SciMedCentral

JSM Clinical Pharmaceutics

Research Article

Toxic Effect of Single Treatment with Kappa-Opioid Agonist, Ru-1205 Compound, on the Neurological Status of Wild Type Mice

Alexander A. Spasov[1,2], Olesya Yu Grechko[1], Natalya V. Eliseeva[1], Roman A. Litvinov[1]*, and Daria D. Shamshina[1]

[1]Volgograd State Medical University, Russia
[2]Volgograd Scientific Medical Centre, Russia

*Corresponding author
Roman A. Litvinov, Volgograd State Medical University, 1, Pl. Pavshikh Bortsov Square, Volgograd, 400131 Russia, Tel: 7-902-361-87-70; Email: litvinov.volggmu@mail.ru

Submitted: 30 August 2017

Accepted: 02 October 2017

Published: 05 October 2017

ISSN: 2379-9498

Keywords
- RU-1205
- Butorphanol
- Kappa-Opioid
- Analgesia
- Neurotoxicity
- Primary observation

Abstract

Using primary observation setting (includes open field, rota-rod, actometer, visual detection of tremor, convulsions, pyloerrection, Streub's symptom, salivation, reflexes, ambivalence, vocalization and passivity) we investigated the neurotoxicological properties of novel kappa-opioid agonist RU-1205 in comparison with butorphanol for the presence of serious neurologic adverse reactions and matched the toxic doses with the analgesic ones. We revealed that the analgesic effect of RU-1205 persists over a wide range of doses (0.001-100 mg/kg) with maximum at 1 mg/kg and ED_{50} 0.02 mg/kg (mice, hot-plate, and 55°C). In contrast, the ED_{50} of butorphanol is 0.2 mg/kg in the same conditions. The serious neurological disruptions after treatment with RU-1205 and butorphanol were observed at 50-100 mg/kg, but paying attention the 10 times greater analgesic efficiency of RU-1205. We concluded that RU-1205 is safer than butorphanol.

ABBREVIATIONS

NL: Nociception Latency; AUC: Area under Curve; VAS: Visual Analog Scale; HFC: Hypolocomotion-Free Coefficient; MAE: Maximal Analgesic Effect

INTRODUCTION

Pain is a significant clinical problem. There is a need for more effective treatments with reduced adverse effects that currently limit the use of mu-opioid receptor agonists. Modern approach to create a new generation of medicinal products including opioid analgesics is to create safe products without any adverse reactions. In the 2015 Ch. Chavkin (University of Washington) - one of the most prominent scientists in the field of researching of kappa-opioid agonists – definitively predetermined the direction of research and development to create a safe analgesic medicinal product on the base of selective kappa-opioid mechanism of action [12]. That is "biased agonism". The main – is direct or indirect activation or inhibition of kappa-opioid pathways, such as partial activation of G-protein associated pathway or inhibition of MAP-kinase p38 (arrestin-associated pathway) [4]. If there are unexpected results of adverse effects evaluation or partial presence of some effects and absence of others, it may be caused by the reason of biased agonism.

It is well known that different CNS functions are regulated by the opioid neuromodulation system. Both unprescribed and therapeutical usage of opioid products may cause serious disturbances in CNS functions, highly probably. The uncorrect neuromediation spikes, ion channels modulation (GIRK or Ca2+ channels), provoked by the opioid receptor activation may be causative event of any psychotropic or vegetative adverse reactions, such as psychoactivation, depersonalization, depression, aversion and mood disorders, hyper- or hypothermia, abstinence etc [5]. There is no complete information about mechanism of each adverse effect developing due to possible differences. For example the role of ventral tegmental area in the aversion development is clear now [12], but mechanisms of hypothermia development is not fully clear for the present day [13]. Adverse effects investigation is a long way prior the approval clinical usage of the new medical product and the first step is a preclinical primary observation tests in a wide range of doses. Primary observation is preliminary for the assessment of the neurotoxicity of new substances, but this is the necessary procedure. The wide use of primary observation tests evolved from S. Irvins paper in 1968 in journal "Psychopharmacologia". That was a primarily visual observation for the reactions of laboratory animals on the given product. Following that the complex of tests was many times reconsidered. Previously we demonstrated that RU-1205 compound has an analgesic activity, mediated by the kappa-opioid receptor activation (electroinduced contractions of the rabbit vas deferens, IC_{50} $2.2*10^{-9}$ M, nor-BNI-invertible) and does not activate mu- and delta-opioid receptors [1,2,3].

