BENI-SUEF UNIVERSITY JOURNAL OF BASIC AND APPLIED SCIENCES 5 (2016) 193–201

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Full Length Article

Modulatory effect of cilostazol on tramadol-induced behavioral and neurochemical alterations in rats challenged across the forced swim despair test

Noha M. Gamil [a], *Yousreya A. Maklad* [b], *Maha A.E. Ahmed* [a,*], *Shahira Nofal* [c], *Amany A.E. Ahmed* [c]

[a] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Misr University for Science and Technology, 6th of October City, Egypt*

[b] *Pharmaceutical and Drug Industries Research Division, Medicinal and Pharmaceutical Chemistry Department, National Research Centre, Dokki, Egypt*

[c] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Helwan University, Helwan, Egypt*

ARTICLE INFO

Article history:
Received 16 March 2016
Accepted 21 March 2016
Available online 30 May 2016

Keywords:
Tramadol
Cilostazol
Forced swim test
Brain monoamines
MDA
SOD
TNF-α

ABSTRACT

Pain-associated depression is encountered clinically in some cases such as cancer, chronic neuropathy, and after operations. Tramadol is an opioid analgesic drug that may modulate monoaminergic neurotransmission by inhibition of noradrenaline and serotonin reuptake that may contribute to its antidepressant-like effects. Clinically, tramadol is used either alone or in combination with other NSAIDs in the treatment of cases associated with pain and depression, e.g. low back pain, spinal cord injury, and post-operative pain management. However, tramadol monotherapy as an antidepressant is impeded by severe adverse effects including seizures and serotonin syndrome. Interestingly, phosphodiesterase-III inhibitors demonstrated novel promising antidepressant effects. Among which, cilostazol was reported to attenuate depression in post-stroke cases, geriatrics and patients undergoing carotid artery stenting. Therefore, this study was carried out to investigate the possible antidepressant-like effects of tramadol and/or cilostazol on the behavioral level in experimental animals, and to examine the neurochemical and biochemical effects of tramadol, cilostazol and their combination in rats, in order to explore the probable mechanisms of action underlying their effects. To achieve our target, male albino mice and rats were randomly allocated into five groups and administered either vehicle for control, fluoxetine (20 mg/kg, p.o.), tramadol HCl (20 mg/kg, p.o.), cilostazol (100 mg/kg, p.o.), or combination of both tramadol and cilostazol. At day 14, mice and rats were challenged in the tail suspension test and forced swim test, respectively. Rats were sacrificed and brains were isolated for determination of brain

* *Corresponding author.* Department of Pharmacology and Toxicology, Misr University for Science and Technology, AlMotamayez District, 6th of October City 12568, Egypt. Tel.: +20 23835486; fax: +20 23835499.
E-mail address: mahapharm@yahoo.com (M.A.E. Ahmed).http://dx.doi.org/10.1016/j.bjbas.2016.03.002

monoamines, MDA, NO, SOD, and TNF-α. The current results showed that concurrent administration of cilostazol to tramadol-treated animals modulated depression on the behavioral level, and showed ameliorative neurochemical and biochemical effects in rats exposed to FST.

1. Introduction

Pain-associated depression is encountered clinically in some cases such as cancer, chronic neuropathy, and after operations (Arbaiza and Vidal, 2007; Attal et al., 2009; Dogar and Khan, 2016). Moreover, patients with cardiovascular diseases such as myocardial infarction are at increased risk of developing depression. The latter is associated with an approximately two-fold increase in cardiac morbidity and mortality (Frasure-Smith et al., 2009; Lett et al., 2004). Forced swim despair test (FST) is an established experimental protocol to induce stress-related depression in laboratory rodents (Barros and Ferigolo, 1998). Stress has been regarded as the most important pathogenic factor in several neuropsychiatric disorders including depression due to its association with several biochemical, hormonal, and behavioral changes (García-Bueno et al., 2008).

