



The Effect of Hyoscine on Serum Serotonin and Acetylcholine Levels, and Their Impacts on Neuro-Behavior in Mice

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Abstract

THE significance of this study stems from the fact that the previously therapeutic indications of Hyoscine to prevent nausea and vomiting may be related to its effect on the nervous system in general, and memory in particular, but the exact mechanism is unknown and requires additional investigations. To study the neurobehavioral effects of Hyoscine and their relationship to serotonin and acetylcholine in mice, the mice divided into 3 groups, each group consisting of 10 animals. The first group was a control, the second group was given a dose of 2 mg/kg, and the third group was given 4 mg/kg. Neurobehavioral experiments were conducted for half of the animals after two weeks of dosing, the rest of animals after 4 weeks. The measured LD50 of Hyoscine was 196 mg/kg when given by i.p. injection. A decrease in motor activity was recorded with doses of 2 and 4 mg/kg and an increase in the number of rearing times as compared to the control group after 2 and 4 weeks of treatment. The dose of 4 mg/kg of Hyoscine indicated a significant increase in serotonin levels compared to the control group. The Hyoscine groups of 2 and 4 mg/kg had significantly lower acetylcholine and COMT enzyme levels than the control group. Hyoscine affects the nervous system by increasing the levels of serotonin, acetylcholine and COMT enzyme in the blood of mice.

Key words: Hyoscine, serotonin, acetylcholine, neuro-behavior, mice.

Introduction

Hyoscine, which is also known as Scopolamine, is an alkaloid extracted from the belladonna plant, a member of the nightshade family, which is essentially the original seasickness remedy and is also used as an anticholinergic substance (anticholinergic) [1].

Acetylcholine is involved in many functions, including memory, learning, and movement (ref.). Endogenous acetylcholine is involved in a variety of functions, including regulation of ionic balance and cellular growth [2]. Although scopolamine works by inhibiting acetylcholinesterase, acetylcholine does not work in the presence of scopolamine because this drug can bound to cholinesterase receptors and block its effects [3].

Hyoscine works by binding to acetylcholine receptors in the central and peripheral nervous systems. Acetylcholine is an important neurotransmitter that plays a role in many functions, including learning, memory, movement, and consciousness [4, 5].

It was found that Hyoscine inhibits muscarinic acetylcholine receptors in the brain as a result of its binding to M1, M2 and M3 receptors, and this association leads to an effect on the function of acetylcholine in the brain [6]. It has been shown that there is a direct relationship between the inhibition of muscarinic acetylcholine receptors and the removal of dopamine and serotonin from the nervous system [7]. The brain function in learning, memory and movement is closely linked to the action of acetylcholine through its interaction with its receptors in the nervous and peripheral systems [8].

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Preventing spasms in smooth muscles is achieved by affecting acetylcholine receptors, and this is the reason behind using Hyoscine to treat colic and painful abdominal cramps [9]. In addition, Hyoscine is used in the form of patches to prevent nausea and dizziness. It is also used to treat cases of vomiting by inhibiting acetylcholine receptors in the inner ear [10]. Previous studies have demonstrated that using Hyoscine can treat vomiting associated with the emergence of some harmful effects on the nervous system that are reflected in behavior and actions [11,12]. The aim of this study is to investigate the neurobehavioral effects of Hyoscine and their relationship to serotonin and acetylcholine levels in mice.

Material and Methods

Materials and working methods

Animals

The study used male albino mice, 1-2 months old with an approximate weight of 28-30 g. The animals were raised in appropriate laboratory conditions in terms of temperature and humidity, and they were provided with the necessary food and water throughout the experiment. The mice were also exposed to light for 12 hours and darkness for 12 hours as well.

Medicines used

Hyoscine as a drug (20 mg/2 ml) was provided by Pioneer Company, Sulaymaniyah, Iraq. Physiological salt was used to dilute the drug according to the amount of dose given depending on the weight of the animal.

Experimental Design and Methods

An experiment to determine the median lethal dose (LD₅₀) was conducted for the intraperitoneal injection of Hyoscine in rats using the Up and Down method.

The Dixon method of ascending and descending was used for the purpose of determining the average lethal dose in the mice, and the dose was increased or decreased according to the outcome of the second day, whether the mouse died or remained alive, and corresponding equations were relied upon to calculate this dose. Initial experiments were relied upon to determine the first dose that will be given to the animals.

