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Biochemical alterations in adult male rat cerebellar cortex after tramadol administration

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Abstract

Tramadol is a artificial centrally acting analgesic mediator used for relieving restrained to unembellished pain as well as postoperative, gynecologic and obstetric pain. Aim of the work: To study the biochemical effects of tramadol on cerebellum of adult male albino rats. Methods: 12-rats were equally divided into two groups: Control group and Tramadol group. Biochemical approach for assessment of serum glutathione (GSH), catalase, malondhyed MDH and brain derived neurotrofic factor BDNF were done. This results thate Tramadol group showed degenerative biochemical changes on the cerebellum. Conclusion of the work is that tramadol had an obvious biochemical alterations on adult male cerebellum.

Keywords: Opiod, biochemical, BDNF and GSH.

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1. Introduction

Tramadol is a synthetically produced opioid and its specific chemical formula is:C_{16}- H_{25}- NO_2 (Carrillo-Munguía et al. 2015; Shah et al. 2013) ,structurally related to codeine and morphine. Tramadol is commercially accessible in the form of a racemic mix, and its core construction contains an aromatic benzene ring with 1,3 substitutions (i.e., methoxyl and a 2-((dimethylamino)methyl) cyclohexanol moiety—DMC), as revealed in Figure 1. Its pharmacological action is mediated by opioid mechanisms through binding to μ-opioid receptors as well as non-opioid mechanisms through inhibition of neuronal reuptake of serotonin (5-HT) and noradrenaline (Miotto, K.; et al., 2017). The widespread use of tramadol and its pharmacological properties raises the need for studies evaluating its effect on malformation risks. Endogenous opioid peptides and receptors are widely expressed by developing cerebellar cells. The involvement of opioids in cerebellar growth regulation has also been revealed by experimentally perturbing the endogenous opioid system (Hauser et al., 2003). Among the several tools which are used to investigate the physicochemical features of compounds are chemistry and theoretical chemistry (Deepa, P. et al., 2012).

The incrimination of tramadol as a drug causing congenital malformations when administered during pregnancy has also become an evolving concern (Källén and Reis, 2015).

1.1: Aim of the Work

The aim of this work is to clarify the possible effects of tramadol administration on the biochemical parameters of cerebellum of adult male albino rate receiving tramadol.

2. Material and Methods

2.1: Animals

This study was conducted in anatomy Department (the Faculty of Medicine), Minia University in Egypt. This work was done on 20 adult non-pregnant female Sprague Dawley rats weighting 200-250 gm, mating of females with males carried out by overnight housing approximately10 days. Pregnancy was detected by the presence of sperms in the vaginal smear by using microscope it was considered day 0 (D0) of gestation

On day 7 of pregnancy (D7), the pregnant female rats were randomly divided in to two groups and were of pathogenically free. The female rates were obtained from the animal house of the Faculty of Agriculture in Minia University from the laboratory animals growing center of the faculty. Rats were living in clean plastic cages and were housed under the standard laboratory conditions, in a controlled temperature average (24–30◦C), in a 12:12-h daylight/darkness cycle and allowed to be free to access the standard lab chow and water ad libitium. Animals were acclimatized for two weeks before the experiment start.

2.2: Chemicals

It was assumed orally through gavage at a dose of 50 mg/Kg/day of tramadol HCL (cis-2-(Dimethylaminomethyl)-1-(3 methoxyphenyl cyclohexanol hydrochloride), which remained accessible as Tamol®, 225 mg pills from Alkan-Pharm®, Egypt (Faria et al., 2017).

2.3: Experimental strategy

Twelve rats were haphazardly divided into two sets, each over six rats: Rats fed a typical laboratory food and water made included Group I, the control group. From the 7th day of pregnancy to day 21, rats in Group II (Tramadol group) received everyday shots of tramadol (50 mg/kg) by a gastric tube [6].

2.4: Animal sacrifice & tissue collection

Blood samples were taken from the tail veins of rats by wounding off the tip of the tail and mildly squeezing the tail to gather blood beforehand execution. The serum was detached from the cells after clot development by centrifugation and sent for chemical study. Rats were sacrificed by decapitation 21 days after labour.The tissues of the cerebellum were rapidly detached. The specimens of the second part were washed immediately with ice-cold saline, dried and stored at−80 ◦C for subsequent homogenization and biochemical assays for Malondialdehyde (MDA), reduced glutathione (GSH).

Tissue homogenates which is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, we sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000×g to get the supernatant.

3. Materials and Method

3.1: Biochemical study

The Minia University Faculty of Medicine's Pharmacology Sector achieved the laboratory examination.

3.2: Antioxidant stress marker

Rat glutathione (GSH) concentration was measured by means of an ELISA tackle (Catalog Number: GR 25 11) from Biodiagnostic, Gizza, in agreement with the manufacturer's commendations.

