

Original Research Article

The Effect of imipramine on the behavior of albino mice in presence of selenium.

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Abstract

Introduction: Imipramine, a tricyclic antidepressant used in the treatment of depression, anxiety, and other mental condition. Selenium is useful in managing depression and anxiety.

Aim: The present study was aimed to investigate the behavior effects of imipramine in presence of selenium on anxiety, spontaneous motor activity and antidepressant behavior.

Methods: Mice were divided into 5 groups of six each. Group 1 (control) was given 5ml/kg 1% Tween 80. Group 2 was given selenium (200 µg/kg). Group 3 was given diazepam (1 mg/kg). Group 4 mice was given imipramine (10 mg/kg). Group 5 was given combined treatment of selenium and imipramine. All drugs were injected as sub-acute (three doses), intraperitoneally and administered at 24, 5, and 1.0 hours before scoring. Animals were tested in the elevated plus maze, open field and forced swim test one hour after drugs injections. All drugs were given by intraperitoneal route.

Results: Imipramine in the dose used had no anxiolytic effect and no effect on motor activity. Selenium has anxiolytic effect in the plus maze and no effect on spontaneous motor activity. The anxiolytic effect of selenium disappeared when given with imipramine. Both imipramine and Selenium alone produced significant antidepressant effect in the forced swim test, this effect disappeared when selenium was administered with imipramine.

Conclusion: Both the anxiolytic effect of selenium and the antidepressant effect of imipramine and selenium was abolished when administered together.

Key-words:

Imipramine, Selenium, Behavior, Plus Maze, Open Field, Forced Swimming Maze.

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INTRODUCTION

Major depressive disorder is a disabling condition that adversely affects a person's family, work or school life, sleeping and eating habits, and general health [1]. Most people with depression are treated by antidepressant drugs [2]. Antidepressants were first developed in the 1950s and have been used regularly since then [3]. Imipramine one of tricyclic antidepressants is used in the treatment of depression, anxiety, attention deficit hyperactivity disorder (ADHD), and other mental conditions [4]. Imipramine is also used in painful conditions due to its significant analgesic effect [5]. Imipramine is a reuptake inhibitor of both serotonin and norepinephrine, and also increases dopamine activity in the brain [6]. Imipramine antagonizes α_2 receptors, which may contribute to its anti-anxiety properties [7].

Selenium is a mineral that is essential for good health but required only in small amounts; it is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes [8,9]. Other selenoproteins help regulate thyroid function and play a role in the immune system [10]. Selenium increases the dopamine activity in the brain, thereby enhancing mood [11, 12]. Selenium deficiency is suggested to play a role in mood swings, depression and aging. A regular dosage of selenium 200 μ g daily can recover a person from acute stage of depression and apathy [13]. Dopamine and serotonin turnover increased; while noradrenaline and 5-hydroxy-3-indoleacetic acid turnover decreased by selenium [14].

This study was carried out to study the interaction between selenium and imipramine in models of anxiety (elevated plus maze) and depression (forced swimming test).

MATERIAL & METHODS

Animals:

The experiments were carried out using male mice (25-40gms weight) bred in the animal house of Faculty of Pharmacy-University of Tripoli. Standard mice food pellet diet and water were freely available. The animals were kept at room temperature (20-25°C), and on 12-hour dark/light cycle. Animals were kept in laboratory for at least 1 day before testing to acclimate with the new environment.

Drugs:

Imipramine hydrochloride was obtained from Novartis Pharma AG, Kurtköy Istanbul; Diazepam was obtained from Roche, Switzerland; Selenium was obtained from Jamieson, Toronto, Montreal, and Vancouver, Canada.

Elevated Plus- Maze:

Elevated plus-maze composed of two open and two close arms (30X05X15cms) that extended from a common central platform (5X5cms). The apparatus was elevated to height of 45 cms above floor level [15]. Mice were gently handled by the right hand and placed on the central platform of the maze facing the close arm. Different parameters were scored to evaluate anxiolytic effect and spontaneous motor activity



in the elevated plus-maze which included: time spent by the mouse in each of the arms, lines crossed in close or open arms, and the number of entries into close or open arms. An arm entry was defined as the entry of all four paws into the arm [16]. The total number of lines crossed and total number of arm entries were calculated. The total number of lines crossed and the total number of arm entries express the spontaneous motor activity [17,18]. Anxiety measure was calculated by dividing the time spent in close arm by the total time of the test [18]. The duration of the test was 4 minutes.