This paper we describe the usage of our primary observation set, designed by researchers of pharmacology department of Volgograd state medical university and the effects of two opioid agonists in it (RU-1205 and butorphanol).

MATERIALS AND METHODS

The object: RU-1205 compound (like a bulk, from South Federal University of Physic and Organic Chemistry, Rostov-on-Don, Russia); reference drug: butorphanol (presentation in solution 2%-1ml in amp. from FSUE "Moscow endocrine plant"). Laboratory animals: outbreed mature white circadian inverted (day to night) mice 20-25 g. Experimental procedures were carried out from 11.00 a.m. to 16.00 p.m. All animals were handled before the testing for 1 month. All manipulations were implemented in accordance with the requirements of Directive 2010/63/EU of the European Parliament and the Council of the European Union for the protection of animals used in scientific purposes (22.09.2010). All experiments with narcotic substances were carried out under the license LO-34-04-000022 (12.10.2012) for working and control of spreading of narcotic drugs, psychotropic substances and precursors, and in accordance with the order of the Volgograd state medical university of the Ministry of Health of the Russian Federation No. 1351-KM of 12.11.2013. All compounds were diluted with distilled water. All measurements were made at 60 minutes time point after injection. If prolonged observation was necessary – it was a period 0.5-3 hours after injection.

The Nociception Testing

The analgesic activity of both compounds was investigated at the hot-plate (t=55°C, Ugo-Basile) with determination of nociception latency. We used "licking of hind paws" pattern of hot plate [6]. Both compounds were tested at the wide range of doses (0.001-100 mg/kg, i.p). The maximal analgesic dose and fields under the "dose-effect" curves were determined.

Nonspecific Neuropsychotropic Activity

Compounds were tested for the presence of psychoemotional, vegetative and muscular coordination disruption adverse effects at range of doses 0.1-100 mg/kg, i.p. All followed procedures are described in the Primary observation protocol.

We investigated the effect of compounds on spontaneous locomotion using actometer (Ugo Basile), also locomotion, searching and emotional behavior using Open-field setting with registration of walking across the center and frequency of prolonged grooming (6-8 seconds). We tested the influence on the coordination of gaits using Rota-rod setting. We evaluated the presence and severity of nonspecific reactions of CNS and neuromuscular hyperactivation including tremor, convulsions, pyloerrection, Streub's symptom, salivation, deflection of time and power of reflexes (ipsilateral leg flexion, corneal and aurical reflexes), and muscle tonus. We tested the changes in rectal temperature, the presence of ambivalence, vocalization and passivity. The methods are referred to the General Russian manuscript for the preclinical investigation of the new medical products under the redaction of AN Mironov, 2012. Early we determined that RU-1205 has no significant effect on breathing and gut motility [1], and we expelled this test from the Experimental schedule and templates. The symptoms were characterized in absolute values and semi-quantitative visual analog scale (VAS, each 25% of visual alteration were respective by one score, evolved by Irwin S. in 1968).

Statistical methods

Statistical analysis was completed using the Kruscal-Walles test (Dunns post-test, <0.05), ANOVA (Tukey post-test, $p<0.05$) and nonlinear and linear regression. When choosing a statistical criterion, we estimate the normality using Kolmogorov-Smirnov test and if it is normal we used ANOVA, if not we used Kruscal-Walles test. The spread of mean values submitted as the standart error of mean (SEM) in all cases.

RESULTS AND DISCUSSION

Comparison of analgesic activities of RU-1205 and butorphanol using the hot-plate test

There were 8.25 ± 0.30 seconds of nociception latency (NL) in non-treated mice. We investigated that in animals, treated with RU-1205 60 minutes before the testing, mean NL level was expectedly greater than in control. It was a dose-depended growing of NL in 0.001-1 mg/kg dose range. At dose-point 1 mg/kg the mean NP level was at 2.7 times greater than in control. However, when doses are about 10-100 mg/kg, we observed the decreasing of NL level before at 1.8 times greater than in control. At the same time the activity peak of butorphanol was observed at dose 10 mg/kg with the full falling of activity when doses are higher (fig.1). Butorphanol makes a peak of action at 10 mg/kg, and RU-1205 – 1 mg/kg – at ten times less, than butorphanol.

