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NEUROBEHAVIORAL AND BIOCHEMICAL EFFECTS OF CHOLINE CHLORIDE ADMINISTRATION IN RATS

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ABSTRACT

Background and Objective:

Choline is an essential nutrient related to vitamin B complex activity. It can be received through different food sources. It is an important component of acetylcholine which is involved in different brain functions. The present study is designed to assess the behavioral effects of choline chloride in animal models.

Methods:

This case-control study was performed in the Department of Biochemistry, Federal Urdu University, Karachi for eight months from May 2022 to December 2022. Twenty-four adult male albino Wister rats were divided into four groups, one control and three test groups. Choline chloride dissolved in saline was given intraperitoneally at 25mg/ml, 50mg/ml and 100mg/ml to the test groups respectively, and saline was given to the control animals for two weeks. Memory and depression-like symptoms were monitored. Morris water maze and brain acetylcholine levels were used for memory functions and force swimming test was performed to monitor depression-like symptoms.

Results:

The present study showed significantly improving memory functions and increase in immobility time exhibited by choline-treated rats indicating depression-like symptoms in rats. The present investigation also shows that serum level as well as glucose level increase in dose dependent manners. Increased depression-like symptoms may be attributed due to onset of diabetes or increased glucose concentration.

Conclusion:

In conclusion choline administration at different doses (25mg/ml, 50mg/ml, and 100mg/ml) produces behavioral and biochemical effects in rats.

Key Words: Choline, Memory, Depression, Acetylcholine

INTRODUCTION

Choline is a water-soluble essential nutrient that has a complex role in the body, required for synthesis of neurotransmitters.¹ Moreover, choline is also required for transport of lipids (lipoprotein), metabolism of methyl group (homocysteine reduction) and signalling through cell membrane. Its role in the body is complex.² As a form of acetylcholine it performs nerve functions.³ It minimizes the neural tube defects during development focusing on brain and memory functionst in the fetus.⁴ Choline synthesis is not sufficient enough to meet the requirement of human body.⁵ Therefore, it

is absorbed through small intestine via choline transporters from foods containing choline.⁶ Fatty liver and muscle damage can be caused by choline deficiency in healthy human consuming adequate amounts of methionine and folate, which can be cured by supplementation of choline through diet.^{7,8}

Since choline has a role in several important functions such as metabolism, synthesis of neurotransmitter and cell structure, it is therefore believed that its deficiency may cause atherosclerosis (through secretion of lipoprotein), non alcoholic fatty liver and even

neurological disorders.⁶ It is therefore suggested to have adequate amount of choline for good health. Scientists reported that choline act as a neuroprotective nutrient that is able to interact with epigenetic mechanism and gene expression and is related to epigenetic alteration which is linked to Alzheimer disease pathogenesis.⁹ Deficiency of choline may contribute to various diseases such as atherosclerosis, liver disorder and also neurological problems.⁹ Inhibition of choline kinase enzyme is effective in inflammatory arthritis treatment.¹⁰ It is widely distributed in different foods and is synthesized by the sequential methylation of phosphatidyl ethanolamine endogenously.¹¹ Reduced intake of choline leads to increase levels of liver fat.¹²

Choline is present in variety of food items but the richest resources are egg and meat. It possesses number of physiological effects in body.⁹ The present study is designed to manipulate the effects of the choline chloride administered intraperitoneally to the male albino wistar rats to evaluate the chronic effects of choline chloride on memory, depression-like symptoms and on other biochemical contents.

METHODS

Study design: Case-control study

Place and duration of study: This study was performed in the Department of Biochemistry, Federal Urdu University, Karachi for eight months from May 2022 to December 2022.

Sample size: Twenty-four albino depression-like rats locally bred (150-200 g) obtained from Aga Khan University Hospital. Animals were caged separately in a quiet room located in Department of Biochemistry, Federal Urdu University Karachi under a 12-hour light-dark cycle and maintained surrounding temperature (22)°C± 2). They all get easy approach to water and standard rodent diet.

Sampling technique: Non-probability consecutive sampling

Data collection: Initially animals were habituated at least three days before experimentation. Choline at doses of 25mg/kg, 50mg/kg, 100mg/kg were injected intraperitoneally (IP) in volumes of 1ml/kg for 15 days. Control animals were injected with saline (1ml/kg).

