Some Biochemical, Neurochemical, Pharmacotoxicological and Histopathological Alterations Induced by Long-term Administration of Tramadol in Male Rats

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ABSTRACT
The present study investigated the effects of repeated tramadol administration in 60 male rats. The animals were randomly divided into 6 equal groups (n=10/group). The first group served as control and administered saline solution only along the study. The remaining groups were administered oral doses of tramadol (tramadol HCl) suspended in saline solution equal to 40, 80, 120,160 and 200 mg/kg/day for 60 days respectively. Liver and kidney functions, sex hormones activity, some biochemical parameters in addition to some histopathological changes of the rat testes and brain tissues were studied. The results of the present experiment revealed a significant gradual increase in the serum ALT, AST and creatinine by increasing the dose of tramadol, the same result obtained for PRL and E₂ in male rats. While tramadol significantly reduced serum LH, FSH and testosterone activities gradually however, 200 mg/kg showed the lowest levels. Repeated administration of tramadol also increased the density of α₁-adrenoceptors in the rat brain cortex, as measured by saturation analysis of [³H]prazosin binding resembling to the effects induced by conventional antidepressants. The histopathological examination of testes revealed severe diffused testicular degeneration, the histopathological lesions were aggravated till testicular tissues calcification according to the dosing of tramadol (40, 80, 120, 160 and 200 mg/kg/day). Brain tissues in all treated groups showed slight congestion of submeningeal blood vessels and neural degeneration. The histopathological alterations of testes and brain tissues and the above findings throw lights on the possible risks of increased hepatic, renal and neurological damages and sexual dysfunction evoked by repeated administration of tramadol for long periods.

Keywords: Tramadol, Biochemical toxicity, Sexual dysfunction, Histopathological changes and Rats.

INTRODUCTION
Tramadol is a centrally acting analgesic which is mainly used for the treatment of moderate to severe pain [1]. It has been postulated that its analgesic activity may be mediated by both opioid and non-opioid (i.e. norepinephrine and serotonin reuptake inhibition) mechanisms [2-4]. Clinically active tramadol is a racemic mixture of two enantiomers that have two distinct but complementary mechanisms of action: the (+)tramadol is a selective agonist for µ-opioid receptor, it preferentially inhibits serotonin reuptake and enhances serotonin efflux in the brain, whereas the (-)enantiomer mainly inhibits noradrenaline reuptake [5, 6]. Being an opioid, tramadol carries all possible risks known from other opiates [7, 8]. Side effects include dizziness, headache, somnolence, nausea, constipation, sweating, pruritus, and central nervous system stimulation [9, 10]. Tramadol causes respiratory depression, psychological and physical addiction similar to that of other opiates and the analgesic efficacy of tramadol can further be improved by combination with a non-opioid analgesic [11-13].
young addicts in our population typically substituted tramadol for heroin. Repeated tramadol administration in such patients might lead to the accumulation of toxic metabolites in the body, increase the risk for pharmacokinetic interactions, and/or decrease the clearance of tramadol, thus increasing its potential for toxicity [14-16].

Therefore, it seemed interesting to explore whether tramadol administrated repeatedly might induce biochemical and neurochemical alterations characteristic of other antidepressant drugs which when administered repeatedly increase the binding to α₁-adrenoceptors in different brain regions, in particular the affinity of these receptors for their agonists (i.e also to noradrenalin, the endogenous neuromediator) [17]. So, the present studies were conducted to assess, the biochemical, pharmacological and histopathological toxicity profiles of this medication (Tramadol HCl) . Liver and kidneys functions, sex hormones activity and some neurochemical parameters were studied on male rats and brain and testes tissues were subjected to histopathological analysis.

MATERIALS AND METHODS

Drug
Tramadol (tramadol HCl), 225mg tablets, was obtained from El-Kahera-pharmaceutical Co., Egypt. Its chemical name is (±) cis-2-[(dimethylamino) methyl]-1-(3-m ethoxy phenyl) cyclohexanol hydrochloride.

