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Mechanistic, Neurobehavioral and Toxicological Study of Scopolamine in Mice

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Abstract

THE aim of the research is to study the mechanism of neurotoxic behavioural changes of scopolamine in the mice. Number of 15 animals, divided into 3 groups, each group consisting of 5 animals. The first group was considered a control group. The second and third groups were given scopolamine in two doses of 10 and 20 mg/kg and were injected once, and then behavioural measurements were taken 24 hours after treatment. Impact of acute high single doses treatment with scopolamine in behavioural tests for higher brain functions there is a significant increase in rearing and decrease activity in crossing numbers in the 10, 20 mg/kg. Both doses significantly affect the Social Interaction behaviour of mice compared to the control group after 24 hours of acute single doses. Both doses of scopolamine significantly effect on swimming scour and tail suspension, both the 10 and 20 mg/kg exhibited significant increases in serotonin levels compared to the control group. Additionally, the 20 mg/kg group showed a significance compared to the control and 10 mg/kg group. Both the 10 and 20 mg/kg exhibited significant decreases in acetylcholine levels compared to the control group. 20 mg/kg exhibited a significant decrease in COMT levels compared to the control group. The scopolamine has an effect on the neurobehavioral of animals. The results showed that these effects have a direct relationship to neurotransmitters, including acetylcholine and serotonin, as well as the effect on the COMT enzyme.

Keywords: neurobehavioral test, neurotransmitters, acetylcholine, serotonin

Introduction

Scoplamine (Escitalopram) is a type of antidepressant medication. Scopolamine is recently used to treat Parkinson's disease, which occurs as a result of a deficiency in dopamine, an important neurotransmitter that plays a role in controlling movement [1].

Scopolamine works by blocking the stimulation of acetylcholine receptors, which helps increase dopamine levels in the brain [2].

Scopolamine binds to all types of muscarinic acetylcholine receptors, not just one type. Which leads to a variety of effects, including dry mouth, blurred vision, imbalance, hallucinations, confusion, and memory loss [3].

Scopolamine can also cause a group of symptoms called anticholinergic syndrome. These symptoms result from inhibiting the activity of acetylcholine receptors, which are receptors found throughout the body and play a role in many functions, including muscle control, organ control, and mental function control [4].

Scopolamine is used as an anesthetic drug in anesthesia, and it is also used as an anti-vomiting and anti-nausea drug as it is a non-selective inhibitor meaning that it binds to all types of receptors, not just one type of them [5].

Risk factors for developing anticholinergic syndrome include taking an overdose of scopolamine or other anticholinergic. Taking scopolamine with other medications that can increase the risk of anticholinergic syndrome, such as antihistamines,

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antidepressants, antipsychotics, and medications to treat Parkinson's disease [6].

The aim of the study is to investigate the underlying toxic effects of scopolamine by linking the neurobehavioral effects to the measurement of some neurotransmitters and the enzyme COMT in mice.

Material and Methods

Animals

This study used adult male mice, 2-3 months old and with an approximate weight of 25-35 grams. The mice were kept in cages specially prepared for them, equipped with water bottles and provided with feed tablets according to their need. The temperature and humidity were controlled, while laboratory lighting was provided to control the number of mice. The hours of light and darkness are 12 hours.

Materials used

Ampoules of the drug Scopolamine from Pioneer Company, Iraq. The concentration fixed on the ampoule was 20 mg/1 ml. The doses were prepared after being calculated according to body weight, diluted with distilled water, and then given to animals by i.p injection.

Experiment design

This study used Swiss mice, numbering 15 animals, divided into 3 groups, each group consisting of 3 animals. The first group was considered a control group and was left without treatment. The second and third groups were given scopolamine in two doses of 10 and 20 mg/kg and were injected once time, then behavioral measurements were taken 24 hours after treatment. At the end of the treatment, the animals were anesthetized for the purpose of drawing blood to separate the plasma and keeping it in Eppendorf tubes until the examination was performed.

Neurobehavioral tests include:

An experiment to evaluate higher brain functions using open field and social interaction tests for mice

Open field test

The general activity and movement of mice inside the open field is calculated.

Social Interaction Test

The mice's social behavior is observed, including the time each handler mouse spends interacting with another handler mouse from the control group.

Experience evaluating the functions of the voluntary nervous system by conducting swimming and tail suspension tests

Swimming test

Swimming rank is determined according to a chart specific to this test according to Mohammad, (1984) [7].

Zero: If the nose is under water.

1: The nose is at or above the water level.

2: The nose and top of the head are at or above the water level, with the ears remaining in the water.

3: As in 2, except that the water reaches the middle of the ear.

4: As in 3 except that the water reaches the base of the ear.

