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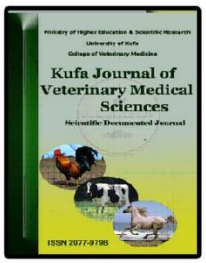

The Effect of Piracetam against Acetyl Choline (Ach) In Behavioral Changes with Some Biochemical Parameters in Adult Mice

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Abstract

The effects of Piracetam against Acetyl Choline (Ach) in adult mice. The Medium Lethal Dose LD_{50} of Piracetam was 455.5mg/kg B.W. orally and Ach 7.25 mg/kg B.W i.p, then an animal's are divided to 4 groups First group serves as control group given normal saline, a second 400 mg /kg B.W. o.p. of Piracetam and third group 5 mg/kg B.W. i.p. Ach. Forth group 400 mg /kg B.W. o.p. of Piracetam and 5 mg/kg B.W. i.p. Ach. The behavioral changes, the Run time, swimming, Time on glass, and biochemical parameters in Ach and Piracetam pretreatment significantly ($P<0.05$) decreased of Run time and swimming, then increased Time on glass when compared with the control. The open field test was significantly ($P<0.05$) increased the termer, depression when compared with the control. The liver function tests was significantly ($P<0.05$) increased the in level of GPT and GOT enzymes in the plasma at forth group.

Key words: Mice , piracetam , Acetyl Choline , Behavioral effect

تاثير الباريسيتام المضاد لأستيل كولين على التغيرات السلوكية و بعض المقاييس الكيموحيوية في الفئران البالغة

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الخلاصة

دراسة تأثير الباريسيتام المضاد لأستيل كولين في الفأران البالغة , تم بتحديد الجرعة المميتة الوسطية لعقار الباريسيتام والذي كان 455,5 ملغ/كغم من وزن الجسم عن طريق الفم وللاستيل كولين 7.25 ملغ/كغم من وزن الجسم عن طريق الحقن بالبريتون, وبعد ذلك تم تقسيم الفأران الى اربعة مجاميع حيث كانت المجموعة الاولى السطيرة تم تجريعها بالماء المقطر والمجموعة الثانية تم تجريعها بعقار البارسيتام بجرعة 400 ملغ/كغم والمجموعة الثالثة تم حقنها بعقار الأستيل كولين بجرعة 5 ملغ/كغم عن طريق غشاء البريتون اما المجموعة الرابعة تم تجريعها بعقار البارسيتام بجرعة 400 ملغم/كغم وحقنها عن طريق البريتون بالأستيل كولين بجرعة 5 ملغم/كغم , التغيرات السلوكية التي حدثت في التجارب كتجربة اختبار قوى السباحة والجري و البقاء على اللوح الزجاجي و بعض المقايس الكيموحيوية في عقار البارسيتام و الأسيتايل والتي كانت قيم القياس عند مستوى معنوية $p< 0.05$ وادى الى انخفاض معنوي في وقت الجري والسباحة مع زيادة معنوية في الوقت على الزجاج مع مقارنتها مع مجموعة السيطرة , اما تجربة الميدان المفتوح كانت قيم القياس عند مستوى معنوية $p< 0.05$ بزيادة معنوية

في حركة الاختلاج والخمول مقارنة مع نتائج مجموعة السيطرة, كما ادى الى زيادة معنوية لمستويات خمائر الكلوتامين الناقلة للامين و الكلوتاميت الناقلة للامين في بلازما الدم لدى المجموعة الرابعة.
الكلمات المفتاحية:- الفئران ، الباريسيتام ، الاستيل كولين ، التاثيرات السلوكية

Introduction

The laboratory animals are used in behavioral and biochemical tests in scientific researches those imperatives to the ethical and health care , the mouse have inbred and out bred stocks is present [1,2]

There are many researches intake of the behavioral activity of lab. mice, which has a good animal model and it's have a developed behavioral sensation for pharmacology and neurosciences for a valid interpretation of results. [3,4]

The research processes can be visualize of a many of stages, involving the description of general appearance, reflexes, species behaviors, locomotion, learning, sensorimotor behavior, and skilled movement. [4,5]

In our excrement we used the piracetam and acetyl choline to study the behavioral change.

