Imbalances of key parameters needed for energy-yielding processes in the serum of tramadol-treated male rats

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ABSTRACT

Background: Addiction is one of the major health challenges in the world. Tramadol is an analgesic that has been widely abused, causing negative health consequences. This study sheds light on the effect of the administration of tramadol hydrochloride (HCl) on the serum levels of key parameters needed for energy production. Male Wistar rats received oral administration of tramadol HCl for 30 days and then were sacrificed to collect blood from the eyes and determine the levels of nutritional measures in the serum by kits. Our findings showed that the daily oral gavage of 50 or 100 mg/kg tramadol HCl for a month decreased the serum levels of ferrous iron and glucose significantly compared to the nontreated rats. Additionally, both doses increased the levels of total protein, triglycerides, and magnesium compared to the nontreated animals. Only the dose of 50 mg/kg tramadol HCl was effective in elevating the serum concentration of vitamin B12. Moreover, tramadol HCl treatment did not trigger any change in the levels of zinc in comparison to the control group. In conclusion, tramadol causes imbalances of key parameters needed for energy production.

INTRODUCTION
Opioids belong to a class of drugs that produce beneficial effects, such as gastric slowing, cough suppression, and analgesia (Schaefer et al., 2007). However, opioid addiction has become a major health problem all over the world. Tramadol is a synthetic analgesic opioid that acts as a weak agonist for μ-opioid receptors (Gillen et al., 2000). Several lines of evidence highlighted that the tramadol exhibits antidepressant effects (Rojas-Corrales et al., 1998). Also, tramadol can produce adverse effects that vary based on several factors. In a case study reported by Osman and Mustafa (2018), it was noted that the prescription of 50 mg tramadol to a female with no psychiatric history was correlated with an elevation in the mood accompanied with acceleration in the flow of speech when prescribed, albeit such a case is rare. Furthermore, it was noted that tramadol overdose causes serotonin syndrome due to its ability to block the reuptake of serotonin (Tashakori and Afshari, 2010).

Despite the fact that the tramadol has low potential to cause addiction compared to other opioids, it is considered as the third abused opioid and results in the symptoms of opioid withdrawal in several cases, even in patients with no history of addiction (De Conno et al., 2005; Pollice et al., 2008). A 61-year-old patient described that she was very agitated if she skipped or delayed tramadol and that the nervousness disappeared after tramadol intake (Pollice et al., 2008). Additionally, several reports showed that the intravenous administration of tramadol causes seizures (Raiger et al., 2012). It is widely accepted that addiction affects the metabolism of biochemical parameters that take part in energy production and fatigue. Thus, the effect of the chronic administration of tramadol on several biochemical parameters was explored in this study.