Tail Suspension Test (TST)

Mice are suspended by their tail, and the time they spend in a state of rest is recorded

Kits used

- Acetylcholine measurement kit from the American company Elabscinse
- A kit for measuring serotonin from the American company Elabscinse
- A kit to measure COMT from the American company Elabscinse

Statically analysis

One-way analysis of variance ANOVA was used to find the significant difference between the groups, and the Cross Cal Well test was used to examine the data in square form with a significant level less than 0.05.

Results

Impact of acute high single doses treatment with scopolamine

Some neurobehavioral tests as behavioral tests for higher brain functions: In table 1

Open Field Test

There is a significant increase in rearing activity and significant decrease in crossing numbers in the 10, 20 mg/kg scopolamine group compared to the control group. There is no significant difference in rearing activity and crossing numbers between the 10 mg/kg and 20 mg/kg scopolamine groups ($p > 0.05$).

Social Interaction Score

Both doses of scopolamine (10 mg/kg and 20 mg/kg) significantly affect the behavior of mice compared to the control group after 24 hours of acute single doses. The higher dose (20 mg/kg) generally has more pronounced effects on behavior,

particularly in terms of rearing activity, crossing numbers, and social interaction. Additionally, there is no significant difference in behavior between the two doses of scopolamine (Table 1).

Evaluate the involuntary nervous systems

Swimming Score

Both doses of scopolamine (10 mg/kg and 20 mg/kg) significantly affect the involuntary nervous system functions of mice compared to the control group after 24 hours of acute single doses. However, the higher dose (20 mg/kg) generally has more pronounced effects on forced swimming duration and tail suspension duration. Additionally, there is no significant difference in swimming score and forced swimming duration between the two doses of scopolamine (Table 2).

This statistical analysis suggests that after a 24-hour treatment of a single dose of scopolamine, both the 10 mg/kg and 20 mg/kg Scopolamine groups exhibited significant decreases in acetylcholine levels compared to the control group. Additionally, the 20 mg/kg group showed a higher level of significance compared to the 10 mg/kg group (Table 4).

This statistical analysis suggests that after 24 hours of a single dose of treatment, the 20 mg/kg Scopolamine group exhibited a significant decrease in COMT levels compared to the control group. However, no significant difference was observed in the 10 mg/kg Scopolamine group compared to the control group (Table 5).

This statistical analysis suggests that after 24 hours of giving single doses of scopolamine, both the 10 mg/kg and 20 mg/kg Scopolamine groups exhibited significant increases in serotonin levels compared to the control group. Additionally, the 20 mg/kg group showed a significance compared to the control and 10 mg/kg group (Table 3).

Discussion

Neurobehavioral tests showed a decrease in the animals' motor activity and an increase in the number of times the animals stood. To link these behaviors with potential mechanisms, it was found that the measurement of acetylcholine showed a decrease compared to the control group, while the increase in the number of times the animals stood was related to an increase in the animal's anxiety and tension, which was explained by an increase in serotonin. The decrease in swimming rank and the period of rest of the animal while hanging its tail are related to a decrease in acetylcholine, as well as a decrease in the COMT enzyme, which causes an increase in serotonin.

Some of the mechanisms by which inhibition of muscarinic acetylcholine receptors in the brain can lead to increased production of serotonin and dopamine can be explained by increased release of serotonin and dopamine from neurons - when muscarinic acetylcholine receptors are inhibited. They lead to increased release of serotonin and dopamine from nerve cells [8, 9].

If high dose scopolamine is administered to mice, this will result in inhibition of muscarinic acetylcholine receptors. This will cause a decrease in the release of acetylcholine from neurons, which may lead to a buildup of acetylcholine in the brain. Acetylcholine buildup can lead to a variety of symptoms, including hallucinations, confusion, and weakness [10]. It increases the sensitivity of nerve cells to serotonin and dopamine receptors. When muscarinic acetylcholine receptors are inhibited, it leads to increased sensitivity of neurons to serotonin and dopamine receptors [11]. Reducing the removal of serotonin and dopamine from the brain occurs when muscarinic acetylcholine receptors are inhibited, which leads to reduced removal of serotonin and dopamine from the brain [12-14].

Scopolamine is a class of selective serotonin reuptake inhibitors (SSRIs) [15, 16]. The mechanism of action of scoplamine is based on increasing the concentration of serotonin in the brain by inhibiting its retrieval from where it was released in the space between neurons [17, 18]. COMT (Catechol-O-Methyltransferase) affects the reaction of neurotransmitters in the brain and can have an effect on behavior and thinking [19, 20].

Conclusions

The study concluded that scopolamine has an effect on the nervous behavior of animals. The results showed that these effects have a direct relationship to neurotransmitters, including Acetylcholine and serotonin, as well as the effect on the COMT enzyme.

