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Intravenous intralipid emulsion treatment of acute tramadol and haloperidol toxicities worsen all Behavioural responses of albino rats possibly via decreasing serum serotonin level

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Abstract

Many studies were designed to assess intralipid emulsion (ILE) efficacy on acute lipophilic drug toxicities. This study was to assess behavioural responses of albino rats towards intravenous ILE treatment of acute tramadol and haloperidol toxicities in relation to serum serotonin level. 60 male adult albino rats divided into four equal groups in 8 days study, the first one administered tramadol 250mg/ kgB.W orally once. The second group received Tramadol 250mg/ kgB.W orally plus 10ml/ kgB.W intravenous ILE once. The third group received intraperitoneal(IP)haloperidol (2.5 mg/Kg) once, while the fourth group received haloperidol (2.5 mg/Kg) IP plus 10ml/ kgB.W IV ILE once behavioural changes were recorded immediately and one week after treatment. Serum was collected for serotonin level estimation. Intravenous ILE treatment of tramadol, worsened movement activities, exploratory behaviour and non-active awakening but improved sleeping time. On haloperidol treatment group, ILE worsened movement activities and exploratory behaviour but improved rearing and increase non active awakening with no effect on sleeping. In addition, head twitch response significantly increased in tramadol+ILE compared to other groups. There was non-significant decrease in serotonin serum levels in tramadol+ILE group comparing to tramadol group, although, a significant ($p<0.05$) decrease in serotonin serum levels in haloperidol+ILE group comparing to haloperidol group. Treating tramadol and haloperidol acute toxicity with ILE worsened behavioral responses by decrease movement activities, exploratory behaviour, comfort behaviour and caused head-twitch response via decreasing serum serotonin levels.

Keywords: Behavioural responses, Acute Toxicity, head twitch response, intravenous lipid emulsion, serotonin.

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1. Introduction

One member of the butyrophenone family of injectable antipsychotics is haloperidol, or HA. According to Meyer-Massetti et al. (2010) [1], it is mostly used to treat schizophrenia and has a lipophilic character. Synthetic analgesic Tramadol hits μ-opioid receptors in the CNS with a high degree of selectivity. According to Pinho et al. (2013) [2], it is often used for the treatment of pain ranging from mild to severe. Acute antipsychotic drug poisoning and tramadol overdose are common causes of hospitalisations of intoxicated people, especially in Egypt and the Middle East [3,4]. Based on case reports and animal trials, intravenous lipid emulsion (ILE) has the potential to counteract lipophilic, harmful xenobiotics [5]. A lot of research has been done on how ILE affects the cardiovascular and hemodynamic systems, but not as much has been done on how it affects toxins that affect the nervous system [6,7]. Academics and public policy organisations have paid a lot of attention to behavioural findings during the last 20 years. There has been a meteoric rise in the amount of behavioural

science literature and policy publications [8]. Injecting rats or mice with serotonergic hallucinogens or other compounds that activate the 5HT2A receptors causes a quick and strong shaking of the head, which is known as the head-twitch response (HTR). With sufficient expertise, the answer could be readily apparent. A useful tools for performing behavioural examinations are the consistent observations made by various workers [9]. Researchers often use the behavioural test for 5HT2A activation to examine the connections between 5HT2A receptors and other systems of transmitters [10]. There has been a lack of research on behavioural responses to ILE toxicity therapy. Our goal was to see how albino rats reacted behaviorally to the acute toxicity of tramadol and haloperidol, with and without intravenous lipid emulsion treatment.

2. Materials and methods

2.1. Drugs

- Tramadol HCL in Tablet form: contains 225mg per tablet of tramadol HCL; purchased it from Illustrious Company of Pharma.
- Haloperidol in ampoule form in a conc. of 5mg/ml was purchased from the Nile pharmaceutical Company in Egypt.
- Intravenous drips of lipid emulsion (ILE): 500 ml of SMOF lipid TM at a conc. of 20% injectable white homogeneous emulsion, purchased from Fresenius Kabi Company cited in Austria. It is composed of 6% soybean oil plus 6% medium chain TAGs plus 5% olive oil and 3% fish oil.

