EXPERIMENTAL PHARMACOLOGICAL MODEL FOR INDUCING AND QUANTIFYING DEPRESSION IN MOUSE

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Abstract

Due to the fact that depression quantification in laboratory animals is hard to realize, an experimental study was conducted in order to create a model of depression induced by administration of reserpine in white male mice NMRI strain. To this end, three doses of reserpine were tested (0.5mg/kg-bw, 0.75 mg/kg-bw, 1.5 mg/kg-bw), a neurosympatholytic agent which produces catecholamine depletion at central and peripheral level. Depression onset evaluation was performed at 2 moments: after 11 and after 21 days of neurosympatholytic administration, both by classical pharmacological tests and also by determining the cerebral activity of monoamine oxidase (MAO), an enzyme involved in catecholamine catabolism.

The experimental data revealed, after 21 days of treatment, for all doses used, modification of investigated parameters towards onset of depressive phenomenon. This way, compared to control group, for the animals submitted to forced swimming test, immobilizing time increased by more than 80% and MAO activity decreased dose-dependently (-39.92% for 0.5 mg/kg-bw dose; -40.31% for 0.75 mg/kg-bw dose; -40.61% for 1.5 mg/kg-bw dose). These effects were in correlation with a significant reduction of motor activity.

Making use of the created model (reserpine 0.75 mg/kg-bw, p.o., 21 days), antidepressant effect of clomipramine was determined as administered in 25 mg/kg-bw p.o. dose. Results were reproducible concerning the parameters used for depression evaluation in laboratory animals and clomipramine has normalized these parameters.

It can be concluded that the model created can help to investigate the antidepressant effect of newly synthesized active substances.

Rezumat

Întrucât cuantificarea depresiei la animale de laborator este un fenomen greu de realizat, am realizat un studiu experimental, pentru crearea unui model de depresie, indusă prin administrarea de rezerpină, la şoareci albi, masculi din suşa NMRI. În acest scop, am testat trei doze de rezerpină (0,5mg/kg corp, 0,75mg/kg corp, 1,5mg/kg corp), neurosimpatolitic ce produce depleţie de catecolamine la nivel central şi periferic.

Evaluarea instalării depresiei s-a realizat la două intervale de timp: după 11 şi respectiv 21 de zile de administrare a neurosimpatoliticului, prin teste farmacologice clasice, dar şi prin determinarea activităţii cerebrale a monoaminoxidazei (MAO), enzimă implicată în degradarea catecolaminelor.

Rezultatele experimentale au evidenţiat după 21 de zile de tratament, pentru toate dozele testate, o modificare în sensul susţinerii instalării fenomenului depresiv, a parametrilor investigaţi. Astfel, faţă de lotul martor, a crescut timpul de imobilizare al animalelor supuse înotului forţat mai mult de 80%, iar activitatea MAO a fost scăzută doză dependent (-39.92% pentru doza de 0,5mg/kg corp; -40.31% pentru doza de 0,75mg/kg corp; -40,61% pentru doza de 1,5mg/kg corp). Aceste efecte au fost corelate cu o reducere semnificativă a activităţii motorii.

Utilizînd modelul creat (0,75mg/kg corp rezerpină p.o, 21 zile), am determinat efectul antidepresiv al clomipraminei, administrată în doză de 25mg/kg corp p.o. Rezultatele au fost reproductibile în ceea ce priveşte parametrii utilizaţi pentru evaluarea depresiei la animale de laborator, iar clomipramina, a readus la normal aceşti parametri.

Putem concluziona că, modelul creat, poate servi pentru investigarea efectului antidepresiv al unor substanţe active nou sintetizate.

Keywords: depression, reserpine, clomipramine, antidepressant

Introduction

Depression is a psychiatric quantitative disorder of the central nervous system (CNS), widely spread in population, which shortens the lifespan in various proportions, according to epidemiological reports from different countries [15, 16, 20].

One of the most supported theories for depression etiology is the monoamine theory which states that the pathology follows decreased levels of one or more of the monoamines (serotonin, noradrenaline, dopamine) involved in maintaining psychic tone at central level [1, 11, 14]. Whilst noradrenaline and dopamine are directly involved in the mechanism, serotonin has the role of regulating the activity of other synaptic transmissions. According to serotonin theory, decreased concentrations at cerebral level may induce disorders in noradrenergic and/or dopaminergic metabolisms [13, 28]. Support for these hypothesis is the antidepressant medication [22], which acts under different mechanisms, that all address central monoaminergic system: inhibition of noradrenaline and adrenaline re-uptake, inhibition of serotonin re-uptake by acting at the level of synaptic active transporters or by direct action on pre-synaptic receptors (α_2 adrenergic receptors). Another therapeutical approach in depression is the inhibition of the activity of cerebral monoamine oxidase (MAO), enzyme involved in biotransformation of the neurotransmitters of the monoaminergic system [18].

