

EFFECT OF ASHTAMANGAL GHRITA ON ACETYLCHOLINESTERASE ACTIVITY IN CEREBRAL CORTEX OF RAT PUPS BRAIN

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ABSTRACT

The effect of *Ashtamangal Ghrita* (AMG) on acetylcholinesterase (AChE) activity of cerebral cortex was examined in undernourished and rehabilitated rats pups. undernutrition produced by restricting their feeding time i.e maternal deprivation started from post-natal day (PND) 6th to 21th. After 21 days of life, pups were rehabilitated by leaving them with the mother and weaning subsequently. The AChE activity was estimated by Ellman's method. The results showed that AMG treated animals from PND21st to PND36th have increased AChE activity by about 42%. AMG treatment in undernourished animals increased the AChE activity by 22% in comparison to undernourished animals at PND21st. The AChE activity was 32% more in AMG treated rehabilitated animals in comparison to without AMG treated rehabilitated animals at PND36th. Thus this experiment suggests that AMG treatment increases the brain AChE activity in developing brain.

Key words- AMG *Ashtamangal Ghrita*, PND postnatal day, AChE acetylcholinesterase.

INTRODUCTION

Research on the neurochemical, neurophysiological and behavioural effects of PEM has shown that restricted diets in the early development of rats and mice influence brain cell division^{1,2}, delay myelination^{3,4} and decrease their performance on learning tasks in comparison with controls fed ad libitum^{5,6}. Likewise, studies of children who have suffered severe protein-calorie malnutrition in early infancy suggest that brain size and intellectual development suffer a degree of damage that is refractor to subsequent rehabilitation^{7,8,9,10}. Results of these studies are difficult to interpret because of the interaction of antecedent and subsequent environmental, cultural, and nutritional determinants.

Thus it has become necessary to rely on animal studies where more precise controls are possible to determine the effect of under nutrition in early life and subsequent brain development. Effect of undernutrition from the birth into post-weaning period on behavior in rehabilitated adult animals¹¹ but little attention has been given to its effect on physical and brain growth as well as acetylcholinesterase concentration in brain. Acetylcholinesterase is often considered to be a marker for nerve ending particles, and by implication, synapses. Various studies have shown that the development of whole brain AChE in suckling rats is somewhat retarded by under-feeding the mother during lactation.^{12,13}

In classical ayurvedic texts *Lehan Karma* (process of chanting by tongue) is done for the child whose mother is diseased, not having good quality of milk, inadequate lactation and child not getting sufficient amount of milk (undernutrition) or inadequate growth and development without having any disease. Health of the child depends on *Lehan*¹⁴. For *Lehan Karma*, many compounds have been prescribed. *Ashtamangal Ghrita* is one of them, which is a polyherbal formulation as it contain eight drugs –*Bramhi* (*Bacopa monneri*), *Vacha* (*Acorus calamus*), *Pippali* (*Piper longum*), *Sariva* (*Hemidesmus indicus*), *Kushtha* (*Saussurea lappa*), *Siddarthaka* (*Brassica campestris*), *Saindhava* (Rock salt) and *Ghrita* (Traditionally prepared butter oil). This formulation is used as *Rakshoghna* (protection from the infection), enhance *Medha*(intellect) and *Smriti*(memory).

Materials and methods

ANIMALS

Rats of Charles Foster strain, originally obtained from central animal house IMS, BHU, Varanasi, UP, India, were used in the present study. Litters of only 8-10 pups were used in the experiments. Animals were fed ad libitum on normal animal diet. They were given free access of mineral water. Temperature of animal room was maintained around 25°C and humidity was varied between 50% -75% as changes occurred in environment during the study period.

Total 40 pups of both sexes (5 males and 5 females in each group) were used in the study. Pups were equally divided into four groups. Two groups, i.e. 3rd and 4th were made undernourished from the post natal day 6th (PND 6th) by the method of maternal deprivation i.e. separated from the mother for 12 hours (8pm -8am), while the pups of group 1st and 2nd were

remained with mother. Drug was administered from the post natal day 6th (PND 6th) to pups of group 2nd and 4th upto postnatal day 21(PND 21st) by curved blunt needle attached with insulin syringe. After post natal day 21st, 5 pups were sacrificed from each group by decapitation to measure acetylcholinesterase activity in cortex, simultaneously whole wet brain weight; wet cortex weight and wet cerebellum weight were measured. After postnatal day 21st, rest pups, which was undernourished, rehabilitated upto postnatal day 36th and all the pups were sacrificed to measure acetylcholinesterase activity as well as whole wet brain weight, wet cortex weight and wet cerebellum weight.

