and decrease in Km value. Honey has therefore acted as a natural antioxidant. Its use for prolonged periods has been suggested to retard the aging process.

KEY WORDS: - Catalase, acetyl cholinesterase, antioxidant, honey, milk, catalytic potential.

INTRODUCTION

Brain has been described a pace maker of ageing as age related changes occurring in the brain leave deleterious impact on the functional efficiency of other tissues. (1). Brain requires minute to minute supply of glucose for its functioning and hence the decline in the uptake of glucose occurring during ageing upsets the chemical architecture of the brain leading to biochemical lesions and functional impairments (2). Age related decline in the neuronal activity has been reported to be due to an increase in the lipid peroxidation (3). Since membrane lipid peroxidation leads to impaired neuronal electrical activity, the activity of enzyme acetylcholinestrase (AChE) which is an index of the electrical activity gets affected substantially during ageing. The present study is aimed to examine the protective action of feeding honey and milk on ageing brain. We have evaluated the beneficial effects of feeding boney on the neuronal activity and the proteins. We have also studied the correlation between the neuronal activity and pro-oxidants antioxidant status in the brain of 18 month old albino rats fed with milk and honey in a restricted diet regime.

MATERIAL AND METHODS

Male albino rats weighing 270 ± 5g (18 months) wistar strain purchased from Central Animal Facility (CAF), Indian Institute of Sciences, Bangalore were housed in polypropylene ages of 3 each at 27 ± 2^0C and 12h/12 h D cycle. They were fed on standard pellet diet (Hindustan Lever Ltd. Mysore) and water *ad. libitium.*

To study the effect of feeding honey with milk, a group of nine animals were bottle fed with 7ml of pure coorg honey with 250 ml of milk (Purchased from Bangalore milk Dairy) once a day for 15 days. No other food was given, experimental animals thus had restricted diet regime. Rats of 3 and 6 months of ages were also used for comparison. After 15 days of feeding with honey and milk, the animals were sacrificed by decapitation. Brain was excised and removed; different regions of the brain viz. cerebral cortex, cerebellum and medulla oblongata were separated at 0^0C cleaned of the

adhering blood vessels and used for the estimation of enzyme catalase and acetyl cholinesterase (AChE).

The animal protocol was approved by the animal ethics committee.Tissues were homogenized (10% W/V) in ice cold 0.51 M KCl. Supernatant was used for the estimation of lipid peroxidation by the method of Ohhawa et.al.,(4).A portion of the homogenate was centrifuged at 105,000 X g for 90 minutes and the resulting supernatant was used for the assay of enzyme catalase (E.C.1.1.1.1.6) which was estimated by titrimetric procedure as given by Colowick and Kaplan (5). AChE activity was determined colorimetrically (6). The data was statistically analysed using students't' test. The $P<0.05$ was considered statistically significant.

RESULTS

The data after statistical analyses is presented in tables 1-2 and fig.1-4. Lipid peroxidation enhanced ($P<0.01$) and catalase activity decreased ($P<0.01$) as a function of age (fig.3-4). Marked depletion of lipid peroxidation was found in the cerebral cortex,cerebellum and medulla oblongata after feeding honey with milk ($P<0.01$.fig.2) Catalase activity was significantly elevated ($P<0.01$) on feeding with honey. The percentage increase was the highest in cerebral cortex (+86%) followed by cerebellum (81%). Medulla oblongata exhibited the least elevation (74.4%).

Age related changes in the neuronal activity and total proteins of cerebral cortex, cerebellum and medulla oblongata illustrate age related changes in the level of total proteins (table 1). Catalase activity and neuronal efficiency as reflected by AchE activity suffered a substantial decrease due to ageing ($P<0.01$: table 1-2 and fig 3). The catalytic potential of catalase and AChE decreased as a function of ageing in all the regions of the brain studied .The energy of activation enhanced and Km decreased with age (table 1-2). Feeding milk and honey reversed substantially all the above adverse effects of age. Catalytic potential of enzyme catalase and AChE enhanced (table 1-2) and per oxidative damage declined (fig-2).

DISCUSSION

Thus, it is clear from the results that lipid per oxidative damage in the different regions of the brain increased with age and the neuronal efficiency as indicated by the activity of enzyme catalase declined during ageing. An inverse relationship was noted between lipid peroxidation and catalase activity with a significant decrease in the catalytical potential with age (fig.1-4, table-2). An increase in lipid peroxidation and a decline in enzyme catalase activity have earlier been reported with age (3) substantiating the results obtained in our studies.

Our results in addition, have demonstrated that age related decline of neuronal activity and catalytic potential of anti-oxidant enzyme catalase correlate inversely with the level of proteins in the ageing brain. This is suggestive of the fact that the decline in neuronal activity is linked to increase in lipid peroxidation and proteins.