Quantification of depression in humans as well as the evaluation of efficacy of antidepressants resorts to questionnaires assessing the mental status: Hamilton Rating Scale for Depression, Beck Depression Inventory [19, 26, 29].

In non-clinical experiments, the evaluation of depressant/antidepressant effect is hard to perform as it uses pharmacological tests that attempt to assess the effect by creating experimental stress environment approaching the symptoms registered in human: *forced-swimming test* [25]*; tail suspension test* [4, 9]; s*ucrose preference* [21]; *intracranial self-stimulation* [21].

The present work employs experiments on laboratory animals aiming at quantifying the depressant effect induced by reserpine administration [5, 6, 10], a neurosympatholytic acting both centrally and peripherally. The pharmacological tests were correlated with determination of cerebral monoamine oxidase activity, starting from the hypothesis that a reduction in the levels of monoaminergic neurotransmitters could lead to decreased enzyme activity. On this model of depression induced by reserpine administration, tests were performed concerning the antidepressant effect of clomipramine, a tricyclic antidepressant acting by non-selective inhibition of the re-uptake of norepinephrine and serotonin [17, 18].

Materials and Methods

Method of inducing depression with reserpine

To a collectivity of 200 white NMRI strain male mice oral reserpine was administered in order to induce catecholamine depletion at central level.

Groups of 40 animals were randomized and treated with reserpine in doses of 0.5 mg/kg-bw, 0.75 mg/kg-bw and 1.5 mg/kg-bw. Duration of reserpine administration was 21 days (a dose daily). A control group was treated with normal saline 0.1 mL/10 g-bw, p.o. (once a day).

In order to quantify the depression induced by reserpine administration in laboratory animals two types of tests were conducted:

a. pharmacological tests for evaluation of motor behavior, of immobility time for animals subjected to forced swimming and of pain hypersensitivity (hot plate test);

b. biochemical tests for determination of cerebral monoamine oxidase (MAO) activity, an enzyme involved in catecholamine metabolism.

These tests were conducted after 11, respectively, 21 days of consecutive administration of reserpine.

For evaluation of cerebral MAO activity, 10 animals from each group were sacrificed in day 11 and 21. In order to compare the

modifications of enzyme's activity under experimental stress within this protocol, a group of 10 mice was initially sacrificed and brain enzyme activity was determined.

As the experiment unfolded a selection was operated regarding the dose and duration of treatment for which the above-mentioned parameters modified themselves according to depression manifestations. The dose of 0.75 mg/kg-bw was therefore chosen since the effects quantifying the depression were statistically significant after 21 days when for the respective dose did not pronounce sedative effect nor ptosis (specific effect of this centrally-acting neurosympatholytic) were recorded.

Method for evaluation of antidepressant effect of clomipramine using the experimental model of depression induced with reserpine

Based on the experimental study data, a new collectivity of 40 white male mice NMRI strain was administered orally 0.75 mg/kg-bw reserpine, once daily, for 21 consecutive days. Following this time interval, clomipramine 25 mg/kg-bw was administered orally, once daily, for 21 days.

Both the depression induced by reserpine after 21 days of administration and also the antidepressant effect of clomipramine administered 21 days after the induction of depression with reserpine were evaluated using the same pharmacological and biochemical tests.

Pharmacological and biochemical experimental tests

Determination of immobility time of mice in forced swimming test employed Porsolt test [7, 24, 25]. The mice subjected to forced swimming release an increased amount of catecholamines in order to cope with the experimental stress to which are submitted. In the following time interval the immobility time was noted, as an expression of decreased central monoaminergic tone.

Mice were introduced in a glass container (30 cm diameter and 30 cm height) containing water at a temperature of 23^0C. Immobility time was determined over 4 minutes following a 2 minutes accommodation with the test environment.

Evaluation of pain sensitivity was performed using modified hot-plate test [2, 8, 12]. The threshold of pain perception was determined by measuring the time until the animal licked his paw (T_{lick}) when on hot plate heated at 56°C.

Determination of motor activity aimed to evaluate the degree of inhibition of CNS by measuring the distance and speed for the animals in the auto-track (device that automatically registers the two above-mentioned parameters).