Neurobehavioral tests are performed after the following periods:

Hyoscine treatment by daily dose for 4 weeks

The mice were divided into 3 groups, each group consisted of 10 animals. The first group was given physiological saline by intraperitoneal injection and considered as a control group, the second group was

given a dose of 2 mg/kg, and the third group was given 4 mg/kg. Neurobehavioral experiments were conducted after two and four weeks of the treatment. Blood samples were taken for the purpose of plasma separation and conducting biochemical tests.

Neurobehavioral tests:

An experiment was performed to evaluate high brain functions using open field and social interaction tests for mice, these include

1. Open field test: The general activity and movement of mice inside the open field was calculated.

2. Social Interaction test:

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

Experiments of evaluating the functions of the voluntary nervous system by conducting forced swimming, tail suspension, and directed behavior tests

Biochemical tests:

-Measuring serum level of serotonin (kit from the American company, Elabscinse)

-Measuring serum level of acetylcholine (kit from the American company Elabscins)

-Measuring serum level of COMT enzyme (kit from the American company Elabscins)

Statistical analysis

One-way analysis of variance ANOVA was used to find the significant difference between the groups, the nonparametric kruskal-wallis H test was used for analysis score with a significant level of p value (less than 0.05).

Results

The LD₅₀ of Hyoscine, determined through intraperitoneal injection in mice, was found to be 196 mg/kg. LD₅₀ represents the dose at which 50% of the test subjects are expected to die as a result of the administered substance (Table 1).

Open Field Test:

Rearing

There was an increase in rearing activity in the 2 mg/kg Hyoscine group compared to the control, but it was not statistically significant ($p > 0.05$). A substantial increase in rearing activity in the 4 mg/kg Hyoscine group was found compared to the control ($p \leq 0.05$). In addition, there was a significant difference in rearing activity between the 2 mg/kg and 4 mg/kg Hyoscine groups ($p \leq 0.05$) (Table 2).

Square Crossing Number

There was a significant decrease in crossing numbers in the 2 mg/kg Hyoscine group compared to the control ($p \leq 0.05$). A substantial decrease in crossing numbers in the 4 mg/kg Hyoscine group was found compared to the control ($p \leq 0.05$). There was no significant difference in crossing numbers between the 2 mg/kg and 4 mg/kg Hyoscine groups ($p > 0.05$) (Table 2).

Social Interaction / Score:

Both doses of Hyoscine (2 mg/kg and 4 mg/kg) significantly affected the behavior of mice compared to the control group. The higher dose (4 mg/kg) generally had more pronounced effects on behavior, particularly in terms of rearing activity and social interaction. Additionally, there was no significant difference in behavior between the two doses of Hyoscine (Table 2).

Open Field Test:

Rearing

There was a significant decrease in rearing activity in the 2 mg/kg Hyoscine group compared to the control group ($p \leq 0.05$). A substantial decrease in rearing activity in the 4 mg/kg Hyoscine group was detected compared to the control group ($p \leq 0.05$). There was a significant decrease in rearing activity in the 4 mg/kg Hyoscine group compared to the 2 mg/kg Hyoscine group ($p \leq 0.05$) (Table 3).

Square crossing Number

There was a significant decrease in crossing numbers in the 2 mg/kg Hyoscine group compared to the control group ($p \leq 0.05$). There was a substantial decrease in crossing numbers in the 4 mg/kg Hyoscine group compared to the control group ($p \leq 0.05$). In addition, there is a significant decrease in crossing numbers in the 4 mg/kg Hyoscine group compared to the 2 mg/kg Hyoscine group ($p \leq 0.05$) (Table 3).

Social Interaction Score:

Both doses of Hyoscine (2 mg/kg and 4 mg/kg) significantly affected the behavior of mice compared to the control group after daily doses for 4 weeks. The higher dose (4 mg/kg) generally had more pronounced effects on behavior, particularly in terms of rearing activity, crossing numbers, and social interaction. Additionally, there was a significant difference in behavior between the two doses of Hyoscine, with the higher dose resulting in more severe impairments (Table 3).

Serotonin levels

The serotonin levels after a two-week daily dosing of Hyoscine (Table 4). After daily doses of Hyoscine for 4 weeks, the 4 mg/kg Hyoscine group exhibited a significant increase in serotonin levels compared to the control group. However, no significant difference was observed in the 2 mg/kg

Hyoscine group compared to the control group. The statistical significance in acetylcholine levels between the control group and both treated groups of Hyoscine, 2 mg/kg and 4 mg/kg exhibited significant decreases in acetylcholine levels compared to the control group. Additionally, the 4 mg/kg group showed a higher level of significance in acetylcholine levels compared to the 2 mg/kg group after 2, 4 weeks (Table 5).