Piracetam was synthesized and clinically used since 1972. Piracetam (2-oxo-1-pyrrolidineacetamide) is a cyclic come from gamma-aminobutyric acid (GABA), that made after the loss of one H_2O molecule of followed by ring formation [1]. It is the first delegate of the i*nootropic*i drugs. [6,7,8]

Piracetam increasing cognition under conditions of hypoxia, and also increased amount of memory and learning behavior. piracetam have is synergistic effect when taken with acetyl choline, and causes a good improvement in memory and learning. Piracetam have a specific pharmacological and therapeutic effect but its mechanism of action still unknown. [8,9]

Acetylcholine (ACh) is an important neurotransmitter communicated between neurons and muscle at the neuromuscular junction, and has been embroiled in consciousness mechanisms , arousal, and attention in the brain. its present as direct neurotransmission in autonomic ganglia, the Cholinergic receptors binding can occur through muscarinic receptor G protein-complex or nicotinic receptor ionotropic mechanism [10,11]

Material and Method

We getting albino mice from (Laboratory of Drug Control) in Baghdad Iraq , either sex weighting between 23-33 g in the same age about 8 week old they housed in groups , temperature was 23-24 ^{0} and continually ventilation , humidity 45-55% RH , lighting about 12-14 hours , with synthetic standard feeding and high quality of waters provide. [1,2,3]

Mice divided in to groups that contain from male and three female putting each group alone in cage.

Determination of acute toxicity (LD50)

The acute toxicity of piracetam (tablet contains 800 mg, GlaxoSmithKline company) and Acetylcholine iodide (India , HIMEDIA) was determination on albino mice either sex male and female with weight average 33 g under standard husbandry condition , The animal administration single dose each 24 hours and observation toxicity effect of drugs , administration of piracetam was orally and Ach was intraperitoneal injection , in piracetam the experience extend to five days we started from dose 50 mg/kg in 1st day to 800 mg/kg in 5th day without toxicity effect but the Ach extend to 3 day started with 5 mg/kg in 1st day and finished with 20 mg/kg in 3rd day by death of animal and toxicity effect on animal with 33g BW in dose 10 mg/kg extend to 40 minuet after injection by drug. [12]

Behavioral experiments

By using four groups each group have one male and four female, this groups placed together in same environment, feeding and water. groups differentiated between them by drug administrated the 1st group was control giving distal water, 2nd was administrated piracetam in dose 400 mg/kg, 200 mg/kg by orally, the 3rd group administrated Ach in dose 5mg/kg, 2.5mg/kg by injection intraperitoneal ,the 4th group administrated each of piracetam in dose 400 mg/kg and Ach in dose 5mg/kg but the administrated of Ach after 1 hour from piracetam giving , and study the behavioral change on groups by

Open field area

The open field test screening efficacy, anxiety-related, and locomotor effects of neurobiological manipulations by using clear cage and record the change that happened on mice in the cage like urination, cleaning coat, movement of animal. **[**12, 13, 14]

Running wheel

By calculate the time of running the animal in the wheel and direction of running. [14]

Marble burying

This test assay the perseverative behavior and anxiety by recording of marbles buried number that mice explored it in clean cage [15,16]

Wire suspension

This measure give forepaw strength of mice front paws and ability to grasp and give the releasing and fall down time . [17]

Forced swim test

This test measure the ability to swim and floating to give an indicator for efficiency of anti-depressant and anxiety. [12, 15]

Visual cliff

The visual cliff is a perception test that can be used to assay a mouse's visual acuity. A test arena with a clear bottom is placed on a table so that half of it is overhanging the edge of the table, providing the appearance of empty space. The mouse is exposed to the test arena, and amount of time spent in the portion over the empty space is recorded. [18]

Liver function test

We study the change of concentration of ALT, AST in the blood of albino mice administrated different doses of piracetam and Ach by using plasma after collecting, to anesthetic animal wet a pieces of cotton by chloroform and putting in jar with mice to few minute after that start to collect blood by heart puncture and put in anticoagulant tube and shacking well after that we will put the tube in centrifuge to separate plasma from blood and put it in Eppendorf tube.

After that we should add SGPT, SGOT end point determination kit (syrbio, Syria) to plasma sample and adding NaOH 0.04 N and put it at 37 c^0 and reading at 546 nm in Spectrophotometer.

Statistical analysis

All data parameters were calculated as average to the baseline values, and were expressed as mean ± standard error. Values were statistically analyzed by One-way ANOVA Values of $P<0.05$ followed by correction were included to identify the statistical differences of behavioral changes calculated by Mann–Whitney *U* test.

Results

The duration of LD_{50} test Table (1) shows the effect of Piracetam on adult mice. The revealed treatments were Piracetam administration pretreatment the LD_{50} was 455.5 mg/ kg B.W. (table. 1).

Table. 1 The LD_{50} of Piracetam on adult mice.