Comparison of non-speciphic Neuropsychotropic Action of RU-1205 and Butorphanol

Our open-field setting was consisted of circle floor with standard perpendicular lines for the locomotion registration. In the cross-points of lines there were grooves with 1.5 cm diameter for the animal detection. It is interesting for "searching something", which can demonstrate the "searching" activity. In locomotion testing we determined, that the mean of crossing-line activity was 15.0 ± 5.8 in non-treated animals. Locomotor activity in the open-field is one of the aspects of nonspecific neuronal oppression. (Figure 2) demonstrates the locomotor activity datas for both compounds. We determined the hypolocomotion-free coefficient (HFC) for both compounds, using a formula: ED_{50} (hypolocomotion)/ ED_{50} (analgesic). Analgesic ED_{50} are 0.02 and 0.2 mg/kg, and hypolocomotion ED_{50} are 1.3 and 1.1 mg/kg for RU-1205 and butorphanol respectively. HFC are 5.5 for butorphanol and 67.0 for RU-1205. So, the safety of RU-1205 is at 11 times higher, than safety of butorphanol for this type of reaction. In the "searching" activity test the number of peeping was 1.4 ± 0.5 in control. We calculated AUC from the graphs of the dependence "dose-number of peepings" for both compounds. The summary AUC was at two times less in butorphanol, than in RU-1205 (Figure 3). "Searching" and locomotion activities are the aspects of detecting of mood disorders when the doses are little and the sign of spreading neuronal oppression when doses are great. In the behavioral testing of the emotional component in the open-field (walking across the center and prolonged grooming) we observed the significant the significant frequency growing of

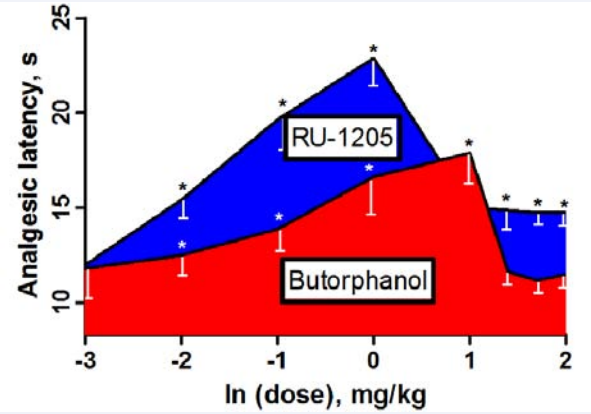

Figure 1 The dependence of mean NL at hot-plate test from dose of RU-1205 or butorphanol (n=8 for each point).

* - statistically significant differences with control group (with 100%), ANOVA, post-test Tukey, p≤0.05

The summary AUC of RU-1205 compound is 45.05, in comparison, the summary AUC of butorphanol is 29.32 (summary AUC was calculated using formula: AUC(RU-1205)-AUC(Control)). The AUC range from the minimal doses to the activity peak of RU-1205 demonstrates the growing of the analgesic potency (27.72 for RU-1205 compound and 12.88 for butorphanol). Minimal value at the "analgesic latency" axis is the base line of analgesic sensitivity in control (8.25 seconds).

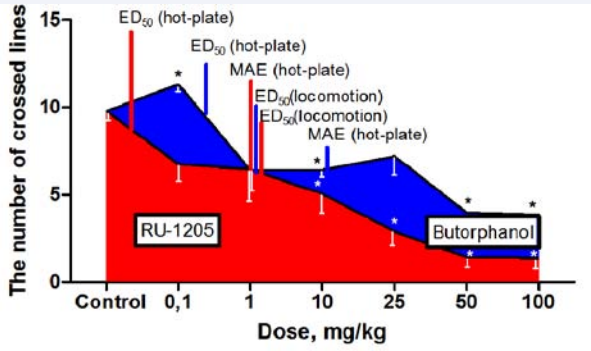

Figure 2 Comparison of crossing-line activity (open-field) of mice treated with RU-1205 and butorphanol (n=8 for each point).

* - statistically significant differences with control group (with 100%), ANOVA, post-test Tukey, p≤0.05.

Vertical red and blue lines are showing the points of analgesic or locomotion ED50, or maximal analgesic effect (MAE). Red lines: RU-1205, blue lines: butorphanol.