Animals and dosing
The experiments were carried out on 60 male (Rattus norvegicus) weighing 180-200g, obtained from the animal research unit of the faculty of veterinary medicine, zagazig university. All animals were housed in a quite non stressful environment for 10 days before study; they were given normal rat chows and water ad libitum through the experiment. The animals were randomly divided into equal 6 groups (n=10/group). The first group served as control and administered saline solution only along the study. The second, third, fourth, fifth and sixth groups were administered oral doses of tramadol (tramadol HCl) suspended in saline solution equal to 40, 80, 120,160 and 200 mg/kg /daily for 60 days respectively. Control and treated rats were sacrificed at the end of the experiment.

Sample preparation
Blood samples were collected in dry centrifuge tubes for serum preparation, sera were separated and preserved at -20 °C till used for analysis. The brain (cortex) for α₁-adrenoceptors binding was dissected out, frozen on dry ice and stored until used for binding experiments after preparation of the tissue homogenate. Brain and testes specimens were isolated and rinsed in phosphate buffer solution (PH 7.5), and fixed in phosphate buffered formalin for histopathological examination.

Biochemical analysis
1- Some liver, kidney function parameters and sex hormones activities:
- Aminotransferases (ALT, AST) activities were measured using the method of Thomas [18].
- Creatinine (creat.) was determined using the method of Fossati et al., [19].
- Luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL), testosterone (Tes) and estradiol (E₂) were determined using enzyme linked immunosorbant assay (ELISA) kits according to manufacture structure.

2- α₁-adrenoceptors binding in the rat brain cortex.
The experiment was carried out according to the method used previously [20], where the tissue was homogenized for 15 s in 10 ml of an ice-cold Tris–HCl buffer (50 mM, pH 7.4) using Ultra-Turrax homogenizer. The homogenates were centrifuged at 30,000xg for 10 min. That step was repeated twice. Final pellets were re-suspended in Tris–HCl buffer to achieve a final concentration of 10 mg wet tissue/ml. Saturation isotherms were generated using seven concentrations (0.01–2 nM) of [³H]prazosin (specific activity 19.5 Ci/mmol, Amersham). The non-specific binding was defined in the presence of 10 μM phentolamine. The 50µl of phentolamine (non-specific) or Tris–HCl buffer (total) and 50 µl of [³H]prazosin were added to a final volume of 900 µl of tissue suspension. The affinity of α₁-adrenoceptors for an agonist was estimated by studying the ability of various concentrations of phenylephrine (0.1 nM–1 mM) to compete for [³H]prazosin binding sites. A total of 50µl of phenylephrine and 50µl of [³H]prazosin (final concentration: 0.3nM) were added to a volume of 900 µl of tissue suspension. Afterwards, the samples were incubated at 25°C for 25 min, followed by a 15-min ice-cold bath. The bound ligand was separated by vacuum filtration through Whatman GF/C filters and washed three times with 5 ml of ice-cold Tris–HCl buffer. Radioactivity was measured with Beckman LS 6500 scintillation counter. All assays were performed in duplicate. The data were analyzed using iterative fitting routines (Graph PAD Prism 2.0).

Histopathological study
Specimens collected from brain and testes which fixed in phosphate buffered formalin, used for preparation of sections stained with hematoxylin and eosin (H&E) processed for histopathological investigation using light microscope according to Bancroft and Gamble [21].

Statistical analysis
Data of the current study was statistically analyzed using the computer program SPSS 11 (2001)[22]. The statistical method was one way ANOVA test.

RESULTS AND DISCUSSION
Data in table (1) showed that administration of tramadol at a dose of 40, 80, 120, 160 and 200 gm/kg
b.wt. daily for sixty days resulted in a gradual significant increase in serum ALT and AST levels at all groups in compared with control one (P≤0.05). Also creatinine activity was significantly elevated with increasing the dose of tramadol table (2).

There was significant increase in E2 and PRL activities compared to control, while the administration of tramadol resulted in a higher significant reduction in testosterone activity accompanied also with a gradual reduction in LH and FSH levels compared to control, these changes became more observable by increasing the dose of the drug, table (3).

Table 1. Effects of tramadol (40, 80, 120, 160 and 200 mg/kg b.wt.) daily dosing orally for 60 days on mature male albino rats in liver functions enzymes (ALT & AST).

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>Tramadol (mg/kg b.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.60±2.11a</td>
<td>56.20±1.20b 61.35±1.60c 66.45±2.35d 75.0±1.69e 79.8±1.25f</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>127.50±2.50a</td>
<td>206.30±2.65b 209.55±2.44c 216.95±2.50d 222.13±2.19e 228.75±1.90f</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E).