The general decline found in the activity of AChE in the brain of aging rat indicates a disruption of the normal metabolism of acetylcholine (Ach) during aging. Such a decrease in AChE activity in the aging rat is due to the inactivation of the enzyme during aging and or due to reduction in the synthesis of enzyme protein. The levels of several enzymes decline during adulthood. This may either be due to a decrease in the transcription of the respective genes or a decrease in the translation of mRNA to enzyme proteins. In view of this the decrease found in AChE activity of the aging brain can be attributed to a decrease in the transcription of the gene coding the enzyme. It may be that during aging a decrease in the translation of mRNA to enzyme protein occurs (7) and hence AChE activity decreased significantly in the aging.

Feeding honey with milk resulted in (1) an augmentation in the activity of the enzymes reflective of neuronal activity (2) and elevation of catalytic potential of the anti-oxidant enzyme catalase and (3) a decreases in the lipid peroxidation. The activity of catalase enhanced due to significant decline in the activation energy and a decrease in Km.Feeding honey has rendered the enzyme AChE of the aging brain more efficient by decreasing the activation energy. These changes were reflected in the concomitant changes in the levels of lipid peroxides (fig 2 & 4).

Increase in lipid peroxidation on aging could be reflective of an age related damage of neuronal integrity. Earlier studies have also demonstrated age related decline in the basal electrical activity of hippocampus which correlated well with lipid peroxidative damage and oxidative stress. Several studies have shown that membrane lipid peroxidative abnormalities may affect production of electrophysiological signals and neuronal activity (1, 3, and 10). Reactive oxygen species (ROS) are generated as an integral part of the "living process". It is assumed that the antioxidant defense capacity of the

Table 1 - Changes in total protein and AChE in different regions of the brain of albino rats on feeding with milk and honey for 15 days

Age in months	Cerebral cortes		Cerebellum		Medulla oblongata	
	Control	Exp	Control	Exp	Control	Exp
			Protein (mg/g)			
3	60.0 ± 1.5	63.8 ± 1.2 NS	58.0 ± 0.6	62.0 ± 0.3 NS	60.1 ± 0.7	62.2 ± 0.5 NS
6	71.8 ± 1.6	74.8 ± 1.8 NS	61.4 ± 1.2	65.2 ± 1.1 NS	61.0 ± 1.0	66.0 ± 0.8 NS
18	70.5 ± 1.2	73.0 ±0.5 NS	62.4 ± 10.0	68.2 ± 0.7 NS	62.0 ± 0.5	65.4 ± 1.0 NS
			AChE (milli moles Ach hydro/mg protein/min)			
3	3.7 ±0.2	4.6 ± 0.1	3.8 ± 0.3	4.5 ± 0.1	3.2 ± 0.3	4.2 ± 0.1
6	4.0 ± 0.2	4.5 ± 0.1	4.5 ± 0.2	5.0 ± 0.2	3.3 ±0.3	3.9 ± 0.1
18	1.8 ± 0.2	3.2 ± 0.3	1.6 ± 0.2	2.8 ± 0.4	1.4 ± 0.3	2.0 ± 0.1
			Km (x M)			
3	5.8	2.5	5.2	2.8	1.8	3.2
18	10.0	5.0	10.8	5.6	12.2	8.6
			Eac (K cal/deg/mole)			
3	2.5	1.8	**4.2**	3.7	6.5	3.8
18	4.2	3.0	**6.5**	3.8	6.9	3.9

NS = Not Significant
P<0.05 (3 m and 18 m) – P<0.01 (6 m and 18 m)

Table 2 - Enzyme catalase kinetics of aging rats (18 months) fed with cichorium milk and honey (for 15 days).

Km (x10^{-2}M)

Age	Cerebral cortex			Cerebellum			Medulla oblongata		
Months	Control	Experiment	% change	Control	Experiment	% change	Control	Experiment	% change
3	0.047 ± 0.002	0.038 ± 0.008	-19	0.05 ± 0.007	0.033 ± 0.006	-34	0.06 ± 0.008	0.05 ± 0.007	-16
6	0.05 ±0.003	.033 ± 0.005	-34	0.076 ± 0.006	0.05 ± 0.008	-34.2	0.05 ± 0.007	0.03 ± 0.006	-40
18	0.0625 ± 0.004	0.055 ± 0.004	-12	0.083 ± 0.003	0.071 ±0.002	-14.4	0.0625 ± 0.004	0.05 ±0.001	-20
Vmax (xM)									
3	566 ± 20	606 ± 32	+78	233.7 ± 22	657.5 ± 33	+181	912 ± 28	921 ± 30	+ 0.9
6	680 ±28	953 ± 24	+ 40	581 ± 31	780 ± 28	+ 34	644 ± 18	654 ± 17	+ 1.5
18	334.5 ± 22	625.0 ± 19	+86	517 ± 17	680 ± 11	+31	726 ± 13	802 ± 14	+ 10.4
(Ea) Energy of activation (Ca/deg/mole subst)									
3	1986 ±24	1947 ± 32	-1.9	4767 ± 38	4397 ± 29	-7.7	1234 ± 18	1143 ± 17	-7.3
6	1588 ± 16	1557 ± 22	-1.95	4764 ± 31	4573 ± 27	-4.0	1280 ± 24	1143 ± 13	-10.7
18	3688 ±14	3049 ± 16	-17.3	3688 ± 18	3049 ±21	-17.3	1829 ± 24	1059 ± 23	-42