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Importantly, the ability of tramadol (similar to other opioids) to cross the blood-brain barrier (BBB) contributes to its benefits and risks (Scharf et al., 2017). Several researches reviewed by Tardy et al. (2020) addressed the role of many vitamins and minerals in energy-yielding processes, oxygen ($O_2$) transport, and brain and muscle functions. Thus, these vitamins and minerals are considered significant contributors to fatigue (a sense of energy depletion) in its physical and mental forms (Ryan and Frederick, 1997). Accumulating lines of evidence demonstrated that the brain consumes 20% of the energy produced from glucose (Herculano-Houzel, 2011). Cellular energy production has many steps starting from the oxidation of macronutrients into acetyl coenzyme A, leading to the generation of pyruvate from glucose followed by the citric acid cycle (CAC) (Alberts et al., 2017). The CAC produces flavin adenine dinucleotide (FADH$_2$) and dihydronicotinamide adenine dinucleotide (NADH) via oxidative processes that require several vitamins and minerals, including vitamin B12, Fe, and magnesium ($Mg^{2+}$) (Alberts et al., 2017). The final process includes the transfer of electrons from FADH$_2$ and NADH electron carriers to the electron transport chain to generate adenosine triphosphate (ATP), a process that also needs some vitamins and minerals such as ferrous (Fe) (Alberts et al., 2017). Notably, triglycerides (TG) contribute to the transfer of blood glucose from the liver (Alves-Bezerra and Cohen, 2017). Besides, $Mg^{2+}$ has a major role in energy production and utilization by forming a complex with ATP (Yamanaka et al., 2016). $Mg^{2+}$ is also needed as a cofactor for kinases in the glycolysis process, in CAC and ATP export from mitochondria to the cytosol (Yamanaka et al., 2016). $Mg^{2+}$ affects muscle strength, cardiorespiratory functions, and neuromuscular performance (Zhang et al., 2017). Another important parameter that contributes to energy production or depletion (fatigue) is the availability of $O_2$ for the brain and muscles as well as the availability of adequate amounts of vitamins and minerals needed for its availability (Tardy et al., 2020). It is worth mentioning that iron in the Fe$^{2+}$ state can bind to the porphyrin ring of heme in cytochromes (the electron carriers needed for energy production), allowing the reversible binding of $O_2$ (Aggett, 2012). In addition, Fe is crucial for the $O_2$ transport in the myoglobin heme protein (Aggett, 2012). Thus, the deficiency of Fe reduces $O_2$ transport and supply to blood and muscles, leading to impairment in the energetic efficiency (Aggett, 2012). In the context of the effect of Fe deficiency on neuronal fatigue, Munoz and Humeres (2012) provided a solid basis for the effect of Fe deficiency on the neurotransmitter system. Also, Fe deficiency can lead to a decrease in the activity of Fe-containing enzymes and $O_2$ delivery to different tissues and thus to fatigue (Yokoi and Konomi, 2017). Additionally, it was noted that vitamin B12 is involved in DNA synthesis, activation of folate, the production of red blood cells, and $O_2$ delivery (Hoffbrand and Jackson, 1993). Moreover, several lines of evidence revealed the antioxidative properties of zinc (Zn), which is a cofactor for several enzymes that take part in energy production (Holt et al., 2012; Mezzaroba et al., 2019). Another aspect that can be considered in energy production is the protein levels. Proteins are fundamental for structural integrity and important biochemical reactions (Tóthová et al., 2018). Proteins have roles in homeostasis, coagulation, energy production, enzymes activity, platelet adhesion, and aggregation (Tóthová et al., 2018). Despite that, the increase in serum protein concentration indicates diseases and health disorders in the body (Anderson and Anderson, 2002).

Aim of the study

In this study, we tested the effect of the oral administration of tramadol hydrochloride (HCl) on serum levels of some biochemical parameters that play key roles in several processes, including energy production and $O_2$ transport. In more detail, we measured the levels of Fe$^{2+}$, glucose, vitamin B12 (cobalamin), total protein (TP), TG, Zn, and $Mg^{2+}$ in rats.

Ethical approval

All experiments were approved by the Animal Ethics Committee at the Applied Science Private University, Amman, Jordan (Ethical approval no. 2021-PHA-11).

MATERIALS AND METHODS

Materials

Capsules of tramadol HCl were purchased from Standardarzneimittel Deutscher Apotheker (Italy), while diethyl ether was from Scharlau, Barcelona, Spain. Kits for measuring the levels of Fe$^{2+}$, Mg$^{2+}$, glucose, TP, TG, and Zn were brought from Abbott Diagnostics (Milano, Italy). The vitamin B12 kit was provided by DiaSorin (Via Crescentino, Italy). Syringes and needles were from Thermo Fisher Scientific, Waltham.

Methods

Animals

Male Wistar rats (150–200 g) were used in this study. The animals were kept at the Animal House of the Applied Science Private University, Amman, Jordan, in a temperature-controlled environment (at 22°C ± 1°C) with a 12/12 hours light-dark cycle. Food pellets and water were provided ad libitum. All experiments were approved by the Animal Ethics Committee at the Applied Science Private University, Amman, Jordan (Ethical Approval number no. 2021-PHA-11).

Treatment of animals

Before starting the experiment, the animals were allowed to acclimatize to the conditions of the experimental room. The animals were divided into control and experimental groups ($n = 8$ in each group). All animals were weighed to ensure the accuracy of the administered doses. Tramadol HCl capsules (100 mg each) were finely ground into a powder using a mortar and pestle and then were dissolved in normal saline to make a stock solution. Tramadol HCl was freshly prepared on the day of treatment. The control group received oral gavage of normal saline, while the experimental groups received 50 or 100 mg/kg tramadol HCl orally. The oral treatments were administered to the animals every day for a total period of 30 days pursuant to El-Gaafarawi (2006). No anesthesia was used during the treatment.