There is a little growing of mean locomotion activity in the «blue» graph (set of butorphanol datas) in the smallest dose, but then it's falling when doses are higher. In the «red» array (RU-1205), there is dose-dependend falling of number of crossed lines.

walking across the center and prolonged grooming in comparison with the control group. We found out that is happens in about 2.4 and 0.7 times respectively at the doses 0.1-1 mg/kg of RU-1205 (Kruscal-Walles test, Dunns post-test, p ≤ 0.05). But when doses of RU-1205 were greater these indicators were loss. So it can be associated with Hypolocomotion. There were no grooving of the prolonged grooming duration and frequency of walking across the center when treated with butorphanol, but the loss of the parameters was equal with RU-1205 when the doses were greater. There were no spontaneous vocalizations in all groups, treated with RU-1205 or butorphanol, and moderate ambivalence was detected in the 20% animals, treated with highest doses of both compounds. In the rota-rod test all mice were trained previously for the holding on the rod for 3 minutes, and all control mice were stayed. Dose range 0.1-25 mg/kg of RU-1205 and butorphanol was significantly ineffective. We set that RU-1205 caused little more deep oppression of the staying on the rod, but significant oppressional effect of both compounds was determined only in dose 25 mg/kg (Figure 4 A & B). When evaluated the neuromuscular hyperexcitability there were no specific signs of it (tremor, cramps, seizures, Schtraubs symptom) in all groups of animals in 3 hours of observation. When the reflexion activity was tested (corneal, aurical and ipsilateral leg flexion reflexes), we observed, that RU-1205 can provide reflex oppression in doses 50 mg/kg and more. Oppression of ipsilateral reflex was detected from 50 mg/kg of RU-1205; oppression of aurical was only when the dose reached 100 mg/kg. Both reflexes are not recovered in 3 hours after the injection of RU-1205. Corneal reflex was normal in all animals. Butorphanol was not tested in this set. There were unexpected results of the rectal temperature testing. In the low and moderately-high doses of RU-1205 (0.1-25 mg/kg), there were no statistically significant deflections of temperature. When the doses were greater, significant loss of the values provoked. At the 50 mg/kg the mean loss of temperature was around 2°C, at the 100 mg/kg it was about 5°C (Table 1). Temperature was not recovered within 3 hours of observation. But there were no significant temperature loss in all doses in animals, treated with butorphanol; there were no significant rectal temperature loss in all doses. Previously similar results have already been obtained for kappa-opioid agonists [8]. Why that did it was unexpected? Early we demonstrated that RU-1205 has no affect the basic vital functions such as breathing or gut motility [1]. There are particularly or fully central regulated functions. It is typical situation if kappa-opioid agent causes hypothermia, mild or moderate affecting of tidal volume (without respiratory depression and serious partial blood V (O_2) lose), slight miorelaxation or mild motor discoordination, aversion and depression signs in the animal testing. However, in case of

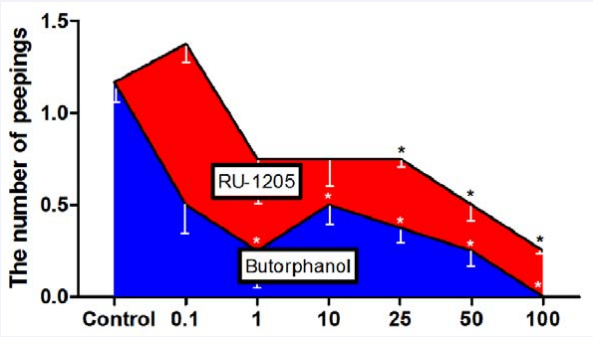

Figure 3 The «searching» activity of mice, treated with RU-1205 (AUC=4.83) or butorphanol (AUC=2.46) (n=8 for each point).

* - statistically significant differences with control group (with 100%), ANOVA, post-test Tukey, p≤0.05. Significant differences were determined from dose 25 mg/kg of RU-1205, and 1 mg/kg of butorphanol before the highest dose of each compound.