Tramadol is a synthetic, centrally acting analgesic agent, which acts as an opioid agonist (Gibson, 1996). Tramadol may also affect monoaminergic systems by inhibiting the reuptake of noradrenaline and serotonin in a mechanism similar to that of antidepressant drugs (Bamigbade et al., 1997). Moreover, it was previously demonstrated that drugs that affect the opioidergic system might show antidepressant effects (Perlikowska et al., 2014). Experimentally, tramadol has shown promising antidepressant effects (Rojas-Corrales et al., 1998). Clinically, tramadol is used either alone or in combination with other NSAIDs in the treatment of cases associated with pain and depression, e.g. low back pain, spinal cord injury, and post-operative pain management (Dogar and Khan, 2016; Tetsunaga et al., 2015). However, tramadol monotherapy as an antidepressant or in combination with other traditional antidepressant drugs resulted in severe adverse effects such as seizures and serotonin syndrome (Boyd, 2005; Sansone and Sansone, 2009). Moreover, long-term use of tramadol developed psychological and physical dependence similar to that of other opiates (Ripamonti et al., 2004).

Cilostazol, on the other hand, is a selective phosphodiesterase-III inhibitor that is therapeutically implicated in the treatment of intermittent claudication. It suppresses platelet aggregation and causes direct arterial vasodilation (Cariski and Lindmayer, 2002). On the experimental level, cilostazol has shown neuroprotective and memory enhancement effects (Yanai et al., 2014; Yoneyama et al., 2015). Interestingly, some clinical studies have shown promising effects of cilostazol in the treatment of geriatric and post-stroke depression cases (Baba et al., 2007; Nishimura et al., 2007). In addition, cilostazol alleviated pre-procedural depression in patients undergoing carotid artery stenting (Tsutsumi et al., 2013). However, up to our knowledge, the effect of cilostazol on brain monoamines balance and depression-induced biochemical alterations has not been studied before.

Therefore, the current study was carried out to investigate the possible antidepressant-like effects of tramadol and/or cilostazol on the behavioral level in experimental animals and to explore the underlying neurochemical and biochemical influences.

2. Materials and methods

2.1. *Animals*

Adult male Swiss albino mice (20–25g) and Wistar albino rats (180–200 g) were used in this study. They were housed in plastic cages under standardized conditions (23 ± 2 °C, 12 h light/ 12 h dark cycle) and were allowed free access to water and standard chow pellets. Animals were left to acclimatize for one week before the experiment. Experiments were performed during the light phase of the cycle, and all procedures were approved by the Ethics Committee of the National Research Centre and in accordance with the international recommendations of the Canadian Council on Animal Care Guidelines (1984) for the proper care and use of laboratory animals.

2.2. *Chemicals*

Fluoxetine was obtained from the Egyptian International Pharmaceutical Industry Co. (EIPICO), Egypt. Tramadol HCl was a gift from October Pharma, Egypt. Cilostazol was obtained from Otsuka Pharmaceutical Co., Japan. All other chemicals were of analytical grade.

2.3. *Experimental design*

Rats or mice were randomly distributed into five groups with 12 animals each. Animals of the control group were daily administered the drug vehicle (7% Tween 80 in normal saline) by oral gavage. Fluoxetine, tramadol, and cilostazol groups were administered fluoxetine (20 mg/kg/day, p.o.), tramadol HCl (20 mg/kg/day, p.o.), and cilostazol (100 mg/kg/day, p.o.), respectively (Ghorpade et al., 2011; Jesse et al., 2008; Yanai et al., 2014). Rats or mice of the last group were treated daily with both tramadol and cilostazol. Treatments were continued for 14 successive days. At day 13, rats were exposed to FST pretest, while on day 14, rats were challenged across the forced swim test (FST) and mice were exposed to tail suspension test (TST), then all animals were sacrificed after 60 min by decapitation. Brains were rapidly isolated on ice and immediately stored at –80 °C until biochemical assays were performed.

2.4. Methods

2.4.1. Evaluation of the behavior of mice in the tail suspension test

This test was performed according to the method described by Steru et al. (1985). On the 14th day of the experimental period, mice were transported from the housing room to the testing area in their own cages and allowed to adapt to their new environment for 1 hour before testing. The vehicle or test drugs were orally administered to mice 60 minutes prior to the experiment. The test started by suspending mice on the edge of a holder 50 cm above a tabletop by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded during a 6 min period. After which, animals were then returned back to their home cages. Mice were considered immobile when they hanged passively and were completely motionless for at least 1 min. A decrease in the duration of the immobility period was considered an index of antidepressant activity.