Determination of cerebral MAO activity was based on the fact that MAO is an enzyme localized at the external mitochondrial membrane and belongs to the class of oxidoreductases (monoamine: O_2 – oxidoreductases, EC: 1.4.3.4). The enzyme is involved in the metabolism of cerebral mono amines: adrenaline, norepinephrine, serotonin, dopamine, tyramine, tryptamine [1, 18].

Following the oxidative deamination, hydrogen peroxide is released, and also ammonia and the aldehyde corresponding to the catabolized substrate.

The method used for determining the present experimental protocol is based on the reduction of tetrazolium salts in an incubation medium containing the enzyme present in fragments of external mitochondrial membrane, NBT (para-nitrobluetetrazolium) and substrate of reaction (serotonin, tryptamine, tyramine, adrenaline, etc.).

Statistical analysis

Statistical calculation used the software GraphPad Prism 5. Statistical evaluation compared the results obtained following the treatment with reserpine (administered in the doses mentioned in the experimental protocol) with initial testing (baseline) and the control group testing. Statistical comparison between groups used the Student *t* test (for normal distribution) or Wilcoxon (for abnormal distribution). Statistical evaluation for multiple groups used the ANOVA test.

Normality of response distribution in collectivity was tested with D'Agostino & Pearson test.

Results and Discussion

Experimental results after depression was induced with reserpine regarding modification of the parameters: immobility time, pain sensitivity, motor activity, cerebral MAO activity.

Table I shows the evolution of immobility time for mice subjected to forced swimming, the variation of this parameter, compared to control group and the statistical significance of this variation. Figure 1 features the evolution of immobility time against baseline while figure 2 features the variation of this parameter against control group.

Experimental results regarding the evolution of motor activity after 11 and, respectively, 21 days of treatment for the groups with reserpine (0.5 mg/kg-bw, 0.75 mg/kg-bw, 1.5 mg/kg-bw) and the control group treated with normal saline 0.1mL/10g-bw, are featured in table II and in figures 3-4.

Experimental results regarding the evolution of time to lick after 11 and, respectively, 21 days of treatment for the group with reserpine (0.5

mg/kg-bw, 0.75 mg/kg-bw, 1.5 mg/kg-bw) and the control group treated with normal saline 0.1 mL/10 g-bw, are featured in the table III and in figures 5-6.

Activity of cerebral MAO for the treated groups and its modifications against control group are featured in table IV and in figure 7. The value of MAO activity, determined in mice not subjected to experimental stress, was of 9.1742 ± 0.4103 µg reduced NBT/mg protein.

According to experimental data, immobility time of animals in control group, subjected to forced swimming, was not statistically significant different compared to baseline, in any of the moments of evaluation (table I; figure 1). Reserpine treatment for 11 days produced an increase of immobility time, statistically significant against the baseline, only at 0.75 and 1.5 mg/kg bw doses. It can be observed, after 21 days of neurosympatholytic administration, that increases occurred for this parameter, of over 80%, statistically significant (table I; figure 1).

Table I

Variation of immobility time after reserpine administration, determined at 11 and, respectively, 21 days of treatment. Statistical significance of the data

Immobility time (s) M±SD	**Control**	**Reserpine 0.5 mg/kg-bw**	**Reserpine 0.75 mg/kg-bw**	**Reserpine 1.5 mg/kg-bw**
Baseline	23.60±5.61	27.80±4.82	33.70± 3.79	23.76±4.21
After 11 days	28.50±4.65	35.20±5.62	54.35±4.11	35.53±3.97
Effect%/baseline	+ 20.76	+26.61	+61.27	+49.51
p/baseline *t* Student	>0.05	>0.05	<0.01**	<0.05*
Effect%/control		+5.85	+40.51	+28.75
After 21 days	30.64±6.01	50.71±6.92	63.71±5.87	43.27±4.98
Effect%/baseline	+29.83	+82.41	+89.05	+82.12
p/baseline *t* Student	>0.05	<0.005**	<0.001**	<0.005**
Effect%/control		+52.58	+59.22	+52.29

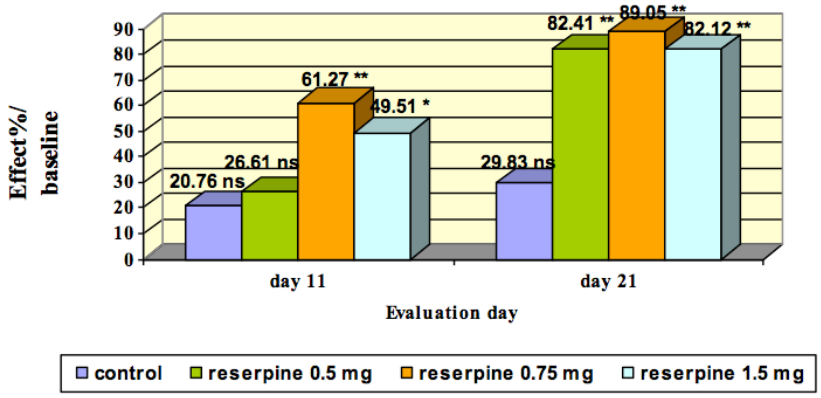

Figure 1

Variation of immobility time for animals in control group and treated with reserpine, against baseline.