Blood collection and serum preparation

The animals were sacrificed under ether anesthesia after 30 days of receiving the oral gavage treatments to collect blood from the eyes. The skin around the eye of the rat was pulled with fingers. After that, a 25-gauge needle was fixed to a syringe and inserted at a 30° angle into the medial canthus of the eye until the
needed volume of blood was withdrawn. The blood was kept to settle for 30 minutes at room temperature to allow coagulation and separate the serum. Samples were then centrifuged at 1,300 g for 15 minutes. The supernatant was preserved at −80°C.

**Determining the levels of serum parameters**

On the day of conducting the biochemical analysis, all serum samples were removed from the deep freeze and were allowed to thaw. The levels of Fe²⁺, Mg²⁺, glucose, TP, TG, and Zn were assayed using the Architect i2000_S analyzer and kits from Abbott Diagnostics (Milano, Italy). Vitamin B12 was determined using the LIAISON vitamin B12 automated assay and kits from DiaSorin (Via Crescentino, Italy). All samples were analyzed according to the kit manufacturer’s instructions.

**Statistical analysis**

Data were presented as the mean ± standard error of the mean (SEM). The normality test was conducted for all groups using the Shapiro–Wilk test. The statistical significance of the difference between groups was assessed by one-way analysis of variance (ANOVA) followed by Dunnett’s test using GraphPad Prism version 7. \( P < 0.05 \) was considered to be significant. Graphs were created using GraphPad Prism version 7.

**RESULTS AND DISCUSSION**

It is widely accepted that addiction affects the metabolism of biochemical parameters that take part in energy production and fatigue (Karam et al., 2004). The effects of addiction vary based on the abused substance, duration of addiction, dose of substance, and type of species used in the experiments. In this study, we investigated the effect of the oral administration of tramadol HCl on different serum parameters in male rats. The results showed that daily oral gavage of 50 or 100 mg/kg tramadol HCl for a month caused noticeable and significant reductions in the levels of Fe²⁺ and glucose as presented in Figure 1a and b. In more detail, the oral treatment of 50 and 100 mg/kg tramadol HCl decreased the levels of Fe²⁺ in the serum by 73.31% and 65.54%, respectively. We chose to test Fe²⁺ as it is the form that binds to heme and ferritin (Lane et al., 2015). Additionally, the decrease in glucose levels reached 52.60% in the group that received 50 mg/kg tramadol HCl and 54.57% in the animals treated with 100 mg/kg tramadol HCl. Notably, only the dose of 50 mg/kg tramadol HCl increased the levels of vitamin B12 in the serum (887 ± 51.88 pg/ml) compared to the control group (699.1 ± 41.03 pg/ml), Figure 2a. On the other hand, the low and high doses of tramadol HCl augmented TP, TG, and Mg²⁺ serum levels significantly compared to the control group (Figure 2b–d). The dramatic and highest rise was found in the TG levels, whereby 115.1% and 96.99% increases were revealed in the animals treated with 50 and 100 mg/kg tramadol HCl, respectively. Notably, the administration of tramadol HCl had no effect on the serum levels of Zn compared to the control group nor on body weight difference (Figure 3a and b). It is noteworthy that the treated animals showed aggressive behavior, which was eliminated upon receiving the daily dose of tramadol.

As in other opioids, the main explanation for the results is that the effects produced by tramadol HCl were due to its ability to cross the BBB and to produce effects on the central nervous system (CNS), thus affecting the metabolic response of various vitamins and minerals in the body (Molina et al., 1994). In this regard, earlier reports indicated that normal Zn levels are important for the suppression of β-amylloid-induced neurotoxicity (Mezzaroba et al., 2019). Moreover, Fe is considered a crucial element for the anabolic processes in mitochondria and for binding to ferritin in heme (Lane et al., 2015). Previous studies reported that massive Fe depletion causes cell death, indicating the importance of Fe for cell survival (Lawn and Lane, 2013). Besides, Fe-starved cells tend to stop other biochemical pathways that rely on Fe as well as storage pathways (Duke University Medical Center, 2008). Importantly, the cells keep tight control for Fe levels, whereby an increase or decrease in its levels is detrimental (Lawn and Lane, 2013). Accordingly, the reduction of serum Fe²⁺ levels after tramadol HCl administration presented in this study suggests that several vital processes (e.g., energy-yielding processes) in the body were affected, leading to detrimental effects. Moreover, the decrease in glucose levels observed in this study can be due to a reshuffling mechanism from the use of Fe to glucose for energy production, a mechanism suggested to be activated during Fe starvation in the cells (Duke University Medical Center, 2008). In more detail, mitochondria that need Fe for its processes shut down energy production during Fe deficiency leading to the utilization of glucose outside mitochondria (Duke University Medical Center, 2008). Despite the fact that the reshuffling mechanism was identified in yeasts, the researchers stressed the fact that these results are predicted to be involved in humans due to conserved mechanisms (Duke University Medical Center, 2008). All of these factors can explain the feeling of tiredness and lethargy during Fe deficiency due to the correlation between Fe, glucose, and energy (Duke University Medical Center, 2008).