Behavioral Methods:

Morris Water Maze Test

The effects on spatial memory were examined by determining the performance of rats in a Morris water maze apparatus. Apparatus of WM used in this study consisted of a circular tank that is made up of transparent glass, in which there is a little platform hidden in opaque water. Initially rats were trained and then test was performed after one hour as well as after 24 hours. Time reached to platform regarded as latency time. Experimentation was carried out by Morris water maze test.⁴

Forced Swimming Test:

This apparatus is designed in a glass tank with specifications and experimentation.¹⁴ During the testing phase swimming behavior of animals were observed in which animals were made to swim throughout the swim chamber. Experiment is based on monitoring of the immobility time. So when the animal is showing immobile status it will only keep its head above the water and attempting no efforts of swimming.

Biochemical Estimation

Neurochemical Estimations

The Hestrin technique was used to determine the ACh levels of the tissue (Batool et al., 2016).¹⁴ The enzyme was inactivated by boiling the tissue sample (whole brain), which also released the associated ACh that reacted to ferric chloride. The brown colour that resulted from this reaction was then measured at 540 nm in comparison to blank. In terms of mol/g of brain-tissues, ACh level was represented.

Estimation of Glucose and cholesterol was by standard lab procedure. Microlab 300 was used for biochemical estimation.

Data analysis: Experimental data were analyzed by one-way ANOVA using SPSS 20 ver. Post Hoc analysis done by Tuckey's method.

Ethical considerations: The procedure was executed in line with National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 2011) and approved by local (Institutional) ethical committee.

RESULTS

Behavioral Effects

Effect of choline chloride (25mg/kg, 50mg/kg, and 100mg/kg) on memory function in rats in water maze is shown in figure 1. Data analyzed by one way ANOVA showed a significant effect of choline chloride administration on memory function ($F=4.17$, df , 3, 20 $p<0.01$). Newman-keuls test showed that all three doses choline chloride significantly improved memory

function in rats in Morris water maze test. Effect of choline chloride (25mg/kg, 50mg/kg, and 100mg/kg) on depression-like symptoms in rats in is shown in figure 2. Data analyzed by one way ANOVA showed a significant effect of choline chloride administration on depression-like symptoms ($F=10.37$, df 3, 20 $p<0.01$). Newman-keuls test showed that all three doses choline chloride significantly produce depression-like symptoms in rats.

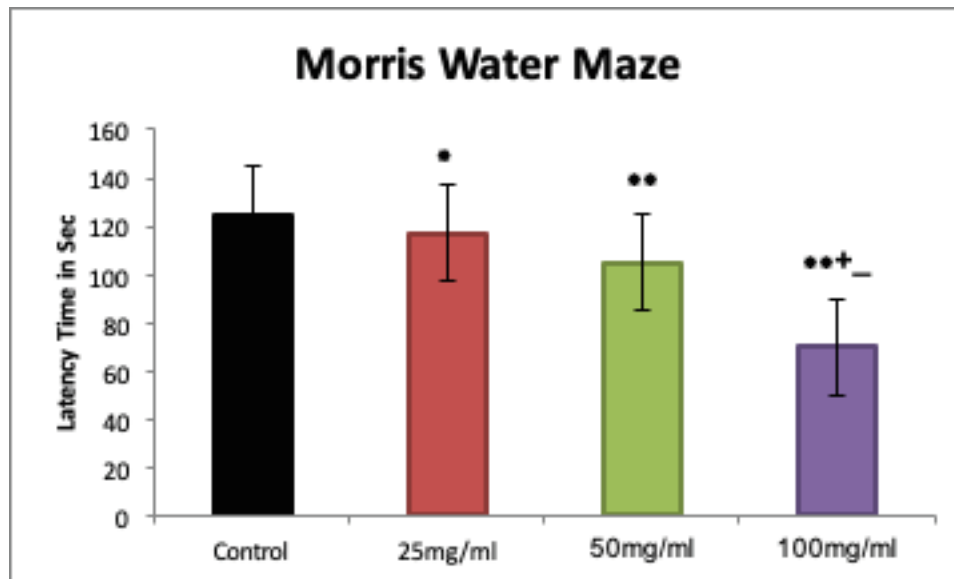


Figure 1: Effect of Chronic Administration of Choline Chloride On Memory by Morris Water Maze in rats. Values are mean \pm SD (n=6). Significant differences by one way ANOVA; ** $p<0.01$ Vs Control, + $p<0.05$ Vs 25 mg/ml, - $p<0.05$ Vs 50mg/ml