The measurement of glutathione (GSH) is based on the reduction of 5, 5 dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione to produce a yellow compound.

3.3: Lipid Peroxidation

Estimation of malondialdehyde (MDA) in the cerebellar tissue was carried out as previously described (Skrzydlewska et al., 2002). Optical densitywas measured at 586 nm after 1 h of incubation at 45°C (Molina-Jijón et al., 2011). Data were expressed as nmol MDA /mg protein calculated using the standard curves All the biochemical assays have been done in triplets.

3.4: Measurement of Inflammatory Markers

Blood samples were left to clot for 2 h and then centrifugation was conducted at 1000× *g* for 20 min to separate sera which were stored at −20 °C till being used. Assays of serum interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were achieved using commercially available enzyme linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) conferring to manufacturer's guidelines.

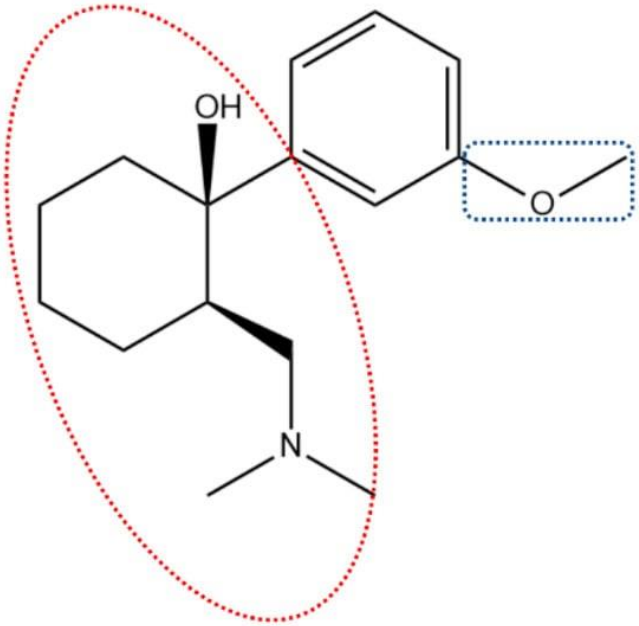

Figure 1. Tramadol chemical formulation show noteworthy moieties, namely: methoxyl unit in the blue dotted square and the tertiary amine-bearing DMC moiety in the red dotted ellipse.

Table 1: The mean values of Rat glutathione (GSH) (mmol/ml) in the studied groups (n=6).

Groups	Mean ± SEM	p-value
Control group	0.35± 0.02	
Tramadol group	0.12 ± 0.016	<0.0001 C*

SEM: standard error of mean, C: vs Con-group: significant at p<

Table 2: The mean values of rat Malondialdehyde (MDA) (mmol/g) in the studied groups (n=6)**.**

Groups	Mean ± SEM	p-value
Control-group	0.28± 0.018	
Tr-group	0.53 ± 0.04	< 0.0001 C* < 0.0001 Q*

SEM: standard error of mean, *: significant C: vs Con-group, Tr: vs Tr-group, at p<0.05.

Table 3 The mean values of rat interleukin-1 beta (IL-1β) (pg/mL), interleukin-6 (IL-6) (pg/mL) and tumor necrosis factor-alpha (TNF-α) (pg/mL in the studied groups (n=6)**.**

	Control Group	tramadol Group
IL-1β (pg/mL)	37.2 ± 7.6	123.6 ± 12.1 **
IL-6 (pg/mL)	18.4 ± 5.5	138.2 ± 5.7 **
TNF-α (pg/mL)	10.3 ± 1.9	27.2 ± 10.1 **

SEM: standard error of mean, *: significant C: vs Con-group, Tr: vs Tr-group, at p<0.05.

4. Results and Discussion

4.1: Laboratory findings

The typical levels of rat glutathione (GSH) in the various study groups were as follows (Table 1). The results of the statistical assessment of GSH levels crossways the groups discovered that the tramadol group's mean GSH values were significantly inferior than those of the Control group (P 0.0001).The mean values of rat Malondialdehyde (MDA) (mmol/g) in studied groups. Statistical analysis of MDA level among groups showed Tr-group showed a significant increase in the mean values of MDA compared to Control group (P<0.0001).

4.2: Measurements of Serum Proinflammatory Cytokines

Measurements of IL-1β and TNF-α demonstrated highly significant increases in tramadol group ($p < 0.05$) when compared to the control group. There was a highly significant increase in tramadol group ($p < 0.001$) compared to the control . Cerebellum is a vital component in the human brain as it plays a role in motor movement regulation and balance control. The cerebellum coordinates gait and maintains posture, controls muscle tone and voluntary muscle activity but is unable to initiate muscle contraction. . It is also involved in some complex cognitive processes like emotions, behavior, learning and memory (Jimsheleishvili S, Dididze M., 2023).