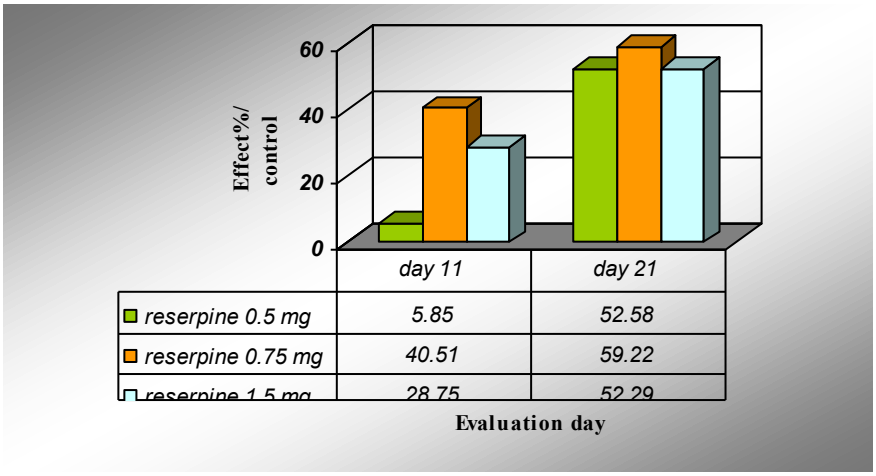

Figure 2
Variation of immobility time for animals treated with reserpine, against control group.

After 11 days of reserpine administration, the distance covered in auto-track has not been modified statistically significant against baseline (table II). Marked and significant decreases of motor activity were noted after 21 days of treatment with neurosympatholytic for the doses 0.75 mg/kg-bw (-32.67%; $p<0.01$) and 1.5 mg/kg-bw (-49.76%; $p<0.005$). Resulted data comply with other literature reporting [3] that supports marked decreases in motor activity, but with no major influence in coordination, induced by reserpine administration.

Table II
Variation of distance (m) covered in autotrack, after reserpine administration, determined at 11 days and, respectively, at 21 days. Statistical significance of the results

Distance (m), 3 minutes; M±SD	**Control**	**Reserpine 0.5 mg/kg-bw**	**Reserpine 0.75 mg/kg-bw**	**Reserpine 1.5 mg/kg-bw**
Baseline	4.12±0.976	5.24±1.012	5.08±0.895	5.62±0.997
After 11 days	4.54	5.97	4.62	4.66
Effect%/baseline	+10.19	+13.93	-9.05	-17.08
p/baseline *t* Student	>0.05	>0.05	>0.05	>0.05
Effect%/control		3.74	-19.24	-27.27
After 21 days	3.89±1.081	4.38±1.002	3.42±0.899	2.84±0.791
Effect%/baseline	-5.65	-16.41	-32.67	-49.76
p/baseline *t* Student	>0.05	>0.05	<0.01**	<0.005**
Effect%/control		-10.76	-27.02	-44.11

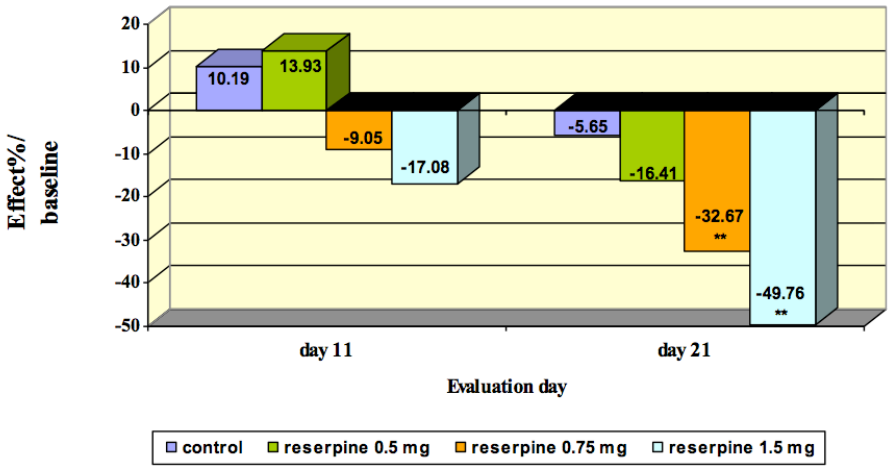

Figure 3

Variation of distance covered in auto track for mice in control group and treated with reserpine, against baseline.