After daily treatment for two weeks, both the 2 mg/kg and 4 mg/kg Hyoscine groups exhibited significant decreases in COMT levels compared to the control group. Additionally, the 4 mg/kg group showed a higher level of significance compared to the 2 mg/kg group. After daily doses of Hyoscine for four weeks, the 4 mg/kg group exhibited a significant decrease in COMT levels compared to the control group. However, no significant difference was observed between the 2 mg/kg Hyoscine and control groups (Table 6).

Discussion

The results showed that Hyoscine is the cause of the decrease in nervous behavior, represented by the lack of activity of the animal, and the reason can be attributed to a decrease in acetylcholine, as was proven in current subsequent results. This result is consistent with the results of other researchers who showed that the decrease in acetylcholine activity causes a decrease in motor activity, while the reason for the increase in the number of standing times is attributed to the elevated levels of animal stress and anxiety due to the increase in serotonin, as proven in our result.

Research indicates that scopolamine is an anticholinergic agent that causes a variety of effects on the neurobehavior of animals [13,14]. Scopolamine binds to acetylcholine receptors, resulting in decreased acetylcholine activity. This can lead to a variety of symptoms, including poor memory, learning, and movement [14,15]. When muscarinic acetylcholine receptors are inhibited, it results in an increased sensitivity of neurons to serotonin and dopamine receptors [16,17]. When muscarinic acetylcholine receptors are inhibited, they lead to an increase in the release of serotonin and dopamine from neurons [18, 19]. Current results have proven the presence of these effects on the animal neurobehavior, which is related to the amount of dose given and the length of the administration period, as Hyoscine affects higher brain functions [20]. This was tested through the open field and social interaction tests of the mice, where it was shown through the open field that there was a decrease in the movement and activity of the mice as well. Animal interaction was reduced in treated animals compared to untreated control mice.

The researchers found that scopolamine causes impairment in working memory, which is associated with increased oxidative stress and neuronal death in

the hippocampus [21]. The effect of scopolamine on learning and long-term memory in rats has been reported by researchers who recorded that scopolamine causes impairment in learning and long-term memory, which is associated with decreased long-term reinforcement potential in the hippocampus [22]. It has recently been proven that Hyoscine has a role in influencing the gene expression of some genes responsible for memory, and that the use of high doses of this drug causes an effect on memory and may cause Alzheimer's disease [23].

In addition, a relationship has been found between the use of Hyoscine and the expression of BDNF in the hippocampus, and this effect is directly reflected in memory [24].

The effect of Hyoscine is compounded by influencing the regeneration of nerve cells in the hippocampus part of the brain, which can lead to Alzheimer's disease in the future. [24,25].

Studies have also shown that Hyoscine works to reduce the expression of dopamine D1 receptors, as well as to reduce the number of GABAergic neurons in the hippocampus [23,25,26].

Conclusion

Hyoscine directly affects the brain by affecting serotonin, acetylcholine, and COMT receptors, and this effect is reflected in memory, learning, and motor behavior in rats.

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Ethical approval

The ethical approval number was UM.VET.2023.035 and dated on 10/15/2023 from vet-medicine university of Mosul.

TABLE 1. Determination of the i.p Median Lethal Dose (LD50) of Hyoscine in Mice

Dose (mg/kg)	Outcome
172	X
138	0
172	X
138	0
172	X
Total animals:5	
Increase or Decrease the Dose: 34	
LD50: 196 mg/kg	
End Result: 195.8 mg/kg	

X represent a died animal, 0 represent a survived animal

TABLE 2. Behavioral tests for higher brain functions after daily doses for two weeks of Hyoscine in mice.

Groups	Open field		Social interaction/ score-3 min
	Rearing\3 min	Square Crossing number\3min	
Control	19.21±2.2	130±20	5
Hyoscine 2 mg/kg	21.11±3.2	73±8 *	4*
Hyoscine 4 mg/kg	45.20±2.5 ^{*A}	50.14 ±3.2 ^{*A}	4*

Data as mean ± SE, score presented as median, $p \leq 0.05$, each group of 5 animals, *Represents a significant difference from control, A: Represent significant difference from 2 mg/kg group.

TABLE 3. Behavioral tests for higher brain functions after daily doses of Hyoscine for 4 weeks in mice.