2.2. Animals

A research was done on sixty male Wistar albino rats weighing between 150-200 g. The rats were kept in a well-ventilated room and given a week to acclimatize to the new environment before the experiment began. The rats were maintained under controlled conditions with a temperature of 21 ± 2 °C, a relative humidity of 45 ± 5%, and a light-dark cycle of 12 hours of light followed by 12 hours of darkness. The feed may be consumed twice daily at no cost, using a commercially available balanced diet. Following the acclimatization phase, the rats were randomly allocated into four groups of similar size (15 rats each). The first group was administered a single oral dosage of tramadol HCL at a concentration of 250mg/kg body weight by oral gavage. The second group was given a single oral dosage of tramadol HCL at a concentration of 250mg/kg body weight. After thirty minutes, they got a single intravenous dose of ILE at a concentration of 10ml/kg body weight, which stands for intravenous lipid emulsion. The third group was administered a single intraperitoneal injection of haloperidol at a dosage of 2.5 mg/kg. Meanwhile, the fourth group got a combination of a single intraperitoneal injection of haloperidol at a dosage of 2.5 mg/kg, followed by an intravenous infusion of 10 ml/kgB.W of ILE after thirty minutes.

2.3. Ethical statement

The experiment was conducted following the principles and procedures outlined by the local research Ethical Committee (approval number 022-408) for the care and use of lab animals in Beni-Suef University, Egypt.

2.4. Serotonin level

Following the process of observation and treatment, blood samples were obtained from the tail vein using clean glass tubes containing heparin to assess the levels of serotonin in the serum. Each animal that died during the observations had the same process, which included taking blood by heart puncture. This ELISA kit is designed for the precise measurement of ST/5-HT concentrations in serum, plasma, and other biological fluids obtained from Elabscience® (USA) by in vitro methods.

2.5. Behavioural observation

The rat was identified via a colour tagging process, in which a special non-toxic dye was put to its back. The animals were separated into two groups, with each group having two identical copies. The behaviour of all rats that were marked was observed for duration of 15 minutes. This observation took place one week following the administration of the medication. The technique used for observation was continuous sampling, and a digital video camera was used for this purpose [11].

2.5.1. Head twitch response

The head twitches were quantitatively measured using the method described by Corne et al., (1963) [12]. This included evaluating the percentage of mice that exhibited at least one head-twitch (a quantal response) within 40 minutes after drug delivery. The test was repeated at the conclusion of the trial, namely 40 minutes after the final dosage was administered.

2.6. Statistical analysis

The resultant data are shown as the mean ± standard error (SE). The data were analysed using one-way ANOVA (analysis of variance). The user employs SPSS version 20, a statistical programme developed by IBM Corp. and launched in 2011. The specific version used is IBM SPSS Statistics for Windows Version 20.0, which is based in Armonk, NY and developed by IBM Corp. Tukey's test was used for the post-hoc analysis. The nonparametric statistical approach was used to compare heteroscedastic data, with significance being determined at $p < 0.05$. The Levene's test was used to assess the behavioural data for conformity to the assumption of homogeneity of variance.

3. Results and Discussions

Intravenous feeding and ILE's usage as a carrier for lipophilic medications to increase absorption are both approved by the Food and Drug Administration (FDA). ILE has been shown to be a successful antidote in studies that have tested it both alone and in combination with other drugs [13]. Overdose deaths and opioid use, especially tramadol, have been on the increase throughout the Middle East. Antipsychotic drugs have also been shown to cause this rise [14]. A tramadol overdose may result in the development of SS, which is characterized by increased serotonergic activity. As per Takeshita and Litzinger (2009) [15], SS symptoms include agitation, heightened reflexes, altered mental status, dilated pupils, and tremors. The purpose of this study was to investigate the relationship between blood serotonin levels and behavioural responses to acute haloperidol and tramadol toxicities as a result of ILE treatment (Table 1).

Table 1: Effect of tramadol and haloperidol injection with or without IL on rat behavior

Groups	Behavior										
	Movement activities and exploratory behaviour					Body care		Comfort behaviour			
	Locomotion	Digging	Exploration	Sniffing	Rearing	Grooming (F)	Grooming (D)sec.	Sleeping (F)	Sleeping (D)	Awake non-active (F)	Awake non-active (D)
Tramadol	89.67±30.88[a]	0.0±0.0	1.0±1.0	15.33±5.84[a]	20.33±3.53[a]	7.67±3.38	26.0±1.0[a]	0.0±0.0	0.0±0.0	2.67±1.76	144.67±122.86
Tramadol then ILE	14.25±10.36	0.0±0.0	0.0±0.0	2.0±1.35[b]	0.0±0.0[b]	0.0±0.0	0.0±0.0[b]	0.5±0.29	47.25±39.95	5.0±1.47	622.5±168.37
Haloperidol	4.5±3.57[b]	1.25±1.25	0.0±0.0	14.75±8.01	0.25±0.25[b]	1.5±0.87	7.25±4.96	0.0±0.0	0.0±0.0	1.75±0.25	790.25±41.01
Haloperidol then ILE	3.5±2.6[b]	0.0±0.0	0.0±0.0	6.25±5.30	0.75±0.48	0.5±0.5	1.5±1.5	0.0±0.0	0.0±0.0	2.5±0.96	831.75±44.09
P value	P<0.05	NS	NS	P<0.05	P<0.05	NS	P<0.05	NS	NS	NS	NS