2.4.2. Evaluation of the behavior of rats in the forced swim despair test

Following the method of Szkutnik-Fiedler et al. (2012), a pretest was performed where rats were placed individually into swim cylinders filled with tap water to a depth of 17 cm. The temperature of water was kept at 25 ± 3°c. After 15 min. of swimming, rats were removed from water, dried with towels and returned to their home cages. No scoring of immobility was performed during the pre-test session. 24 hours after the pretest session, animals were deprived of food and water for 4 hours before the experiment. The swim cylinders were filled to the required depth with fresh tap water adjusted to 25 ± 3°c. Treatments were orally administered to rats 60 minutes prior to the session. Each rat was placed individually in the swimming cylinder and observed for 5 min, and then it was removed from water, dried with towels and returned back to its home cage. The water was changed with fresh one after each rat. At the end of each 5 sec. period during the test session, the behavior of each rat was monitored as one of the following four behaviors: 1) *immobility*: a rat was considered immobile when it remained floating in the water without struggling and making only those movements necessary to keep its head above water. The duration of immobility was scored by adding the total time spent immobile, 2) *swimming*: was defined as horizontal movements of the rat throughout the swimming cylinder including crossing into another quadrant, 3) *climbing*: observed as active movements of the rat with its forepaws in the water, usually directed against the walls, 4) *head twitching/shaking*.

2.4.3. Quantitative determination of brain monoamines in rats

Brain monoamines were estimated using rat-specific ELISA kits (Labor Diagnostika Nord GmbH & Co., Germany for norepinephrine and serotonin, and Uscn Life Science Inc., China for dopamine). The procedures were strictly followed as per the manufacturers' instructions.

2.4.4. Determination of brain total lipid peroxides content measured as malondialdehyde (MDA) in rats

Brain malondialdehyde level was estimated following the colorimetric method of Uchiyama and Mihara (1978).

2.4.5. Determination of brain nitric oxide content measured as total nitrites/nitrates metabolites in rats

Nitric oxide was determined colorimetrically in brain tissues according to a previously described method (Miranda et al., 2001).

2.4.6. Determination of brain total reduced glutathione (GSH) level in rats

Brain GSH was estimated according to the method of Ellman (1959).

2.4.7. Determination of brain superoxide dismutase (SOD) activity in rats

Brain SOD activity was determined spectrophotometrically using assay kit from Trevigen, Inc. (USA). The instructions of the kit were followed as mentioned by the manufacturer.

2.4.8. Determination of brain tumor necrosis factor-alpha (TNF-α) level in rats

Rat-specific TNF-α ELISA kit was used (RayBiotech, Inc., USA) for this assay. The steps were followed as per the manufacturer's instructions.

3. Results

3.1. Effect of tramadol and/or cilostazol on the immobility time of mice exposed to TST

As shown in Fig. 1, oral daily treatment with either fluoxetine, or tramadol decreased the immobility time of mice exposed to TST in comparison to the control group; however, no statistical significance was observed. On the other hand, mice treated with cilostazol showed significant reduction in immobility time by 51% as compared to the control group ($p < 0.05$).

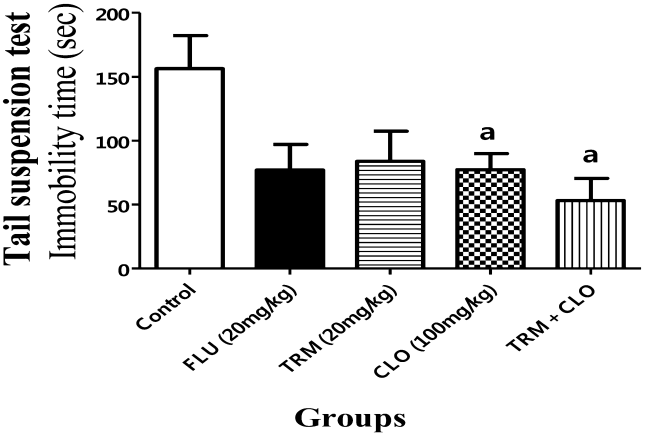

Fig. 1 – Effect of tramadol and/or cilostazol on the immobility time of mice exposed to TST.
Data are represented as mean ± SEM (n = 6). Statistical analysis was performed using t test followed by Mann–Whitney U test. FLU: fluoxetine (20 mg/ kg, p.o.), TRM: tramadol (20 mg/ kg, p.o.), CLO: cilostazol (100 mg/kg, p.o.). Treatments continued for 14 successive days. a: Significantly different from the control group at $p < 0.05$.