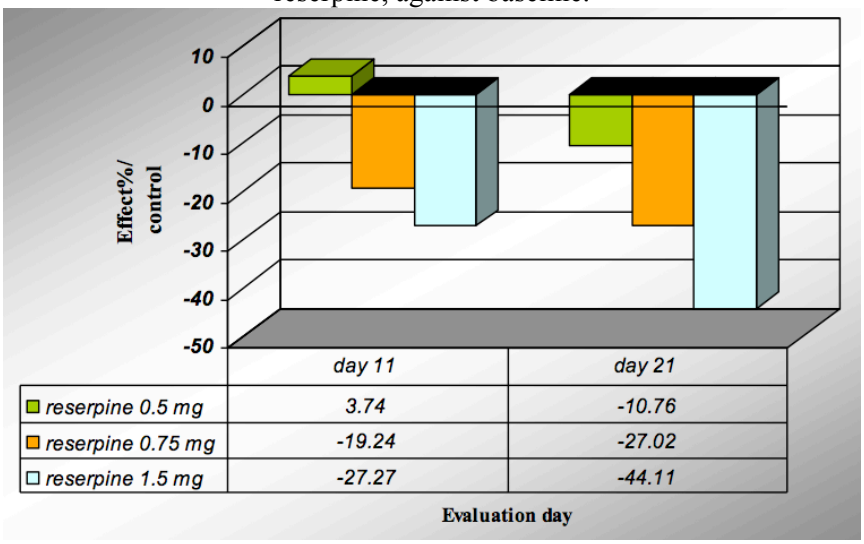

Figure 4

Variation of distance covered in auto track for mice treated with reserpine against control.

T_{lick} for tested groups did not modify statistically significant against baseline in evaluation day 11 (table III; figure 5). At evaluation day 21, T_{lick} has increased statistically significant against baseline for all doses tested.

This modification in positive sense of the parameter was possibly the consequence of catecholamine depletion at central level accompanied by consecutive prevalence of opioidergic system or was the result of sedative effect of reserpine (table III).

In the present experimental model no correlation could be noted between animal responses to thermal stimulus following reserpine treatment and the decrease of immobility time in forced swimming test. Although it is acknowledged that depression can yield somatic and/or visceral pain, the sedative effect of reserpine [23, 27] could have masked, in the hot plate test, the pain hipersensitivity. This hypothesis is also supported by results regarding motor activity evaluation (table II) which account for marked decrease of this parameter after 21 days of neurosympatholytic administration.

Table III

Variation of T_{lick} after reserpine administration, determined at 11 days and, respectively, 21 days of administration. Statistical significance of results.

T_{lick} (s) M±SD	**Control**	**Reserpine 0.5 mg/kg-bw**	**Reserpine 0.75 mg/kg-bw**	**Reserpine 1.5 mg/kg-bw**
Baseline	4.80±0.364	5.80±0.421	6.25±0.501	5.53±0.362
After 11 days	4.18	5.40	5.50	5.40
Effect%/baseline	-12.91	-6.89	-12.00	-2.35
p/baseline *t* Student	>0.05	>0.05	>0.05	>0.05
Effect%/control		6.02	+0.91	10.56
After 21 days	5.25±0.413	9.85±0.376	8.57±0.493	10.27±0.371
Effect%/baseline	9.37	69.82	37.12	85.71
p/baseline *t* Student	>0.05	<0.0001***	<0.05*	<0.0001***
Effect%/control		60.45	36.75	76.34