Open Field:

Open field was constructed from plywood (painted white) and measured 72x72 cms, with 36 cms high walls. Blue lines were drawn on the floor. The lines divided the floor into 16 (18x18 cms) squares; these lines were used to measure spontaneous motor activity [19]. Each mouse was placed in the center of the squares, the horizontal, ambulatory, non-ambulatory and number of movements was recorded for 4 minutes.

Forced Swimming Maze:

Mice were placed individually in glass cylinders (height 27 cms, diameter 15 cms) filled with water to a height of 16 cms (maintained at 23-25°C). The duration of the test was 6 minutes. Behavior parameters (duration of immobility and duration of climbing) were recorded during last 4 min of the 6 min testing period [20]. Immobility behavior is defined as the animal floated on the surface with front paws together and made only those movements with hind limbs that were necessary to keep float.

Climbing behavior is defined as upward-directed movements of fore paws along the side of the swim chamber [21].

Drugs treatments:

Pilot study for sub-acute effect was performed to choose selenium dose, the dose used was 200 µg/kg [22]. Imipramine administered at a dose of 10 mg/kg [23]. Diazepam was administered at a dose of 1 mg/kg [24], which was used as positive control for anxiolytic behavior. All drugs were injected as sub-acute (three doses), intraperitoneally and administered at 24, 5, and 1.0 hrs before scoring. All drugs administered as suspension in 1% Tween 80 (T80) [25]. It was injected in volume of 5ml/kg [26], and was prepared freshly prior to use.

Mice were divided into 5 groups (n=6); group 1 (control) was administered 5ml/kg of 1% T80; group 2 was administered selenium (200 µg/kg); group 3 was administered diazepam (1 mg/kg); group 4 was administered imipramine; group 5 was administered combined treatment of selenium and imipramine. Diazepam treated group was used as positive control for anxiolytic behavior.

Statistical analysis:

Descriptive statistical analysis was performed using computer program SPSS (version 13), also to verify whether the data were normally distributed by using Kolmogorov-Smirnov test maximum deviation test for goodness of fit. If the parameters were normally distributed, treatments were compared by one-way ANOVA, Post-Hoc test (LSD and Duncan test). If the parameters were not normally distributed,



The Effect of imipramine on the behavior of albino mice in presence of selenium

treatments were compared by the Mann-Whitney U test for unmatched sample. The differences were considered significant at the P value < 0.05 . The values are expressed as the mean \pm standard error.

RESULTS

Elevated Plus Maze

Anxiety measure decreased after administration of selenium or diazepam

($P < 0.05$) compared to the control treated group. Anxiety measure was significantly decreased in group treated with diazepam compared to the group treated with selenium ($P < 0.05$). Imipramine treated group and the combined treatment of selenium with imipramine did not show any changes in anxiety measure ($p > 0.05$) compared to the control group, but both groups showed significantly higher anxiety measure compared to selenium or diazepam treated groups (figure 1).

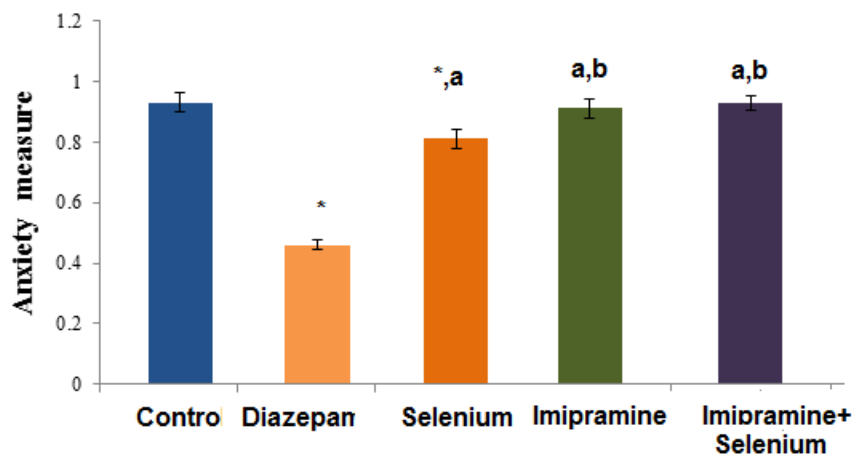


Figure 1: Anxiolytic effects of diazepam, imipramine, selenium, and combined treatment of imipramine with selenium using elevated plus maze. (*) Significantly different from control. (a) Significantly different from diazepam treated group. (b) Significantly different from selenium treated group.