Both the density (Bmax) and the affinity (Kd) of α1-adrenoceptors in the rat brain cortex were significantly and gradually increased with increasing the dose of tramadol where rats administered 200 mg/kg.b.wt. showed the highest density and affinity (35.45±0.20 and 0.45±0.35) respectively comparing to control followed by the group administered 160 mg/kg.b.wt. and 120 mg/kg.b.wt which were nearly equal to each other than the 80 mg/kg.b.wt and 40 mg/kg.b.wt groups where they also showed non significant changes between them. Affinity of α1-adrenoceptors for an agonist (Ki) as measured using phenylephrine competition for [3H] binding sites was not changed upon administration of tramadol even with increasing the doses of the drug, table (4).

Table 2. Effects of tramadol (40, 80, 120, 160 and 200 mg/kg b.wt.) daily dosing orally for 60 days in the kidneys functions creatinine level of mature male albino rats.

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>Tramadol (mg/kg b.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>0.42±0.19d</td>
<td>0.57±0.25c 0.59±0.29b 0.60±0.32a 0.62±0.41c 0.63±0.35e</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E).

Table 3. Effects of tramadol (40, 80, 120, 160 and 200 mg/kg b.wt.) daily orally dosing for 60 days on gonad activities of mature male albino rats.

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>Tramadol (mg/kg b.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (ml U/ml)</td>
<td>2.40±0.20a</td>
<td>1.90±0.50b 1.65±0.42c 1.35±0.35d 0.95±0.22e 0.76±0.15f</td>
</tr>
<tr>
<td>FSH (ml U/ml)</td>
<td>2.20±0.15a</td>
<td>1.50±0.40b 0.99±0.44c 0.85±0.30d 0.76±0.21e 0.55±0.11f</td>
</tr>
<tr>
<td>Testosterone (mg/ml)</td>
<td>4.40±0.44a</td>
<td>2.95±0.56b 2.10±0.45c 1.80±0.60d 1.26±0.19e 0.85±0.13f</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>46.30±1.55a</td>
<td>47.15±0.35c 50.85±0.20d 53.95±0.19e 59.10±0.33f 62.0±0.25g</td>
</tr>
<tr>
<td>Prolactin (pg/ml)</td>
<td>6.90±1.35a</td>
<td>10.15±0.55b 12.65±0.65c 15.95±1.12d 17.85±1.45e 19.00±1.55f</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E).

Table 4. Effects of tramadol (40, 80, 120, 160 and 200 mg/kg b.wt.) daily orally dosing for 60 days on the binding of (3H) prazosin to α1-adrenoceptors - in the mature male albino rat.

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>Tramadol (mg/kg b.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bmax (Fmol/mg protein)</td>
<td>21.15±0.85a</td>
<td>24.20±0.15b 27.35±0.35 c 29.90±0.65d 30.45±0.50e 35.45±0.20f</td>
</tr>
<tr>
<td>Kd (µM)</td>
<td>0.29±0.24a</td>
<td>0.31±0.55b 0.34±0.45c 0.37±0.75d 0.40±0.25e 0.45±0.35f</td>
</tr>
<tr>
<td>Ki (µM)</td>
<td>2.37±0.47a</td>
<td>2.39±0.48b 2.38±0.47c 2.40±0.49d 2.37±0.47e 2.39±0.48f</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E).

Mean values with different superscript letters in the same column are significantly different at (P<0.05).

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Histopathological findings
In all treated groups with tramadol the testes revealed severe diffused testicular degeneration with numerous spermatocyte and spermatid giant cell formation without spermatogenesis especially in group dosed 40 mg/kg b.wt. tramadol (Fig. 2) in comparative with normal testicular tissues (Fig. 1). The spermatocytes were mostly necrotic with an attempt of regeneration. The regenerated cells were seen abundant nuclei. These cells were fused together to form such giant cells (Fig 3). Microspermatooza and dystrophic calcification were found inside the lumen of same seminiferous tubules (Fig. 4). The calcification was gradually invaded almost all the tubular structure with necrosis of the lining epithelium and leucocytic infiltration (Fig. 5). The histopathological lesions were aggravated till testicular tissues calcification according up grading dosing of tramadol (40, 80, 120, 160 and 200 mg/ kg.b.wt.). In all treated groups with tramadol evoked in brain tissues, slight congestion of submeningeal blood vessels and neural degeneration (Fig. 7). In comparison with negative control brain tissues (Fig. 6). In addition to focal areas of haemorrhages focal areas of cytois and satellitosis (Fig 8) in addition of neural degeneration and degenerations of purkinje cells of cerebellum.
Figure 7. Brain tissues of adult male albino rat of control group showing normal cerebrum tissues. (H & E) (Bar = 100 µm).