Table 1 – Effect of tramadol and/or cilostazol on the behavior of rats exposed to FST.

Group	Immobility time (sec)	Swimming time (sec)	Climbing time (sec)	Head twitching (counts/5 min)
Control	132.60 ± 14.80	17.17 ± 3.46	42.25 ± 5.76	35.33 ± 4.08
FLU (20 mg/kg)	74.09[a] ± 6.84	64.55[a] ± 4.18	34.18 ± 5.96	40.45 ± 3.82
TRM (20 mg/kg)	68.33[a] ± 7.80	41.50[a,b] ± 5.72	37.17 ± 7.68	32.08 ± 6.71
CLO (100 mg/kg)	73.42[a] ± 7.56	35.25[b] ± 9.59	57.75 ± 11.55	40.50 ± 6.54
TRM + CLO	59.67[a] ± 8.06	45.50[a,b] ± 9.98	43.58 ± 5.52	38.08 ± 5.59

Data are represented as mean ± SEM (n = 6). Statistical analysis was performed using t test followed by Mann–Whitney U test. FLU: fluoxetine (20 mg/ kg, p.o.), TRM: tramadol (20 mg/ kg, p.o.), CLO: cilostazol (100 mg/kg, p.o.). Treatments continued for 14 successive days.
[a] Significantly different from the control group at $p < 0.05$.
[b] Significantly different from fluoxetine group at $p < 0.05$.

Similarly, co-administration of tramadol and cilostazol for the same duration showed significant reduction in immobility time by 66% as compared to the control group ($p < 0.05$).

3.2. *Effect of tramadol and/or cilostazol on the behavior of rats exposed to FST*

As shown in Table 1, administration of fluoxetine, tramadol, cilostazol, or a combination of tramadol and cilostazol to rats significantly decreased immobility time by 44%, 48.5%, 45%, and 55% as compared to the control group ($p < 0.05$), respectively. On the other hand, rats treated with fluoxetine, tramadol, or a combination of tramadol and cilostazol showed a significant increase in the swimming time by 276%, 142%, and 165% as compared to the control group ($p < 0.05$), respectively. Interestingly, treatment of rats with either tramadol, cilostazol, or both drugs significantly decreased swimming time by 36%, 45%, and 29.5% as compared to the fluoxetine group ($p < 0.05$), respectively. Tramadol and/or cilostazol did not induce significant changes in the climbing time, or head twitches as compared to that of the control or fluoxetine groups.

3.3. *Effect of tramadol and/or cilostazol on total brain monoamines content in rats exposed to FST*

As shown in Fig. 2, administration of fluoxetine, tramadol, or cilostazol to rats significantly increased brain dopamine content