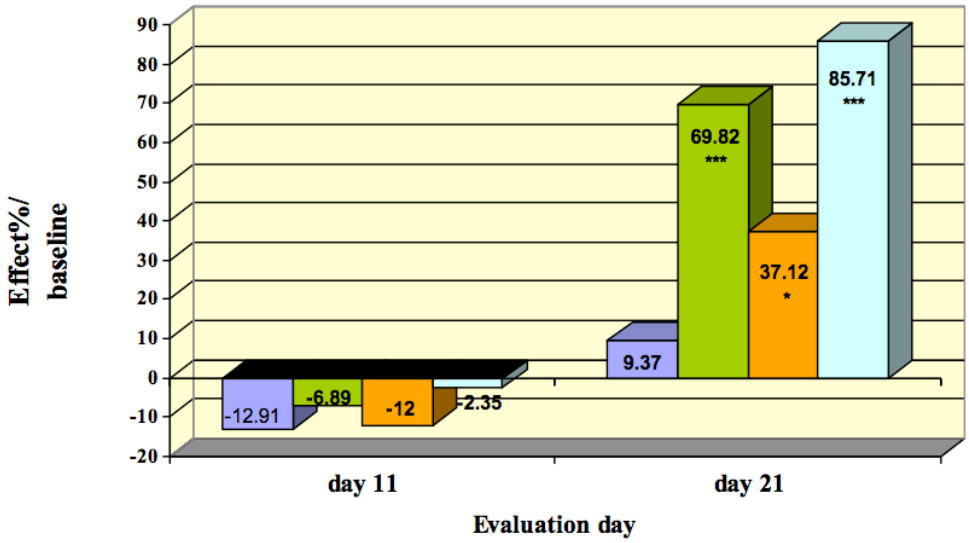

Figure 5

Variation of T_{lick} for mice in control and reserpine groups, against baseline.

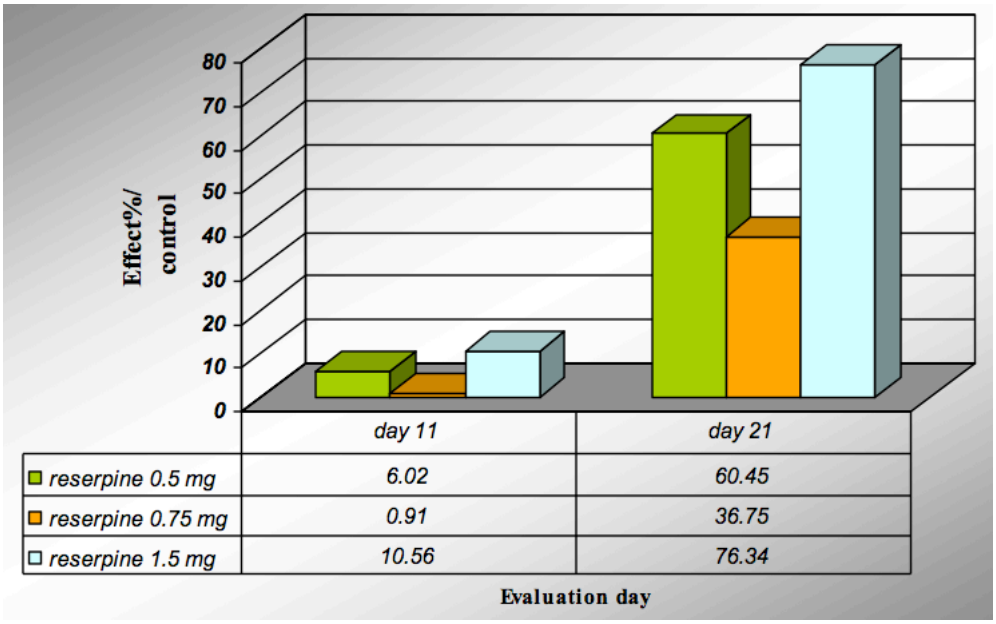

Figure 6

Variation of T_{lick} for mice treated with reserpine against control group.

For control group, treated with normal saline, the cerebral MAO activity has not been modified under experimental conditions (table IV). For the groups treated with reserpine, the reduction in activity of this enzyme, as an expression of catecholamines depletion, was directly proportional with the dose and duration of reserpine administration (table IV; figure 7).

Table IV

Variation of cerebral MAO activity against control group. Statistical significance of the results: Student *t* test, ANOVA.

MAO activity -µg reduced NBT/mg protein; **M±SD**	**Control**	**Reserpine 0.5 mg/kg-bw**	**Reserpine 0.75 mg/kg-bw**	**Reserpine 1.5 mg/kg-bw**
After 11 days	10.08±0.3210	7.79±0.1721	7.43±0.2165	7.20±0,3402
Effect%/control		-22,71	-26,28	-28,57
p/control *t* Student		<0.0031**	<0.0016**	<0.001**
p ANOVA	<0.0001***			
After 21 days	10.12±0.1987	6.08±0.3001	6.04±0.1921	6.01±0.1168
Effect%/control		-39.92	-40.31	-40.61
p/control *t* Student		<0.0001***	<0.0001***	<0.0001***
p ANOVA	<0.0001***			