Fig. 1 Changes in the activity level of catalase activity in different regions of the brain in control and Honey fed 18 Months old albino rats

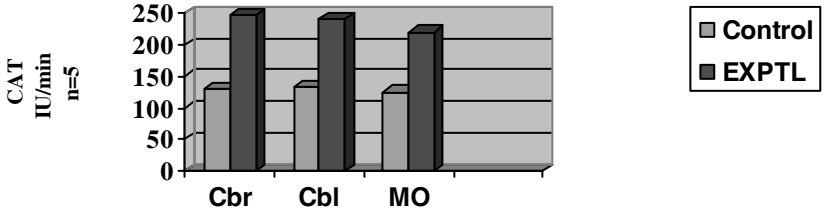


Fig. 2 Changes in the level of lipid peroxidation (MDA) in different regions of the brain in control and Honey fed 18 Months old albino rats

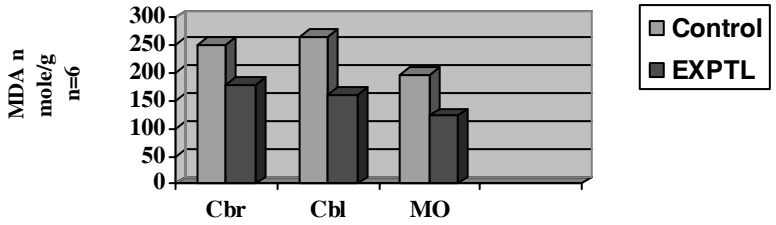


Fig. 3 Changes in the activity level of catalase activity in different regions of the brain as a function of age in albino rats

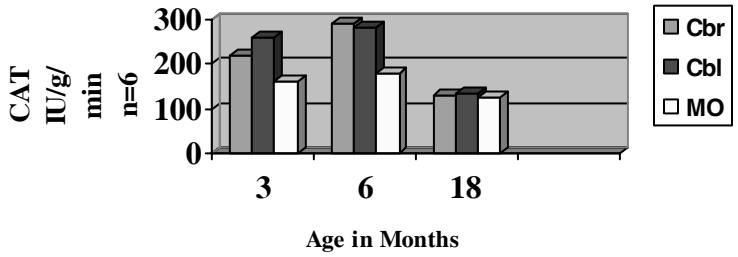
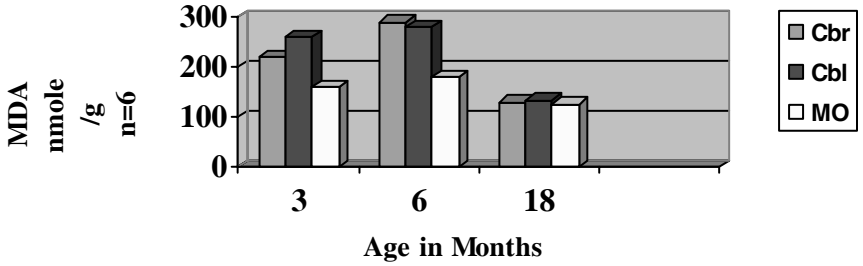


Fig. 4 Changes in the level of lipid peroxidation(MDA) in different regions of the brain as a function of age in albino rats



cells is insufficient to provide complete protection. As a result oxidative molecular damage is a continuous process and a variety of molecular products of free radical reactions accumulate with age. The life-span of an organism depends on its ability to counteract oxidative threat (7, 8, 9).

Central nervous system (CNS) is highly susceptible to oxidative stress. Most of the ROS dependent central nervous disorders have been observed to be actually triggered by the presence of free iron. Free radical generation during brief periods of cerebral ischemia has been suggested to induce delayed neuronal death (10).Antioxidant therapy has proved to be remarkably beneficial to combat ROS induced injury in the CNS. Significant changes were seen in the kinetic parameters of enzyme activities. The Vmax of the enzyme catalase and AChE on feeding honey showed substantial increase indicating that honey and milk when fed 15 days have assisted in making the functional groups of the active sites more accessible for enzyme-substrate complex formation. The Km value of the experimental enzymes showed decline suggesting enhanced enzyme substrate affinity, rendering the enzyme catalytically more potential. Since enzyme catalase is an active member of the detoxification system and *AChE a bio-marker of neuronal activity*. Feeding honey has rendered the brain functionally more efficient by retarding the ageing effects.

The activation energy values of AChE and catalase of honey and milk fed animals decreased indicating that the energy barrier for the enzyme activation declined under protective action of honey. (Table1-2). Thus honey when orally administered, prevented the increase in the peroxidation, enhanced the activity of enzyme catalase and also AChE rendering them catalytically more efficient. This has been medicated through free radical scavenging anti-oxidative and

membrane stabilizing properties of honey. Hence fed along with milk has acted as natural antioxidant and retarded the aging process by suppressing oxidative damage and potentiated the antioxidant catalase and the enzyme AChE involved in neuronal transmission.

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