Results are expressed as mean ± SE

Table 2: Head-twitch response in tramadol and haloperidol with or without IL injected rat

Groups	Head twitch%	
	After first injection	After week of injection
tramadol	0.0	0.0
tramadol+ILE	16.6	0.0
haloperidol	0.0	0.0
haloperidol+ILE	0.0	0.0

Results are expressed as percent

Table 3: Effect of lipid emulsion on Serotonin level after acute tramadol and haloperidol poisoning in different groups of animals:

Parameter / Groups	Serotonin
Tramadol	34.36±7.9[b]
Tramadol +ILE	28.50±1.74[b]
Haloperidol	47.71±11.16[a]
Haloperidol +ILE	20.91±2.18[b]
Significance	P<0.05

Results expressed as mean ±SE

Our study's findings demonstrated that neither the tramadol nor the tramadol+ILE groups saw a statistically significant reduction in blood serotonin levels. Nevertheless, when comparing the haloperidol+ILE group to the haloperidol group, we found that serotonin serum levels were significantly lower ($p<0.01$). In 2011, Lin LCh et al. [16] discovered that 5HT levels in brain tissue homogenates were significantly reduced after 21 days of haloperidol injections. The competitive medicine haloperidol influences the neurotransmitters known as catecholamines in the brain's central and peripheral areas. According to Gajre et al. (2012) [17], it blocks a variety of receptors, including those for serotonin and muscarinic, as well as D1, D2, H1, H2, alpha 1, and alpha 2 receptors. A recent study by Saleem et al. (2021) [18] found that haloperidol lowered serotonin levels. In order to alleviate pain, tramadol HCL blocks the reabsorption of serotonin, which increases brain serotonin levels [19]. After administering ILE to young, healthy men, Sondermeijer et al. (2012) [20] found that their serotonergic response decreased. Aviram and Deckelbaum (1989) [21] found a reduction in serotonin release in their inquiry on the effects of intralipid infusion on human platelets. Elevated blood levels of FFAs theoretically could reduce the central serotonergic system's reactivity. Plasma FFA go up after ILE delivery, but it's not clear why the serotonin response is slowed down in these situations [20]. The group that was given haloperidol had less movement, according to the examination of behaviour patterns. Consistent with this result is the research of Bernardi et al. (1981) [22], which found that mice given 2.5 mg/kg of haloperidol once had fewer frequent bouts of movement. Arruda et al. (2008)23 stated that the control of locomotor activity is significantly influenced by dopaminergic pathways. The mesocortical, mesostriatal, mesolimbic, and tuberoinfundibular pathways are the main ways that DA gets into the brain, which may help explain this result [22]. But because of its higher relationship with motor function, the mesostriatal system is the best alternative (Table 2). According to the data in the table, the group given haloperidol had significantly shorter grooming sessions and less frequent grooming sessions overall. Bernardi et al. (1981) [22] found that grooming time increased on days 3, 5, and 6 following haloperidol injection, whereas our results show the opposite. Metzger et al. (2007) [24] found that rats' behaviour was significantly suppressed after receiving a haloperidol implant. The haloperidol group showed much less movement than the control group, according to Saleem et al. (2021) [18]. The effects of tramadol on locomotion behaviour were similar to those of the control group, according to research by Antiorio et al. (2022) [25]. Examining how tramadol affected the serotonergic system was done by keeping an eye on the HTR. Based on our results, the only group that did not experience HTR was the tramadol+ILE group. In the absence of serotonin, no head twitch response was seen in the group of mice treated with tramadol, which was in agreement with the results of the research by Cha et al. (2014) [26]. Like morphine, tramadol attenuated 5-HTP-induced head twitches dose-dependently (Table 3).

4. Conclusions

Serotonin levels, movement activities, exploratory behaviour, body care behaviour, and comfort behaviour were all reduced in the tramadol and haloperidol groups after ILE administration. The injection of tramadol with ILE, on the other hand, increased the reaction to head twitching. Reductions in movement activities, exploratory behaviour, body care, and comfort behaviour were seen when ILE was administered to treat acute toxicity caused by tramadol and haloperidol. A decrease in serum serotonin levels occurred along with these alterations. A higher head-twitch response was seen in patients with tramadol intoxication who received ILE. Studying for a longer period of time is recommended.

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