Open Field

Ambulatory, non-ambulatory and the number of movements decreased significantly after the administration of diazepam and after the combined treatment of selenium with imipramine ($p \leq 0.05$) compared to control,

selenium, or to imipramine treated groups (figure 2).

Administration of selenium or imipramine each alone, did not show any change on ambulatory, non-ambulatory and number of movements ($p > 0.05$) compared to control mice (figure 2).



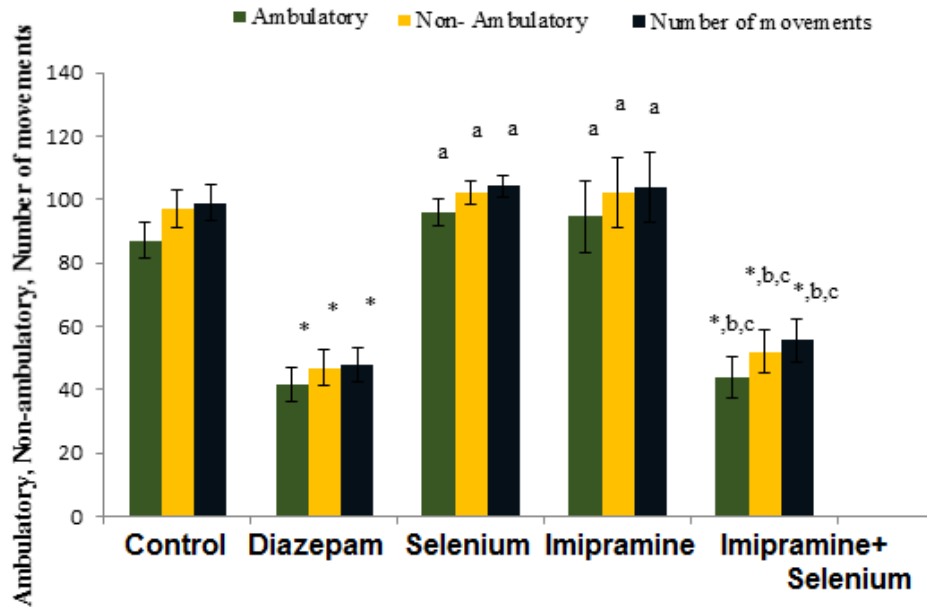


Figure 2: Locomotor activity of albino mice following diazepam, imipramine, selenium, and combined treatment of imipramine with selenium using open field. (*)Significantly different from control. (a)Significantly different from diazepam. (b)Significantly different from selenium. (c) Significantly different from imipramine.

Forced Swimming Maze

Administration of selenium or imipramine, each alone, produced significant decrease in the duration of immobility ($P < 0.05$) compared to control group; while the administration of combined treatment of selenium with imipramine did not show any change in the duration of immobility ($P > 0.05$) compared to control group; but showed significantly higher values in the duration of immobility compared to selenium or imipramine treated groups (figure 3).

The duration of climbing was significantly increased after administration of selenium or imipramine each alone ($P < 0.05$) compared to control group; where selenium showed more significant increase in the duration of climbing compared to imipramine treated group. The combined treatment of selenium with imipramine did not change the duration of climbing ($P > 0.05$) compared to control group; but showed significantly lower duration of climbing compared to selenium or imipramine treated groups (figure 3).