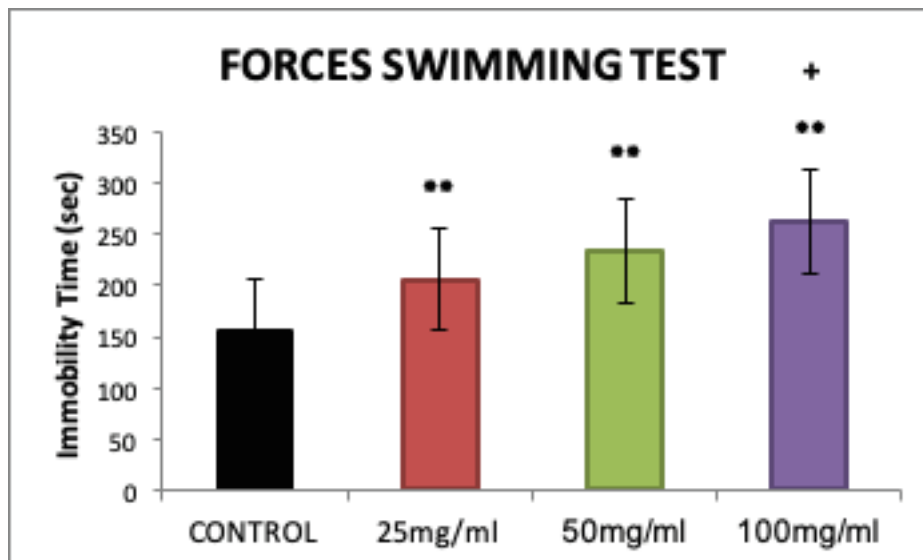


Figure 2: Effect of Chronic Administration of Choline Chloride On Depression-like Symptoms assessed by Forces Swimming Test. Values are mean \pm SD (n=6). Significant differences by one way ANOVA; ** $p<0.01$ vs Control, + $p<0.05$ vs 25 mg/ml

Neurochemical Estimations

Effect on ACh of acute choline chloride treated rats is shown in figure 3. Data analyzed by one-way ANOVA represented a significant treatment effect of choline

chloride ($F=8.06$, $df 3, 20$ $p<0.01$). The result indicates that administration of choline chloride increases acetylcholine levels significantly as compared to control.

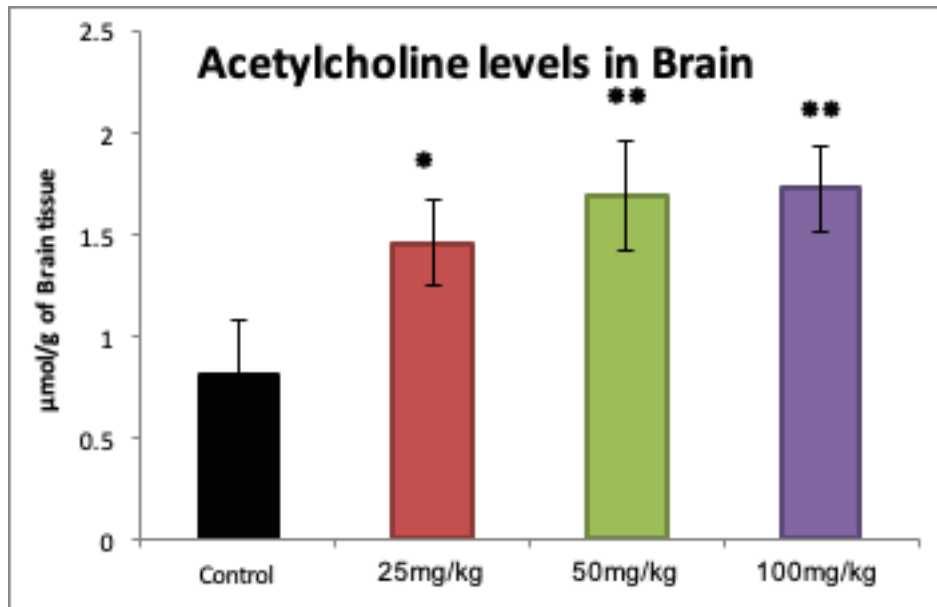


Figure 3: Effect of Chronic Administration of Choline Chloride on Brain acetylcholine levels. Values are mean \pm SD (n=6). Significant differences by one way ANOVA; ** $p<0.01$ Vs Control

Biochemical Effects

Effect of choline chloride treated rats on blood glucose and cholesterol level are shown in figure 4 and 5. Data analyzed by one-way ANOVA represented a significant treatment effect of choline chloride ($F=13.11$, $df 3, 20$

$p<0.01$) and ($F=3.29$, $df 3, 20$ $p<0.01$) respectively. The result indicates that administration of choline chloride significantly increased biochemical molecules in blood.