METHOD FOR PRODUCING UNDERNUTRITION

Rat pups were made undernourished during their brain growth spurt period by restricting their feeding time, i.e., maternal deprivation¹⁵. Pups were separated from the mothers for 12 hours every day while rest of the pups remained with the mother throughout. Undernutrition procedure was started from postnatal day 6th to 21st. After 21 day of life, pups were rehabilitated by leaving them with the mother and weaning subsequently on the same during experiment.

SELECTION OF DRUG:

The selection of *Ashtamangala Ghrita* was based on the textual indication (*Yoga Ratnakara-Balaroga Chikitsa Adhyaya 6*)¹⁶ and preparation of Ghrita based on textual indication (*Bhaishajya Ratnavali Balarogadhikar 71*)¹⁷.

Calculation of dose in rat pups :

The following method was used for calculation of drug dose. The average weight of pups was 8.75 gm on PND 6th. Dose of AMG in human is 0.5 ml/kg/day¹⁸. The dose for pups was considered 10 times higher. i.e. 0.5 x 10 =

5ml/kg/day. For the 8.75gm pups, the volume of AMG shall be -

$$8.75\text{ gm} \Rightarrow \frac{5}{1000} \times 8.75$$

Calculated dose \Rightarrow 0.044 ml/pups on PND 6th. According to above formula weight wise dose was calculated on every day. Drug was given by curved blunt needle with insulin syringe.

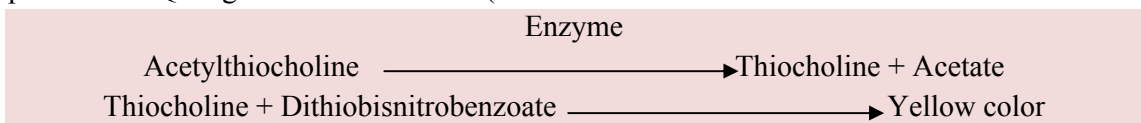
CHEMICALS

All the chemicals used were of highest purity available and preferably of analytical (AR) grade. Most of the inorganic and organic chemicals were products of Sisco Research Laboratories (India) or Qualigens Fine Chemicals (India). Sodium hydroxide and Sodium chloride were products of Qualigens Fine Chemicals (In-

dia). Potassium dihydrogen orthophosphate, Sodium phosphate dibasic dihydrate, Dithiobis nitrobenzoic acid and Acetylthiocholine were products of Sisco Research Laboratories, Mumbai, India.

PRINCIPLE OF ACETYLCHOLINESTERASE ACTIVITY MEASUREMENT¹⁹

The principle of the method was the measurement of the rate of production of thiocholine as acetylthiocholine was hydrolyzed. This was accomplished by the continuous reaction of the thiol with 5 : 5-dithiobis-2-nitrobenzoate ion to produce the yellow anion of 5-thio-2-nitro-benzoic acid. The rate of color production is measured at 412 nm in a photometer.



PREPARATION OF SOLUTIONS

(a) Preparation of 0.1M Phosphate buffer

Solution A: 5.34 g of Sodium phosphate dibasic dehydrate and 4.08 g of Potassium dihydrogen orthophosphate were dissolved in 300 ml distilled water. (0.2M) Solution B: 6.2g Sodium hydroxide was dissolved in 150 ml of distilled water.

Solution B was added to solution A to get the desired pH (pH 8.0 or 7.0) by pH meter and then finally the volume was made up to 600ml with distilled water.

(b) Preparation of DTNB Solution (0.01 M)

39.6 mg of DTNB were dissolved in 10 ml pH 7.0 phosphate buffer (0.1 M) and 15 mg of sodium bicarbonate were added. The reagent was made up in buffer of pH 7 in which it was more stable than in that of pH 8.

(c) Preparation of Acetylthiocholine Iodide (ATC) Solution (0.075 M)

21.67 mg of ATC was dissolved in 1 ml of distilled water. It was used as substrate.

Procedure for Acetylcholine esterase measurement

The pups were decapitated; brain was removed quickly and placed in ice cold saline. Cortex and hippocampus were quickly dissected out on a petri dish chilled on crushed ice. The tissues were weighed and homogenized in 0.1M phosphate buffer pH 8.0. 0.4ml aliquot of the homogenate was added to a cuvette containing 2.6ml phosphate buffer (0.1M, pH 8) and 0.1 ml of DTNB. The contents of the cuvette were mixed thoroughly by bubbling air and absorbance was measured at 412 nm in a spectrophotometer. When absorbance reaches a stable value, it is recorded as the basal reading. 0.02ml of substrate i.e, acetylthiocholine was added and change in absorbance was recorded for a period of 10 minutes at intervals of 1

minute. Change in the absorbance per minute was thus determined.