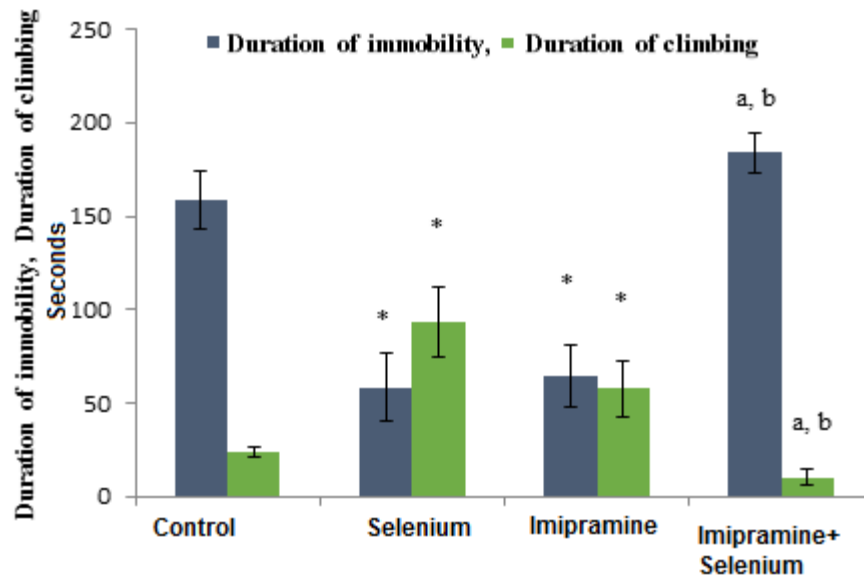


Figure 3: Antidepressant effects of, imipramine, selenium, and combined treatment of imipramine with selenium using forced swimming maze. (*) Significantly different from control. (a) Significantly different from selenium treated group. (b) Significantly different from imipramine treated group.

DISCUSSION

Antidepressants were first developed in the 1950s and have been used regularly since then [3]. Selenium is an antioxidant and is essential for brain function; increases in dietary selenium, especially in deficient people, improve mood and depressive symptoms [27].

The present study used three different models for the measurement of anxiety (elevated plus maze), locomotor activity (open field) and antidepressant effect (forced swimming test).

Diazepam, produced anxiolytic and sedative effect and it has been used in this

work as positive control [28]. Diazepam at the dose used, produced anxiolytic effect (decrease anxiety measure) without affecting locomotor activity (no effect on total lines or total entries) by using plus maze model; while decreased the locomotor activity using open field model. The decrease in spontaneous motor activity shown in the open field and not in plus maze, was due to the fact that open field is more sensitive to the changes in spontaneous motor activity than plus maze [29].

The subacute doses of imipramine did not induce anxiolytic effect, as indicated by the no change in anxiety measure. In accordance with our results Takeuchi et al., [30] did not



find an anxiolytic effect for imipramine. However, it was reported that the acute administration of imipramine produced anxiety effect while chronic administration showed anxiolytic action [31]. Therefore, in our case 3 doses were not enough to show any anxiolytic activity.

Selenium induced anxiolytic effect; this effect has been previously investigated by Ghisleni and Kazlauskas [32]; they found that diphenyl diselenide produced signs of an anxiolytic action. Selenium may be involved in the synthesis of GABA [33]; other study concluded that there is a strong relation between defect in dietary selenium and anxiety [34].

Imipramine counteracted the anxiolytic effect of selenium when administered together. Imipramine inhibits Na⁺-K⁺ ATPase leading to inhibition of noradrenaline and serotonin reuptake [35]. It was found that selenium interferes with the mechanism of action of imipramine, where selenium activates Na⁺-K⁺ ATPase leading to activation of noradrenaline and serotonin reuptake [36]. Imipramine is metabolized by CYP4502D6, at the same time it accelerates the metabolism of selenium by increasing CYP450 isoenzymes contents [37] which is responsible for the metabolism of selenium leading to decreasing its concentration.

Imipramine did not produce effect on spontaneous motor activity which is evaluated by both models elevated plus maze and open field. Imipramine might decrease spontaneous motor activity by increasing the dose, where imipramine has relatively higher

affinity as an agonist for H₁ histamine and α₁ adrenergic receptors when used chronically at dose of 20 mg/kg [38].

Selenium did not produce any significant change on spontaneous motor activity and that agrees with previous study concluded that selenium has antidepressant action on mice without accompanying changes in spontaneous motor activity [39]. Using open field test, the combined treatment of selenium and imipramine showed significant decrease in spontaneous motor activity, this might be due to the effect of selenium and imipramine by increasing GABA neurotransmitter levels. Selenium helps in the synthesis of GABA [33], while imipramine enhances GABA release through increased GABAB binding receptors [40]. The spontaneous motor activity was reduced using open field but not plus maze, indicate that open field might be more sensitive to the changes in spontaneous motor activity compared to plus maze.

Imipramine induced antidepressant effect which is indicated by a significant decrease in the duration of immobility and an increase in the duration of climbing. This agrees with previous study using forced swimming test [41]. Imipramine antidepressant effect was suggested to be due to serotonin and noradrenaline reuptake inhibition [42]. On the other hand, the increase in duration of climbing was suggested to be due to its effect on noradrenaline neurotransmitter [43].