Groups	Open field		Social interaction \ score
	Rearing\3 min	Square Crossing number\3min	
Control	19.21±2.2	130.5±20.23	+5
Hyoscine 2 mg/kg	15.21±3.0*	75.13±8.42 *	+3*
Hyoscine 4 mg/kg	7.19 ±2.03 ^{*A}	35.13±3.12 ^{*A}	+1 ^{*A}

Data as mean ± SE, score presented as median, $p \leq 0.05$, each group of 5 animals,

*Represents a significant difference from control, A: Represents a significant difference from 2 mg/kg group.

TABLE 4. Serotonin levels after a two-week daily dosing of Hyoscine

Groups	Serotonin\2 weeks	Serotonin\4 weeks
Control group	213.458± 50.31	213.458± 40.22
Hyoscine 2mg/kg	488.866 ±65.12*	225.101± 39.34
Hyoscine 4mg/kg	696.911±67.25* ^A	446.452±56.29* ^A

Data as mean ± SE, score presented as median, $p \leq 0.05$, each group of 5 animals,

*Represents a significant difference from control, A: Represents a significant difference from 2 mg/kg group.

TABLE 5. Levels of acetylcholine after a daily doses of Hyoscine for two and four weeks of treatment

Groups	Acetylcholine\2 weeks	Acetylcholine\4 weeks
Control group	187.5±20.13	187.5±19.50
Hyoscine 2mg/kg	150.647±91.35*	145.029±40.10*
Hyoscine 4mg/kg	123.435±19.6* ^A	90.799±20.30* ^A

Data as mean ± SE, score presented as median, $p \leq 0.05$, each group of 5 animals,

*Represents a significant difference from control, A: Represents a significant difference from 2 mg/kg group.

TABLE 6. levels of COMT after a daily doses of Hyoscine for two weeks

Groups	COMT\2 weeks	COMT\4 weeks
Control group	0.156± 0.014	0.156±0.020
Hyoscine 2mg/kg	0.130±0.020*	0.140±0.030
Hyoscine 4mg/kg	0.080±0.021* ^A	0.086±0.021* ^A

Data as mean ± SE, score presented as median, $p \leq 0.05$, each group of 5 animals,

*Represents a significant difference from control, A: Represents a significant difference from 2 mg/kg group.

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تأثير الهيوسين على مستويات السيروتونين والأسيتيل كولين وتأثيرها على السلوك العصبي في الفئران

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الملخص

تتبع أهمية هذه الدراسة من أن المؤشرات العلاجية الموصوفة سابقاً للهيوسين للوقاية من الغثيان والقيء قد تكون مرتبطة بتأثيره على الجهاز العصبي بشكل عام، والذاكرة بشكل خاص، لكن الآلية الدقيقة غير معروفة وتتطلب المزيد من البحث. هدف الدراسة لبحث التأثيرات السلوكية العصبية للهيوسين وعلاقتها بالسيروتونين والأسيتيل كولين في الفئران. تم تقسيم الفئران إلى 3 مجموعات، كل مجموعة تتكون من 10 حيوانات. المجموعة الأولى مجموعة السيطرة، والمجموعة الثانية أعطيت الهيوسين جرعة 2 ملغم/كغم، والمجموعة الثالثة أعطيت 4 ملغم/كغم. وأجريت تجارب سلوكية عصبية على نصف الحيوانات بعد مرور أسبوعين، والبقية بعد 4 أسابيع. الجرعة المميّنة الوسطية LD50 للهيوسين هو 196 ملغم / كغم عند الإعطاء بالحقن i.p. سجل انخفاض في النشاط الحركي في جرعة 2 و 4 ملغم/كغم كما سجل زيادة في عدد مرات الوقوف في نفس المجاميع مقارنة بالمجموعة الضابطة بعد مرور 2 و 4 أسابيع من العلاج. أظهرت مجموعة الهيوسين 4 ملغم/كغم زيادة كبيرة في مستويات السيروتونين مقارنة بمجموعة السيطرة. سجلت مستويات الأسيتيل كولين في مجموعتي الهيوسين 2 و 4 ملغم/كغم انخفاضاً ملحوظاً في مستويات الأسيتيل كولين وانزيم COMT مقارنة بمجموعة السيطرة. يؤثر الهيوسين على الجهاز العصبي من خلال زيادة مستوى السيروتونين والأسيتيل كولين وانزيم COMT في دم الفئران.

الكلمات الدالة: الهيوسين، السيروتونين، الأسيتيل كولين، السلوك العصبي، الفئران.