It receives inputs from the sensory system of the spinal cord and from different parts of the brain and integrates this information to fine-tune motor activity. Damage to this area in humans results in a loss in the ability to control fine movements, maintain posture and motor learning (Asan AS, et al., 2022). The cerebellum is particularly susceptible to developmental and environmental injury because of its long developmental period (Magar et al., 2020).

Because of the increase in users of opiate and the use of analgesics as they are the most commonly used drugs worldwide this study aimed to shed light on the biochemical effects of tramadol in the cerebellar cortex of adult male albino rat after Exposure to tramadol (Barbosa,J. et.al., 2020)

;. tramadol hydrochloride (opioid) is a synthetic centrally acting analgesic agent used for treating moderate to severe pain including postoperative, gynecologic and obstetric pain, as well as pain of various other Its uptake can lead to bad effects on the nervous system,with less side effects than traditional opioid medications Although tramadol is thought to have low dependence potentials (Motawea, S., 2020). Tramadol is considered a class IV drug by the FDA and has been since July 7th, 201.when used in the management of moderate to sever pain should be only used when the physicians decide that the benefits outweighed the potential risks or when the other drugs fail to control the pain (Dhesi M,et al., 2022).Exposure of rats to tramadol causes cerebellar anomalies (Ezi S, et al., 2021).

It was stated by (Spoto Giulia, et. al., 2021) that in humans, a rapid growth in cerebellum development takes place in the third trimester. This is in contrast to the development of the cerebellum in the commonly used animal model system, the rodent, in which the cerebellum is relatively immature at birth, and the proliferation of the external granular layer and the formation of the internal granular layer occur postnatal (Binda Francesca,et al., 2020). Therefore, the rat was the animal of choice in the current study as the neurodevelopment in the early postnatal days in rat is correspondent to the human development in the mid to late gestational periods.

In the current study the Tramadol administration increases the oxidative stress as appeared by decreased GSH level with tramadol administration as reported by (Aboulhoda, B E.,et.al.,2018). tramadol act as catalyst that directly produces ROS especially in the presence of oxygen. In addition, tramadol itself has strong affinity for sulfur present in cellular protein (Xu L. et al., 2012) The present study proposed oxidative stress as an adverse effect of tramadol exposure. We recorded enhancement of we revealed a significant enhancement of NF-κB protein immune expressions in nano-silver treated groups. We also found statistically significant increases of the serum inflammatory cytokines; IL-1β, IL-6 and TNF-α following Ag-NPs administration in a dose-dependent manner. This could be explained by the excess release of ROS. .

Perrone et al. declared that *Txnip* gene up-regulation stimulates the activation of NF-κB protein and the release the pro-inflammatory mediators such as TNF-α and IL-1β (Perrone L.,et al., 2009). In line with our results, in-vivo oral nano-silver (22, 42, 71 nm) given 1 mg/kg for 14 days elevated the serum levels of TGF-β, IL-1, IL-4, IL-6, IL-12 and increased delivery of B cells and natural killer cells [Park E.-J.,et al., 2010]. Nano-silver (10 and 75 nm) motivated inflammation in vitro by stimulating NF-κB and AP1 (activator protein-1) pathways [Prasad R.Y.,et al., 2013]. Interestingly, JNK is activated by inflammatory mediators such as IL-6 and TNF-α; activated JNK phosphorylates c-Jun (a component of AP-1 complex); AP-1 complex controls the transcription of inflammation-related genes [Assi K.,et al., 2006]

Lipid peroxidation has been used as an indirect marker of oxidant-induced cell injury, when liver is damaged by some chemical toxin; hepatocytes generate a large number of free radicals, causing lipid peroxidation of the cytomembrane to produce MDA. Malondialdehyde levels indirectly reflect the extent of cellular damage by free radicals and are widely used as an index of free radical mediated lipid peroxidation (Mansour, 2000). The oxidative stress induced by tramadol in the brain was reported by Lemarie Hussein et al, 2017 (BVMJ-33(2): 149-159 155 and Grimm, (2009); Mohamed et al., (2013). They explained this by those complexes I, III, and IV of electron transfer chain in mitochondria were found to be inhibited by tramadol at high doses. Inhibition of complex III resulted in the generation of ROS as a consequence of the intrinsic characteristics of the electron transfer process to this complex from reduced ubiquinone. The brain is particularly susceptible to oxidative damage due to its high levels of oxygen consumption, increased levels of polyunsaturated fatty acid and relatively low levels of enzymatic antioxidants (Butterfield et al., 2002). Chronic administration of tramadol to mice resulted in oxidative stress in brain tissues; and this effect was associated with a significant decrease in brain non-enzymatic antioxidant, intracellular reduced glutathione level and in enzymatic antioxidant, glutathione peroxidase activity (Abdel-Zaher et al., 2011).

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