Selenium produced antidepressant effect that may be through noradrenergic mechanism, due to the increase in the



duration of climbing. This is supported by other studies that simple selenium containing molecule significantly reduced the immobility time and has antidepressant-like action using the same model that used in our study [39, 44]. Furthermore, another study showed that organo selenium compound produces antidepressant-like effect in forced swimming test that seems to be dependent on its interaction with noradrenergic and dopaminergic systems, but not with serotonergic system [45].

When selenium and imipramine were combined together, their antidepressant effect disappeared. This unexpected paradoxical response is difficult to explain. It might be possible that imipramine accelerated the metabolism of selenium by increasing CYP450 isoenzymes activity [37], whereas selenium interfered with the mechanisms responsible for antidepressant effect of imipramine by activation of noradrenaline and serotonin reuptake through activation of Na⁺-K⁺ ATPase enzyme [36].

CONCLUSIONS

Imipramine in the dose used had an antidepressant effect, no anxiolytic effect, and did not change the spontaneous motor activity. On the other hand, selenium had antidepressant and anxiolytic effects with no effect on the spontaneous motor activity. The combined treatment with imipramine and selenium resulted in loss of antidepressant effect of both drugs and the anxiolytic effect of selenium.

It is recommended that selenium can improve anxiety and depressive behavior, but its co-administration with imipramine should be avoided.

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None

COMPETING INTERESTS

Authors declare that there are no competing interests with others.

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The Effect of imipramine on the behavior of albino mice in presence of selenium

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ملخص باللغة العربية

تأثير الإيميبرامين على سلوك الفار الأبيض في وجود السيلينيوم

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مقدمة:

إيميبرامين مضاد للاكتئاب ثلاثية الحلقات يستخدم في علاج الاكتئاب، والقلق، وحالات عقلية أخرى. السيلينيوم مفيد في معالجة الاكتئاب والقلق. تهدف الدراسة الحالية إلى تحقيق آثار سلوك الإيميبرامين في وجود السيلينيوم على القلق، والنشاط الحركي العفوي، والسلوك المضاد للاكتئاب.

الطرق:

تم تقسيم الفئران إلى 5 مجموعات كل منه تتكون من ستة حيوانات. ثم اعطاء المجموعة الأولى حجم 5 ملي ليتر لكل كيلوجرام من وزن الجسم 1% توين 80. أعطيت المجموعة الثانية السيلينيوم (200 ميكروغرام لكل كيلوغرام). وأعطيت المجموعة الثالثة الديازيبام (1 ملجرام لكل كيلوغرام). وأعطيت المجموعة الرابعة من الفئران إيميبرامين (10 ملي جرام لكل كيلوجرام). وأعطيت المجموعة الخامسة كل من السيلينيوم والإيميبرامين متزامنين في نفس الوقت. تم حقن كل مجموعة بثلاث جرعات (شبه حادة) عن طريق الحقن الصفاقي وذلك قبل 24 و 5 و 1.0 ساعة من القياس. تم اختبار الحيوانات في متاهة زائد المرتفعة، الحقل المفتوح والسباحة القسرية وذلك بعد ساعة واحدة من الحقنة الأخيرة لكل دواء.

النتائج:

لم يكن للإيميبرامين في الجرعة المستخدمة أي تأثير مزيل للقلق كما أنه لم يكن له تأثير على النشاط الحركي للحيوان. كان للسيلينيوم تأثير مزيل للقلق في متاهة زائد وليس له تأثير على النشاط الحركي العفوي. اختفى التأثير المزيل للقلق للسيلينيوم عندما أعطي مع إيميبرامين. كل من الإيميبرامين والسيلينيوم له تأثير مضاد للاكتئاب في اختبار السباحة القسرية، ولكن هذا التأثير اختفى عند حقن الحيوانات بكلا الدواءين في نفس الوقت.

الخلاصة:

كل من التأثير المزيل للقلق للسيلينيوم والتأثير المضاد للاكتئاب لكل من الإيميبرامين والسيلينيوم ألغيت عندما حقنا معا في نفس الوقت

الكلمات المفتاحية:

إيميبرامين، سيلينيوم، السلوك، متاهة زائد المرتفعة، الحقل المفتوح، السباحة القسرية.