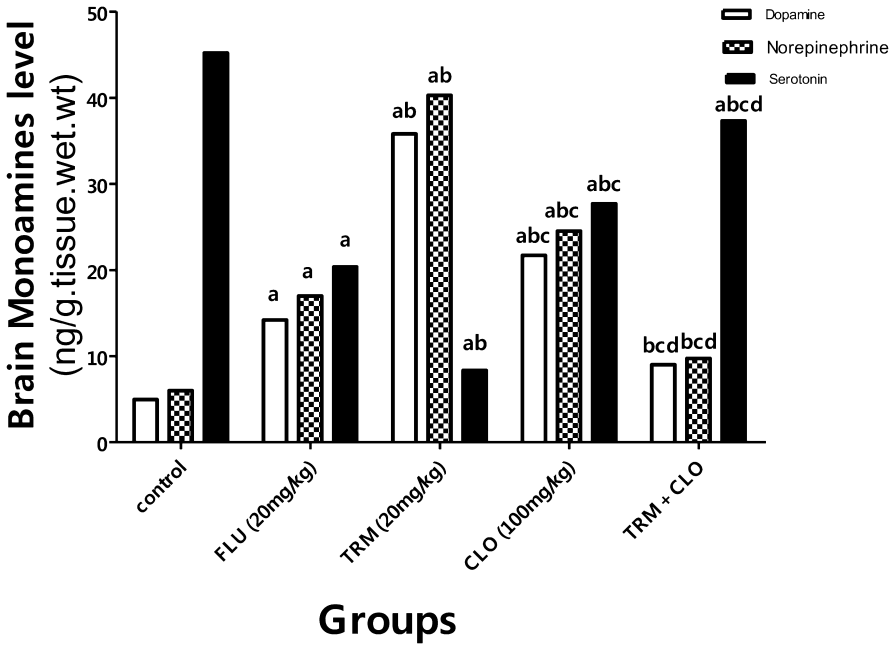

Fig. 2 – Effect of tramadol (TRM) and/or cilostazol (CLO) on total brain monoamines content in rats exposed to FST.
Data are represented as mean ± SEM (n = 6). Statistical analysis was performed using ANOVA followed by Tukey's post hoc test. FLU: fluoxetine (20 mg/ kg, p.o.), TRM: tramadol (20 mg/ kg, p.o.), CLO: cilostazol (100 mg/kg, p.o.). Treatments continued for 14 successive days.
a: Significantly different from the control group at $p < 0.05$;
b: Significantly different from fluoxetine group at $p < 0.05$;
c: Significantly different from tramadol group at $p < 0.05$;
d: Significantly different from cilostazol group at $p < 0.05$.

by 185%, 620%, and 336% as compared to the control group ($p < 0.05$), respectively. Likewise, a significant increase in brain dopamine by 153%, 53% was observed by either tramadol, or cilostazol groups as compared to fluoxetine group, respectively ($p < 0.05$). However, the combination of both tramadol and cilostazol induced a significant decrease in brain dopamine by 36.5% as compared to fluoxetine group ($p < 0.05$). On the other hand, cilostazol significantly reduced brain dopamine content by 39.4% as compared to tramadol group ($p < 0.05$), whereas the combination of tramadol and cilostazol significantly decreased brain dopamine by 75%, and 58.5% as compared to tramadol or cilostazol groups, respectively ($p < 0.05$).

Similarly, administration of fluoxetine, tramadol, or cilostazol to rats induced a significant increase in brain norepinephrine content by 182.5%, 572%, and 308% as compared to the control group ($p < 0.05$). Moreover, a significant increase in brain norepinephrine content by 138%, 44.5% was observed by either tramadol, or cilostazol as compared to the fluoxetine group, respectively ($p < 0.05$). However, cilostazol significantly lowered brain norepinephrine content by 39.2% as compared to tramadol group ($p < 0.05$). Likewise, tramadol and cilostazol combination showed significantly reduced norepinephrine content by 43%, 76% and 60% as compared to fluoxetine, tramadol and cilostazol groups, respectively ($p < 0.05$).

Administration of fluoxetine induced a significant reduction in brain serotonin by 55% as compared to the control group ($p < 0.05$). Likewise, tramadol-treated rats exhibited significant decrease in brain serotonin by 81.5% and 59% as compared to the control and fluoxetine groups, respectively ($p < 0.05$). Similarly, cilostazol significantly reduced brain serotonin content by 39% as compared to the control group ($p < 0.05$). However, cilostazol induced a significant increase by 36% in brain serotonin content as compared to the fluoxetine group ($p < 0.05$). On the other hand, cilostazol exhibited significant increase in brain serotonin content by 232% as compared to the tramadol group ($p < 0.05$). Co-administration of tramadol and cilostazol induced a significant decrease in the brain content of serotonin by 17.5% as compared to the control group ($p < 0.05$). Meanwhile, it significantly increased serotonin content by 83%, 347% and 35% as compared to fluoxetine, tramadol and cilostazol groups, respectively ($p < 0.05$).

3.4. *Effect of tramadol and/or cilostazol on total brain malondialdehyde (MDA), nitric oxide (NO), and glutathione (GSH) contents, and superoxide dismutase (SOD) activity in rats exposed to FST*