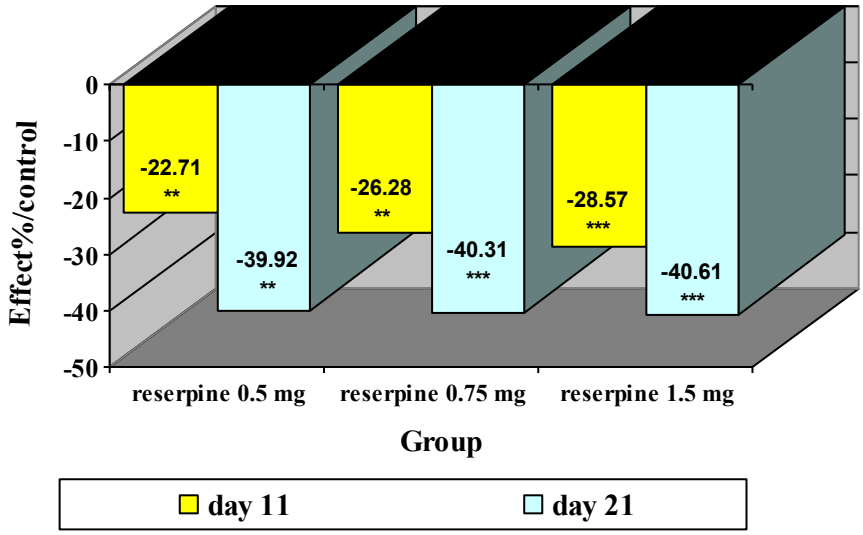

Figure 7
Variation in cerebral MAO activity after reserpine treatment against control group.

In the experiment it was observed that following reserpine administration, none of the doses administered determined any decrease in mice body weight (effect due to neurosympatholytic action of increased speed of bowel movements). Body weight for mice treated with reserpine remained similar as for mice in control group treated with normal saline.

Experimental results for the evaluation of antidepressant effect of clomipramine (25 mg/kg-bw, p.o.) in mice with depression induced by reserpine.

The variations of the parameters determined in order to both evaluate depression induced with reserpine and also to evaluate the antidepressant effect of clomipramine are featured in table V and figures 9-10. Reserpine in 0.75 mg/kg-bw, p.o., for 21 days, has induced the similar effect against baseline compared with the resultsdescribed for the experiment above, thus supporting the reproducibility of the experimental model of depression. Clomipramine, as an antidepressant non-selectively inhibiting norepinephrine and serotonin re-uptake, has brought the evaluated parameters back to values close to baseline determinations (table V; figure 8). Clomipramine administration following depression induction with reserpine (figure 9) has decreased immobility time of mice subjected to forced swimming by 40.79% (p=0.0002), increased distance covered in auto

track by 117.72% (p<0.0001) and increased cerebral MAO activity by 37.87% (p=0.0001).

Table V

Variation of parameters quantifying reserpine-induced depression and also the antidepressant effect of clomipramine. Statistical significance of the results (Student *t* test).

Parameter	**Immobility time (s)**	**Distance (m)**	**T_{lick} (s)**	**MAO activity - µg** reduced NBT
Baseline - M±SD	37.10±6.013	5.93±1.076	6.50±0.742	9.137±0.164
Reserpine 0.75 mg/kg-bw (21 days) - M±SD	66.73±5.416	3.16±0.981	7.17±0.698	6.348±0.371
Effect%/baseline	79.86	-46.71	10.30	-30.52
p/baseline	<0.0001***	0.0001***	p<0.05*	0.0031**
Clomipramine 25 mg/kg-bw (21 days after reserpine - M±SD	39.64±5.971	6.88±1.047	6.75±0.570	8.752±0.199
Effect%/baseline	6.84	16.02	3.84	-4.21
p/baseline	p>0.05	p>0.05	p>0.05	
Effect%/reserpine	-40.59	117.72	6.46	37.87
p/reserpine	0.0002***	<0.0001***	p>0.05	0.0001***