Figure 8. Brain tissues of adult male albino rat daily orally dosing 40 mg/kg b.wt. of tramadol suspension for 60 days, showing aggregation of the inflammatory cells in the cerebrum mainly lymphocytes and macrophage with degenerated neuron. (H & E) (Bar = 100 µm).

Figure 9. Brain tissues of adult male albino rat daily orally dosing 80 mg/kg b.wt. of tramadol suspension for 60 days, showing focal aggregation of the glia cells (focal gliosis) in the cerebrum tissue. (H & E) (Bar = 100 µm).

Figure 10. Higher magnification of Figure (8).

Figure 11. Brain tissues of adult male albino rat daily orally dosing 120 mg/kg b.wt. of tramadol suspension for 60 days, showing extravasated RBCs (hemorrhage). (H & E) (Bar = 100 µm).

Figure 12. Brain tissues of adult male albino rat daily orally dosing 120 mg/kg b.wt. of tramadol suspension for 60 days, showing degenerated neurons. (H & E) (Bar = 100 µm).

Figure 13. Brain tissues of adult male albino rat daily orally dosing 160 mg/kg b.wt. of tramadol suspension for 60 days, showing severe extravasated RBCs. (H & E) (Bar = 100 µm).

Figure 14. Brain tissues of adult male albino rat daily orally dosing 160 mg/kg b.wt. of tramadol suspension for 60 days, showing congested meningeal blood vessels. (H & E) (Bar = 100 µm).
The above observations may be confirmed by the suggestions of Wu et al., [29] and Jassen-ortho inc.[30] who stated that liver and kidney are responsible for tramadol metabolism and excretion, so it may cause hepatotoxicity and nephrotoxicity.

The opiate use is known to decrease the levels of sex hormones in both sexes and this lowered hormonal level is thought to be responsible for the diminished fertility of both male and female opiate users [32]. In the present study, gonadal examinations revealed that administration of tramadol, 80, 120, 160 and 200 gm./kg b.wt. for two months influenced reduced sex hormones activity of male rats compared to control group while, 40 mg / Kg b. wt. tramadol influenced this activity to a lesser extent compared to other treatments where there was a reduction in the levels of LH, FSH and testosterone with induction of PRL and E2 levels.

Previous studies concerned with gonadal activity during drugs abuse have been supported the present results where Chowdhury [33] reported decreased levels of LH and testosterone with increased prolactin hormone after morphine and methadone administration. Also El-Gaafarawi., [34] observed the reduction of serum levels of LH, FSH and testosterone and the induction of prolactin hormone (PRL) and E2 secretions after paroxetine and tramadol treatment respectively. Similar results for reduced testosterone and elevated E2 have been reported [35]. The results concerning sexual dysfunction could be supported by the histopathological findings in testicular tissues of tramadol treated groups compared to normal tissues.

The current work also conducted to examine whether tramadol induces neurochemical alterations similar to the changes characteristic of conventional antidepressant drugs and the results have shown that upon treatment with tramadol the density and the affinity of α1 adrenoceptors in the rat brain cortex was increased, these findings were in accordance with Heal, [36] and Stockmeier et al.,[37] who reported that repeated administration with tricyclic antidepressants or electroconvulsive shock increases either the density or affinity of the brain α1 adrenoceptors for agonist and also are similar to those of Gorecka et al., [38] who found that repeated administration of tramadol (20 mg/kg,i.p. for 21 days) increased the density of α1 adrenoceptors in the rat brain cortex, as measured by saturation analysis of [3H]prazosin binding . Our results are confirmed by the histopathological alterations observed in the brain tissue of rats from all treated groups compared to control rats. The data obtained in the present study, together with the data obtained by others; show that tramadol manifests many of the actions previously observed for the recognized antidepressants.

REFERENCES


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