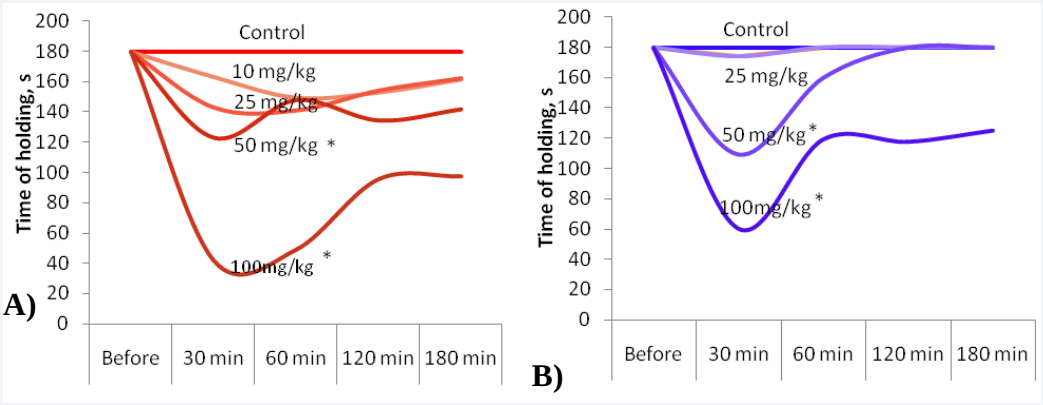

Figure 4 (A) – The activity of RU-1205 in the Rota-Rod test. (B) - The activity of butorphanol in the Rota-Rod test (n=8 for each point).
* - statistical significant, ANOVA, post-test Tukey, p<0.05.

Table 1: Results of the rectal temperature measurement after treating with RU-1205 or butorphanol (Δ %) (n=8 for each dose and control).

Group	RU-1205	Butorphanol
0.1 mg/kg	100.5±1.1	99.2±1.8
1 mg/kg	101.1±3.0	99.2±1.5
10 mg/kg	97.8±0.9	99.7±0.7
25 mg/kg	96.8±2.4	100.3±3.1
50 mg/kg	92.7±0.7*	99.5±0.8
100 mg/kg	87.6±0.3*	100.0±2.2

* - statistically significant differences with control group (with 100%), ANOVA, post-test Tukey, p≤0.05

Table 2: Semi-quantitative presentation of investigated results for RU-1205/butorphanol.

Functional part of CNS		Dose, mg/kg					
		0.1	1	10	25	50	100
Full locomotion	Crossing-line	N/N	N/N	0-↓/N	↓/↓	↓/↓	↓/↓
	Searching	N/N	0-↑/0-↓	N/↓	↓/↓	↓/↓	↓/↓
Neuro-muscular hyperexcitability, coordination, tonus	Tremor	N/N	N/N	N/N	N/N	N/N	N/N
	Seizures	N/N	N/N	N/N	N/N	N/N	N/N
	Schtraub symptom	N/N	N/N	N/N	N/N	N/N	N/N
	Rota-Rod	N/N	N/N	0-↓/N	↓/0-↓	↓/↓	↓/↓
Reflexes	Ipsilateral leg flexion	N/N.A.	N/N.A.	N/N.A.	N/N.A.	↓/N.A.	↓/N.A.
	Aurical	N/N.A.	N/N.A.	N/N.A.	N/N.A.	N/N.A.	↓/N.A.
	Corneal	N/N.A.	N/N.A.	N/N.A.	N/N.A.	N/N.A.	N/N.A.
Vegetative reactions	Rectal temperature	N/N	N/N	0-↓/N	0-↓/N	↓/N	↓/N
	Exophtalmus	N/N	N/N	N/N	N/N	N/N	N/N
	Ptosis	N/N	N/N	N/N	N/N	N/N	N/N
	Piloerrection	N/N	N/N	N/N	N/N	N/N	N/N
	Hypersalivation	N/N	N/N	N/N	N/N	N/N	N/N
	Skin color and condition	N/N	N/N	N/N	N/N	N/N	N/N
Emotional condition	Prolonged grooming	0-↑/N	↑/N	0-↑/0-↓	N/↓	↓/↓	↓/↓
	Walking across the center	0-↑/N	0-↑/N	N/N	N/0-↓	↓/↓	↓/↓
	Spontaneous vocalisation	N/N	N/N	N/N	N/N	N/N	N/N
	Ambivalency	N/N	N/N	N/N	N/N	N/N	0-↑/0-↑

Abbreviations: «N» normal feature, **0-↓** mild oppression, «↓» moderate oppression, **0-↑** mild growth, «↑» moderate growth, N.A. - not applicable.

RU-1205 there is only hypothermic reaction, except other vital or behavior function alterations. Different studies suggest the hypothermic effect of kappa opioid receptor agonists is modulated by a number of neurotransmitter systems, including glutamate, serotonin, dopamine and norepinephrine and G-protein-gated potassium (GIRK) channels, associated with kappa-opioid receptors [9]. The same neuromediator systems are include in the other adverse consequences of kappa-opioid agonists using. But only hypothermia was manifested. It is possible that RU-1205 is effected like biased kappa-opioid agonist producing analgesic effect without aversion and alteration of breathing [10-12], but different vital ganglions such as respiratory center and thermoregulation center are has different mechanisms of transmitter and second messenger regulation. There were no expressed ptosis, exophthalmos, hypersalivation, piloerrection and no visual abnormalities in the color or condition of skin in animals, treated with RU-1205 or butrophanol. There is a good sign of absence of serious vegetative disruptions.