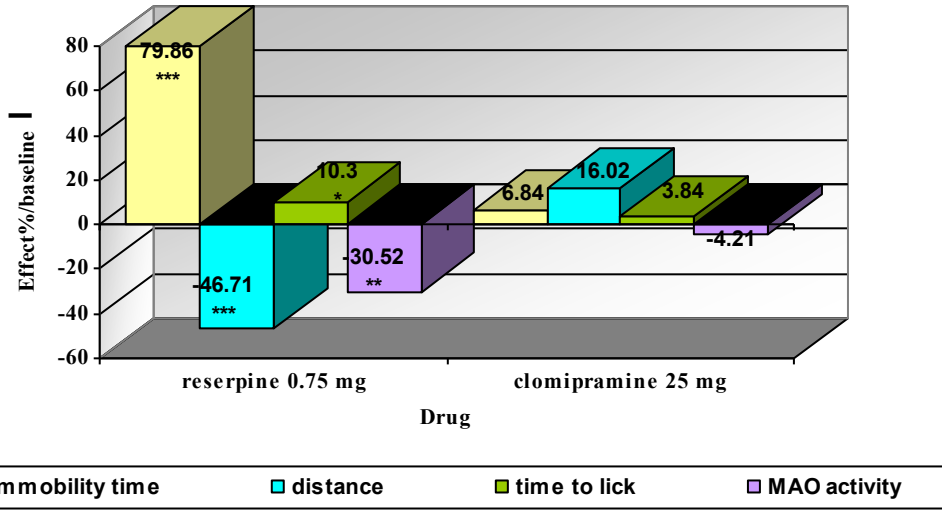

Figure 8

Modification of parameters immobility time, distance covered in auto track, time to lick, cerebral MAO activity following reserpine administration (0.75 mg/kg-bw) and clomipramine (25 mg/kg-bw), against baseline.

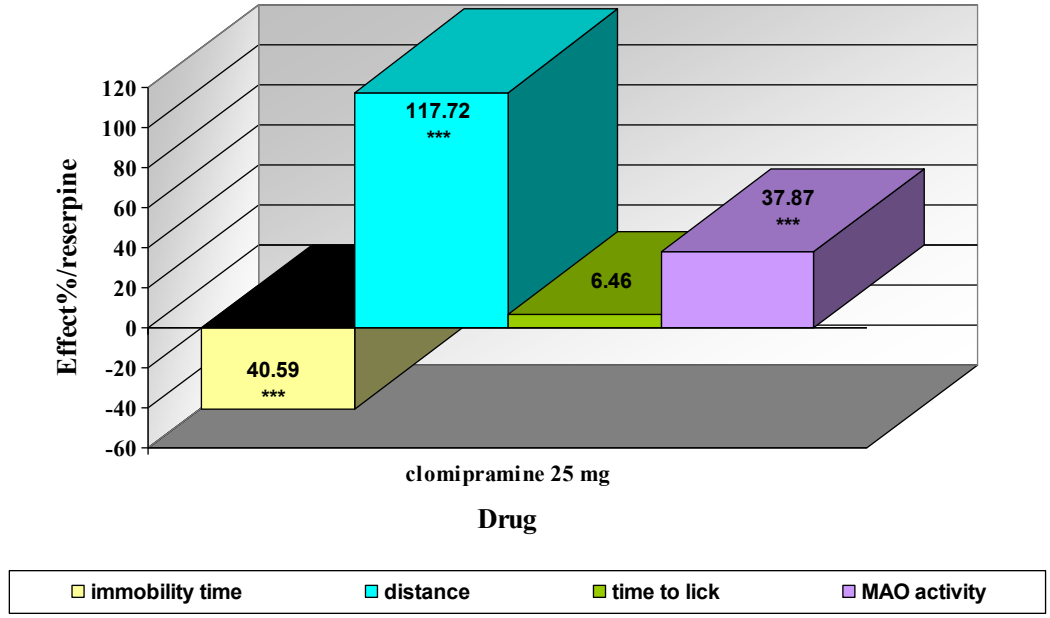

Figure 9

Modification of the parameters: immobility time, distance covered in auto track, time to lick, cerebral MAO activity, following clomipramine administration (25 mg/kg-bw), against reserpine.

In the evaluation of antidepressant effect of clomipramine, experimental results support the reproducibility of the depression model induced with reserpine while also demonstrating the capacity to normalize the same parameters used for quantifying depression (as classic antidepressant).

Conclusions

An experimental model of depression was designed, induced by reserpine administration, a neurosympatholytic producing catecholamine depletion. For effect evaluation several pharmacological tests were performed (aiming at determining the immobility time in forced swimming test, the thermal pain sensitivity in hot-plate test and motor activity in auto-track test) together with a biochemical test (aimed at determining the activity of cerebral MAO, enzyme involved in degradation of monoamine neurotransmitters)

The experimental results have demonstrated that, after chronic administration of reserpine, motor activity and cerebral MAO activity

registered statistically significant decreases and immobility time of mice under experimental stress conditions increased.

The reproducibility of the experimental model was verified by determining the antidepressant effect of a well-established such drug, clomipramine. The tricyclic antidepressant antagonized the effects induced by reserpine at an oral dose of 25 mg/kg-bw, expected result which supports the viability of the experimental model imagined for future researches on antidepressant efficacy of newly synthesized drug molecules.

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