In addition, the hypoglycemic effect of tramadol HCl shown in the current study can be related to the decrease in the

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**Figure 1.** Tramadol HCl decreases Fe²⁺(a) and glucose (b) serum levels. Data presented as mean ± SEM, \( n = 8 \). *Significant compared to control group, \( p < 0.05 \). One way ANOVA followed by Dunnett’s test post-hoc test.
Hallucinations not detected.
can be a contributing factor to the increase in Mg^{2+} that can enhance glucose availability and decrease O_2 requirements. The role of Mg^{2+} is emphasized by the translocation of Mg^{2+} to the locations of energy production (Zhang et al., 2017). Also, it was noted that the levels of plasma Mg^{2+} decreased in alcohol abusers compared to healthy people (Nechifor, 2011). Additionally, the findings of this study proved that tramadol HCl treatment caused an increase in the levels of TG. It is well established that the brain uses glucose as a major energy fuel compared to muscles that utilize glycogen (Bordone et al., 2019). However, when glucose levels reduce, the brain can use alternative substrates for energy, such as medium-chain TG and other substrates (Bordone et al., 2019). Accordingly, the increase in the levels of TG after tramadol HCl treatment can be a compensatory mechanism for the decrease in glucose that is needed for energy production.

With respect to TP, it is noteworthy to mention that the serum TP measures the amount of albumin and globulin proteins. High levels of serum protein may indicate cancer, inflammation, chronic kidney disease, liver disease, or dehydration (Tóthová et al., 2018; Wu et al., 2019). As serum proteins include albumin and globulin, there are two possibilities for explaining the increase in their levels. The first possibility is the increase in albumin-to-globulin ratio, a matter that can refer to the occurrence of leukemia or underproduction of antibodies (Bushier, 1990). The other possibility includes high globulin levels compared to albumin, which may indicate the existence of an autoimmune disease, liver cirrhosis, nephrotic syndrome, multiple myeloma, gastrointestinal diseases, or internal parasitism (Diogenes et al., 2010; Tóthová et al., 2018; Wu et al., 2019).

Notably, the dose of 50 mg/kg tramadol HCl produced an increase in vitamin B12 levels that were not exhibited in the group treated with 100 mg/kg. In fact, by considering the Food and Drug Administration guidelines (2005) on converting the doses used in humans and animals, the dose of 50 mg/kg tramadol HCl used in rats is equivalent to the ones consumed by humans. On the other hand, although calcium (Ca^{2+}) and phosphorus (P) levels were not measured in this study, a change in their levels is expected as Mg^{2+} is involved in the active transport of Ca^{2+} and potassium across cell membranes (Kirkland et al., 2018). Also, phosphorus is involved in the phosphorylation of many sugars and proteins, activity of enzymes, intracellular storage of energy, and maintenance of pH (Heaney, 2012). Further investigation into the effect of tramadol HCl on other biochemical parameters is recommended in order to apply strategies that can help in decreasing fatigue among addicted people.

CONCLUSION

Taken together, the results of this study showed that oral gavage of tramadol HCl caused a reduction in the levels of Fe^{2+} and glucose accompanied by a noticeable enhancement effect for vitamin B12, TP, TG, and Mg^{2+} concentrations. Tramadol HCl treatment had no change in Zn levels. We strongly recommend a periodic nutritional follow-up for addicted people to avoid fatigue symptoms due to malnutrition. Based on the outcome of biochemical analysis, vitamin and mineral supplementations can contribute to a better health status for addicted people.

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CONFLICT OF INTEREST

The authors had no conflicts to declare.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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