The summary of the testing results for RU-1205 and butorphanol are submitted in the (Table 2). Thus, we assumed that RU-1205 is safer than butorphanol in terms of serious neurological adverse reactions. So, this easy primary observation tests we used, help us to identify the preferable way to finding and studying the possible adverse effects of RU-1205 compound.

CONCLUSION

We found out that RU-1205 has lower neurotoxicological potential, than well known partial opioid agonist butorphanol. We consider that there were no serious neurologic, behavioral or vegetative adverce reactions in RU-1205 0.1-50 mg/kg treated mice, except mild hypothermia and coordination loss (which are still less pronounced than in butorphanol treated animals due to higher analgesic activity of RU-1205). When the greater doses there were symptoms of nonspecific CNS oppression with greater doses, such as hyporeflexia and Hypolocomotion in both groups of treatment both in RU-1205 and butorphanol treated mice. We assumed that the potency of RU-1205 to produce antinociception is greater than the potency to produce nonspecific neurotoxicological adverse effects in comparison with butorphanol. In addition, signs of weak anxiolytic actions were determined for low doses of RU-1205 compound.

REFERENCES

1. Grechko OY, Litvinov RA, Spasov AA, Rashchenko AI, Shtareva DM, Anisimova VA, et al. Study of μ- and δ-Opioid Activities in Agents with Various κ-Receptor Selectivity. Bull Exp Biol Med. 2017; 162: 632-635.
2. Grechko OY, Eliseeva NV, Spasov AA, Litvinov RA. Analgetic Activity of a Benzimidazole Derivative on Models of Inflammatory Pain. J Volg SMU. 2016; 58: 101-103.
3. Spasov AA, Grechko OY, Shtareva DM, Rashchenko AI. Study of the interaction of RU-1205 with neurotransmitter analyzers. J Volg SMU. 2014; 50: 120-122.
4. Bruchas MR, Land BB, Aita M, Xu M, Barot SK, Li S, et al. Stress-Induced p38 Mitogen-Activated Protein Kinase Activation Mediates κ-Opioid-Dependent Dysphoria. J Neurosci. 2007; 27: 11614-11623.
5. Pasternak GW. Opiate receptors. NJ: Humana Press, 2011; 497.
6. Cahill CM, Taylor AM, Cook C, Ong E, Morón JA, Evans CJ. Does the kappa opioid receptor system contribute to pain aversion? Front Pharmacol. 2014; 5: 253.
7. Espejo EF, Mir D. Structure of the rat's behaviour in the hot plate test. Behav Brain Res. 1993; 56, 171-176.
8. Handler CM, Geller EB, Adler MW. Effect of mu-, kappa-, and delta-selective opioid agonists on thermoregulation in the rat. Pharmacol Biochem Behav. 1992; 43:1209-1216.
9. Rawls SM, Benamar K. Opioid, cannabinoid, and transient receptor potential (TRP) systems: effects on body temperature. Front Biosci (Schol Ed). 2011; 3: 822-845.
10. Brust TF, Morgenweck J, Kim SA, Rose JH. Biased agonists of the kappa opioid receptor suppress pain and itch without causing sedation or dysphoria. Sci Signal. 2016; 9: 117.
11. Bruchas MR, Land BB, Aita M, Xu M, Barot SK, Li S, et al. Stress-induced p38 mitogen-activated protein kinase activation mediates κ-opioid-dependent dysphoria. J Neurosci. 2007; 27: 11614-11623.
12. Ehrich JM, Messinger DI, Knakal CR, Kuhar JR, Schattauer SS, Bruchas MR, et al. Kappa Opioid Receptor-Induced Aversion Requires p38 MAPK Activation in VTA Dopamine Neurons. J Neurosci. 2015; 35: 12917-12931.
13. Minervini V, Dahal S, France CP. Behavioral Characterization of κ Opioid Receptor Agonist Spiradoline and Cannabinoid Receptor Agonist CP55940 Mixtures in Rats. J Pharmacol Exp Ther. 2017; 360: 280-287.

Cite this article

Spasov AA, Grechko OY, Eliseeva NV, Litvinov RA, Shamshina DD (2017) Toxic Effect of Single Treatment with Kappa-Opioid Agonist, Ru-1205 Compound, on the Neurological Status of Wild Type Mice. J Clin Pharm 3(1): 1014.