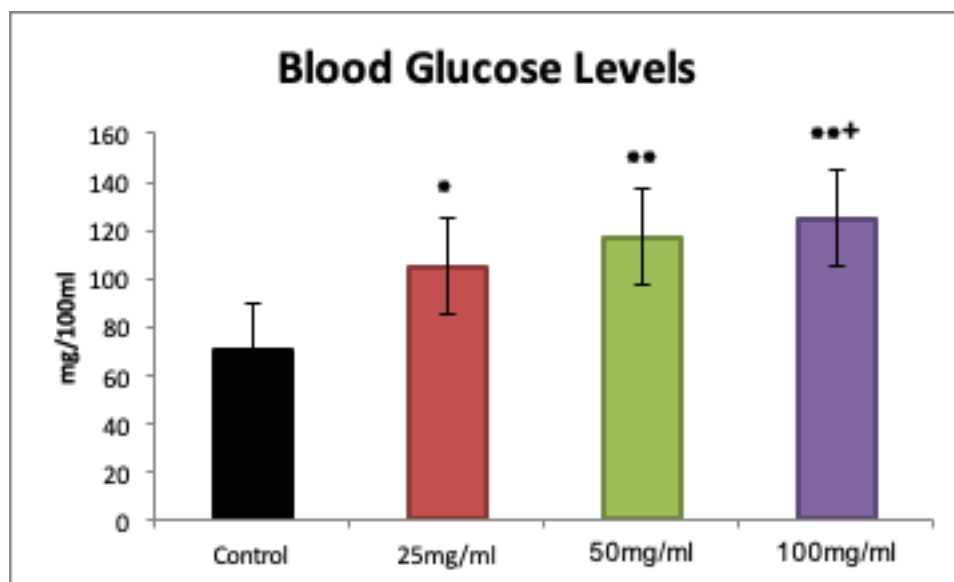


Figure 4: Effect of Chronic Administration of Choline Chloride On Blood Glucose levels in rats. Values are mean \pm SD (n=6). Significant differences by one way ANOVA; ** $p<0.01$ Vs Control, + $p<0.05$ Vs 25 mg/ml

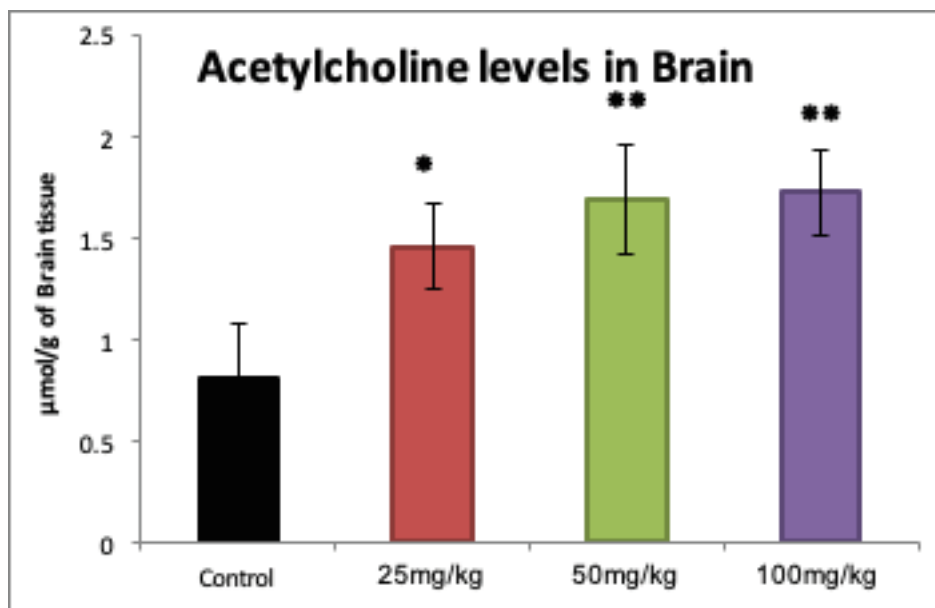


Figure 5: Effect of Chronic Administration of Choline Chloride On Blood Cholesterol level in rats. Values are mean \pm SD (n=6). Significant differences by one way ANOVA; **p<0.01 Vs Control, + p<0.01 Vs 25 mg/ml