Acknowledgment

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Conflicts of interest

The authors declare that no conflict of interest

Ethical considerations

Ethical approval date: 10/15/2023 and number: UM.VET.2023.035

TABLE 1. Behavioural tests for higher brain functions after 24 hour of acute single doses of scopolamine in mice

Groups Control	Open field		Social interaction\score-3 min
	Rearing\3 min	Square Crossing number\3min	
Scopolamine 10 mg\kg	11.21±2.11	140.13±22.51	+5
Scopolamine 20 mg\kg	16.14±5.10*	119.42±7.19*	+3*
Groups	20.40±3.12*A	100.11±12.31*A	+3*

TABLE 2. Evaluate the involuntary nervous systems after 24 hour of acute single doses of scopolamine in mice

Groups	Swimming score\3 min	Tail suspension\ Immobility time-Sec
Control	4	140.82±12.12
Scopolamine 10 mg\kg	4	50.16±21.08*
Scopolamine 20 mg\kg	3*	50.41±12.72*

Data as mean ± SE score precent as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg

TABLE 3. Serotonin level after 24 hours of giving single doses of scopolamine

Groups	Serotonin
Control group	213.458±20.35
Scopolamine 10 mg\kg	248.581± 32.11
Scopolamine 20 mg\kg	373.959± 51.20*A

Data as mean ± SE, score precent as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg

TBLE 4. Level of acetylcholine after 24 hours of treatment of single doses of scopolamine

Groups	Acetylcholine
Control group	187.5±35.20
Scopolamine 10 mg\kg	157.336±43.11*
Scopolamine 20 mg\kg	104.703±50.20*A

Data as mean ± SE score precent as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg

TABLE 5. The level of COMT after 24 hour of single doses of treatment

Groups	COMT
Control group	0.156±0.031
Scopolamine 10 mg\kg	0.164±0.041
Scopolamine 20 mg\kg	0.050±0.033*A

Data as mean ± SE score precent as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg

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دراسة ميكانيكية وسلوكية عصبية سمية للسكوبولامين في الجرذان

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[1] طالب دراسات عليا في علم الأدوية البيطرية وعلم السموم - فرع الفسلجة والكيمياء الحيوية وعلم الأدوية - كلية الطب البيطري - جامعة الموصل - الموصل - العراق.

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الملخص

الهدف من البحث هو دراسة آلية التغيرات السلوكية السمية العصبية للسكوبولامين في الفئران. قسمت الفئران إلى 3 مجاميع، كل مجموعة مكونة من 5حيوانات. المجموعة الأولى اعتبرت مجموعة سيطرة تم إعطاء المجموعتين الثانية والثالثة السكوبولامين بجرعتين 10 و 20 ملغم/كغم حقنا بالخلب لمرة واحدة، ثم تم أخذ القياسات السلوكية بعد 24 ساعة من العلاج. اظهر العلاج الحاد بجرعات مفردة عالية من السكوبولامين في الاختبارات السلوكية لوظائف المخ العليا ان هناك زيادة معنوية في عدد مرات الوقوف وانخفاض كبير في النشاط الحركي في كل من جرعة 10 و 20 ملغم / كغم. تؤثر كلتا جرعتي السكوبولامين (10 مجم / كجم و 20 مجم / كجم) بشكل كبير على سلوك التفاعل الاجتماعي لدى الفئران مقارنة بمجموعة السيطرة بعد 24 ساعة من الجرع المفردة الحادة. كلا جرعتي السكوبولامين (10 ملغم/كغم و 20 ملغم/كغم) تؤثران بشكل كبير على مرتبة السباحة ومدة تعليق الذيل السكوبولامين، أظهرت كل من مجموعتي السكوبولامين 10 ملغم/كغم و 20 ملغم/كغم زيادات كبيرة في مستويات السيروتونين مقارنة بمجموعة السيطرة. كما أظهرت مجموعة 20 ملغم/كغم مقارنة بمجموعة السيطرة ومجموعة 10 ملغم/كغم. أظهر كل من 10 ملغم / كغم و 20 ملغم / كغم انخفاضًا ملحوظًا في مستويات الأسيتيل كولين مقارنة بمجموعة السيطرة. أظهرت مجموعة السكوبولامين 20 ملغم/كغم انخفاضًا ملحوظًا في مستويات COMT مقارنة بالسيطرة.خلصت الدراسة إلى أن السكوبولامين له تأثير على السلوك العصبي للحيوانات. وأظهرت النتائج أن هذه التأثيرات لها علاقة مباشرة بالنواقل العصبية، بما في ذلك الأسيتيل كولين والسيروتونين، وكذلك التأثير على إنزيم COMT .

الكلمات الدالة: الاختبارات السلوكية العصبية، النواقل العصبية، استيل كولين، سيروتونين.