Calculation

The enzyme activity was calculated using the following formula;

$$R=5.74 \times 10^{-4} \times A/CO$$

Where, R = Rate in moles of substrate hydrolyzed/minute/gm tissue

A = Change in absorbance/min

CO = Original concentration of the tissue (mg/ml)

STATISTICAL ANALYSIS

The data collected were transferred on master chart showing various items/variables in columns and subjects in rows. The analysis of data was done using statistical software SPSS version 16.0.

Inter-group comparison (Between the group)

To compare the means of more than two independent groups, one-way ANOVA (Analysis of Variance) was applied and value of F test was determined. Wherever F test resulted statistically significant, post-hoc test was applied for multiple comparison, identifying significant pairs of groups.

RESULTS

As per the collected data of Acetylcholinesterase activity (rate, in moles substrate hydrolyzed per min per gm of tissue) in healthy and undernourished pups after the study of 21 days and 36 days, either received or not received the AMG during first 21 days, maximum mean rate was observed in group 1st and 4th in healthy and undernourished pups respectively, but no significant change was observed after 21 days in any group. Maximum mean rate was observed in 2nd and 4th group after 36 days of study in healthy and undernourished AMG treated pups respectively. While the minimum rate was observed in 3rd group after 21 days and 36 days of study in 3rd. [table no. 1 (a)]

By the LSD Post Hoc test no significant change in Acetylcholinesterase activity was observed after 21 days of study in all group sets. After 36 days of study, significant changes were observed between the

group 2nd Vs 3rd and 3rd Vs 4th. While in other sets of groups no significant change was observed.[Table no.1(b)].

DISCUSSION

Effect of undernutrition on brain acetylcholinesterase activity- Brain AChE activity in all the experimental groups of animals was measured as described in the 'Method' section. AChE activity was found to be 3.0 ± 0.561 at PND 21 in control animals (group1). At PND 36, it was found to be 4.008 ± 0.165 . This shows that AChE activity increases during development. This increase in activity from PND 21st to PND 36th was about 33% and significant.

Undernutrition from PND 6th to 21st (group 3) reduced the brain AChE activity by about 11% but statistically it was not significant. Rehabilitation increases the AChE activity by 28%. However, even after 15 days rehabilitation AChE activity was still found to be lower by same amount (11%) in comparison to control animals (group 1). This shows that 15 days undernutrition decreases the brain AChE activity by 11% which could not be caught up even after 15 days of rehabilitation.

Effect of AMG on brain AChE activity in undernourished and rehabilitated animals

AMG treated normal animals (group2) did not show any change in brain AChE activity at PND 21st in comparison to control animals (group1). AChE activity increased significantly by about 42% in AMG treated animals from PND 21st to PND 36th. AChE activity was found to be 4.245 ± 0.109 in AMG treated animals at PND 36th. This value was about 6% higher than control animals (group1) of same age. This shows that AMG treatment has a positive effect on

brain AChE activity. However this effect was statistically not significant.

Brain AChE activity was found to be increased by AMG treatment in undernourished animals. AMG treatment in undernourished animals (group 4) increased the AChE activity by 22% in comparison to undernourished animals at PND 21st. Brain AChE activity in AMG treated undernourished animals (group 4) was higher by 13-14% in comparison to control and control + AMG treated animals (group 1&2). This shows that AMG treatment in undernourished animals had a higher positive effect on brain AChE activity. Thus, AMG treatment is more effective in undernourished animals in comparison to normal animals.

Brain AChE activity increased about 31% from PND 21st to PND 36th in AMG treated rehabilitated animals. AChE activity of rehabilitated animals without AMG treatment was found to be 3.567 ± 0.893 at PND 36th, while AChE activity of AMG treated rehabilitated animal was 4.450 ± 0.103 . This shows an increase of 32% in AChE activity by AMG treatment which was statistically significant.

CONCLUSION

The present study was a preliminary study experiments suggest that AMG treatment increases the brain AChE activity in developing brain. This effect was more pronounced in undernourished animals.

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Table no. 1 : Intergroup comparison of mean Acetylcholinesterase activity (rate, in moles substrate hydrolyzed per min per gm of tissue) of pups after first 21 days i.e. drug trial period and 36th days i.e. rehabilitation period -

1 (a) :

Group	After 21 days	After 36 days
1	3.00 ±.561	4.008 ±.165
2	2.972 ±.349	4.245 ±.109
3	2.772 ±.692	3.567 ±.893
4	3.392 ±.681	4.450 ±.103
F value	0.975	3.374
P value	0.429 NS	0.049

1 (b) : Post Hoc Test/multiple comparison (LSD) p value

Group compared	After 21 days	After 36 days
1 Vs 2	NS	0.425
1 Vs 3	NS	0.149
1 Vs 4	NS	0.126
2 Vs 3	NS	0.043
2 Vs 4	NS	0.489
3 Vs 4	NS	0.008

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