Drug	No. of mice	Dose range	First dose	Last dose	Range about stander table	LD_{50} mg/ kg B.W.
Piracetam	5	400	50	800	OOOOO	455.5
Ach	4	5	5	20	OOXO	7.26

The duration of open field test Table (2) shows the effect of Piracetam, Ach and Piracetam & Ach on adult mice. The revealed treatments were Ach administration pretreatment significantly increased in Tremor and Depression. The revealed treatments were Piracetam & Ach both administration pretreatment significantly increased Tremor and Depression (table. 2).

Table. 2 The effect of Piracetam, Ach and Piracetam & Ach on open field test

Groups	Control	Piracetam 400 mg/kg	Ach 5 mg/kg	Piracetam 400 mg/kg & Ach 5 mg/kg
Movement	1	1	0.75	0.75
Urination	0.2	0.25	0.75	0.5
Deification	0.2	0.25	0.5	0.75
Tremor	0	0	0.75 *	0.75 *
Depression	0	0	0.75 *	0.75 *
Convulsion	0	0	0.5	0.5
Stay in angle of cage	0.25	0.25	0.75	0.75

*significantly different from the respective control (0 concentration) $p<0.05$

The duration of behavioral tests Table (3) shows the effect of Piracetam, Ach and Piracetam & Ach on adult mice. The revealed treatments were Ach administration pretreatment significantly decreased in Run time, Swimming and Time on glass. The revealed treatments were Piracetam & Ach both administration pretreatment significantly increased Time on glass (table. 3).

Table. 3 The effect of Piracetam, Ach and Piracetam & Ach on behavioral tests

Groups	Control	Piracetam 400 mg/kg	Ach 5 mg/kg	Piracetam 400 mg/kg & Ach 5 mg/kg
Groups	Control	Piracetam	Ach	Piracetam & Ach
Marble burying No.	13.75 ± 1.194	19.00 ± 0.447	8.75 ± 0.791	12.00 ± 1.366
Run time sec.	157.50 ± 0.9	157.50 ± 0.2	50.00 ± 0.2 *	128.50 ± 0.6
Wire suspension sec.	59.75 ± 0.3	90.00 ± 0.9	37.00 ± 0.6	44.75 ± 0.1

Swimming sec.	117.50 ± 0.1	202.25 ± 0.7	81.75 ± 0.1 *	123.00 ± 0.5
Time on glass sec.	94.25 ± 0.2	67.25 ± 0.4	109.50 ± 0.2 *	120.25 ± 0.2 *

*significantly different from the respective control (0 concentration) *p<0.05*

Biochemical Analysis Table (4) shows the effect of administration Piracetam and Piracetam & Ach on adult mice. There have significant difference elevation between groups at Piracetam & Ach in both GPT and GOT enzymes in following biochemical parameters (table. 4)

Table. 4 The effect of Piracetam and Piracetam & Ach on biochemical Analysis

Groups	Control	Piracetam 400 mg/kg	Piracetam 400 mg/kg & Ach 5 mg/kg
GPT Ul /L	0.98 ± 0.085	1.44 ± 0.256	6.02 ± 1.071 *
GOT UI/L	0.84 ± 0.052	1.34 ± 0.063	1.41 ± 0.026 *

*significantly different from the respective control (0 concentration) *p<0.05*

Discussion:

In this study determent of acute toxicity of drug Piracetam was safely drug and don't show any toxicity sings during the five day conversely with Ach that show acute toxicity in three day due to fast action on the CNS.[8,11] mice exploratory animal they movement any part of cage to explore and identification on object, all most of mice urination as marking to their area and that is normal behavior especially when change the mice cage as the result in table (2) first group of control, but by administration Ach the parameters changed In table (2) group of Ach that happened due to effect of the Ach on the mice CNS that will increase involuntary urination and deification [6,7,8] also duo to effect of Ach increase of muscle tremor , depression , convulsion , stay in angle of cage , that present also when administrate Ach and Piracetam [10,11], because the administration root was I.P injection and fast absorption that giving acute Ach effect in open field area but in other experiment we can see the effect of Piracetam on the behavioral in table (3) when compare with control except in Visual cliff that was duo to effect of drug on brain and support nerves system and we can see the agonist effect of Ach in the same table when giving together and compare the rustle with Ach [11] And when examination the effect of drugs on liver the result was normal when compare with normal range GPT (17.5-30.2 U/L), GOT (45.7-80.8 U/L). [19]

Conclusion

Piracetam medication significantly (P <0.05) reduced the effect of Ach toxicity, we suggest uses of Piracetam to increase against effect to depression termer can combine with Ach neurotoxicity or related substances caused that sings.

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