DISCUSSION

Choline is an essential nutrient which is involved in different body metabolisms. Its metabolites are also involved in different functions, from the cell structure to neurotransmitter and deficiency of choline can cause serious problems.⁹ Choline is present in milk, meat as well as in almonds, kidney beans, spinach and fish.¹⁵ Studies reported that choline in the form of phospholipids is involved in cell structure; in the form of neurotransmitter it is precursor of the acetylcholine which is important for learning and memory.¹⁵ Deficiency of choline causes Alzheimer's disease, non-alcohol fatty liver, oxidative stress etc.¹⁵ Our study showed that acute and chronic administration of choline chloride at different doses (25mg/kg, 50mg/kg, and 100mg/kg) significantly improved memory and produced depression-like symptoms in rats in dose-dependent manner while memory significantly improved. Present result also revealed that repeated administration of choline chloride significantly increased serum cholesterol level. Serum glucose concentration also increased but statistically it was non-significant in choline treated rats.

Previous studies showed that choline in the form of neurotransmitter (acetylcholine) plays an important role in the learning and memory.¹⁶ It has been also reported earlier that choline supplementation increase learning and memory function in the fetal that exposes with

alcohol.¹⁷ In the present study we used water maze to assess memory function. In current study memory functions monitored following chronic administration of choline chloride in rats and it was observed that tests took less time to reach the platform than the other test and control groups. It means that latency time of choline chloride treated rats is less than the control group more significantly in the dose of 100mg/kg. Improvement in memory attributed to acetylcholine neurotransmitters and behavioral tests results are also supported by brain acetylcholine levels showing more acetylcholine levels at 100mg/kg dose.

Choline possesses an important role in neurotransmitter functions specifically on acetylcholine and dopaminergic functions. Different studies suggest that choline supplements enhance dopamine receptor densities giving improving effects on memory impairment.¹⁵ It was studied that low concentration of choline may cause anxiety but not depression-like symptoms.¹⁸ It was also reported that over-supplementation of choline caused depression-like symptoms but not in normal doses.¹⁶ Number of epidemiological studies demonstrated previously that the onset of diabetes or increasing glucose level may cause the depression-like symptoms.¹⁹ In this present study we noted the depression-like symptom by using Forces swimming

test in which we found the significantly increased immobility time, meaning the dose-dependent choline chloride treated rats (25mg/kg, 50mg/kg, 100mg/kg) showed the depression-like symptoms. This report justifies the previous study that depression may occur because glucose level increases. Earlier studies reported that choline is related to the high-fat-diet and produces obesity and is insulin resistant. Previous investigations also described that deficiency of choline causes the low plasma glucose level with increasing fatty liver.^{20,21} It has been reported that dietary choline reduces body fat by enhancing triglyceride-rich lipoprotein derived fatty acids by brown fats that could result in improving hypercholesterolemia.²² In present investigations it was described that serum glucose concentration elevated in dose dependent

manner and concentration of serum cholesterol was also found significantly increase which assured the previous studies that glucose and cholesterol level increased in serum with choline supplementation.

CONCLUSION

It is concluded in the present study that effect of choline administration at different doses (25mg/ml, 50mg/ml, and 100mg/ml) on behavioral models produced depression-like symptoms and also improved memory function. Serum glucose and cholesterol concentration was also found increased after chronic intraperitoneal administration of choline chloride. Further experiments are needed for evaluation of choline role in different neurological disorders.

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Authors' contribution:

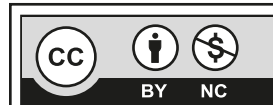
Lubna Anis; Design, data collection, data analysis, manuscript writing

Irfan Sajid; Concept, data analysis, manuscript writing

Shoaib Ahmad; Concept, manuscript revision

Saida Haider; Data interpretation, manuscript revision

All the authors approve the final version to be published, and agree to be accountable for all aspects of the work.



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