As shown in Table 2, administration of fluoxetine to rats induced a significant increase by 323% and 91% in brain MDA and NO contents, and a significant decrease in brain SOD activity by 51% as compared to the control group ($p < 0.05$), respectively. On the other hand, administration of tramadol induced a significant increase in the brain content of MDA, NO, and GSH by 174%, 272%, and 38.5% ($p < 0.05$) as compared to the control group, respectively. A significant increase by 95% was also observed by the same treatment in brain NO level as compared to the fluoxetine group ($p < 0.05$). On the contrary, tramadol induced a significant decrease by 15% in brain MDA level as compared to the fluoxetine group ($p < 0.05$). Interestingly, administration of tramadol induced a significant decrease by 77% and 53% in brain SOD activity as compared to the control and fluoxetine groups, respectively ($p < 0.05$). Interestingly, rats treated with cilostazol exhibited a significant increase in the brain content of MDA by 363%, 43.5%, and 69% as compared to the control, fluoxetine, and tramadol groups, respectively ($p < 0.05$). Meanwhile, cilostazol induced a significant increase in brain GSH content by 47.5% as compared to that of the control group ($p < 0.05$). In a similar manner, treatment with cilostazol induced a significant increase in brain NO content by 138% and 25% as compared to the control and fluoxetine groups, respectively ($p < 0.05$). On the other hand, a significant decrease by 35.9% in brain NO was observed by cilostazol group as compared to the tramadol group ($p < 0.05$). Likewise, rats treated with cilostazol showed significant decrease in brain SOD content by 65% and 28% as compared to the control and fluoxetine groups, respectively ($p < 0.05$). However, a significant increase in brain SOD activity by 51.4% was observed by cilostazol group as compared to the tramadol group ($p < 0.05$). Co-administration of tramadol and cilostazol induced a significant increase in brain MDA, and NO by 62% and 45.5% as compared to the control group, respectively ($p < 0.05$). However, the same combination showed a significant decrease in brain MDA content by 50%, 41% and 65%, a significant

Table 2 – Effect of tramadol and/or cilostazol on brain oxidative stress markers in rats exposed to FST.

Groups	MDA (nmol/g wet tissue)	NO (μmol/g wet tissue)	GSH (mg/g wet tissue)	SOD (U/g wet tissue)
Control	6.57 ± 0.45	15.22 ± 0.81	11.37 ± 0.64	12.55 ± 0.48
FLU (20 mg/kg)	21.20[a] ± 0.78	29.05[a] ± 0.78	13.04 ± 1.24	6.17[a] ± 0.40
TRM (20 mg/kg)	18.00[a,b] ± 0.68	56.62[a,b] ± 3.04	15.75[a] ± 1.23	2.92[a,b] ± 0.14
CLO (100 mg/kg)	30.43[a,b,c] ± 1.22	36.28[a,b,c] ± 1.12	16.77[a] ± 1.06	4.42[a,b,c] ± 0.20
TRM + CLO	10.67[a,b,c,d] ± 0.42	22.15[a,b,c,d] ± 0.64	13.76 ± 0.90	9.17[a,b,c,d] ± 0.39

Data are represented as mean ± SEM (n = 6). Statistical analysis was performed using ANOVA followed by Tukey's post hoc test. FLU: fluoxetine (20 mg/ kg, p.o.), TRM: tramadol (20 mg/ kg, p.o.), CLO: cilostazol (100 mg/kg, p.o.). Treatments continued for 14 successive days.

[a] Significantly different from the control group at $p < 0.05$.

[b] Significantly different from fluoxetine group at $p < 0.05$.

[c] Significantly different from tramadol group at $p < 0.05$.

[d] Significantly different from cilostazol group at $p < 0.05$.

reduction in brain NO content by 24%, 61% and 39%, and a significant enhancement of brain SOD activity by 49%, 214% and 107.5% as compared to the fluoxetine, tramadol and cilostazol groups, respectively ($p < 0.05$). On the other hand, the combination group induced a significant decrease in brain SOD activity by 27% as compared to the control group ($p < 0.05$).

3.5. *Effect of tramadol and/or cilostazol on total brain TNF-α content in rats exposed to FST*

As illustrated in Fig. 3, administration of fluoxetine induced a significant increase in brain TNF-α content by 258.5% as compared to the control group ($p < 0.05$). Similarly, administration of tramadol induced a significant increase in the brain content of TNF-α by 748% and 136.5% as compared to the control and fluoxetine groups, respectively ($p < 0.05$). In the same manner, rats treated with cilostazol showed a significant increase in the brain content of TNF-α by 396% and 38% as compared to the control and fluoxetine groups, respectively ($p < 0.05$). On the other hand, cilostazol induced a significant decrease in brain TNF-α as compared to the tramadol group ($p < 0.05$). Combined treatment of tramadol and cilostazol induced a significant increase in brain TNF-α by 101.5% as compared to the control group, and a significant decrease by 44%, 76% and 59% as compared to the fluoxetine, tramadol and cilostazol groups, respectively ($p < 0.05$).

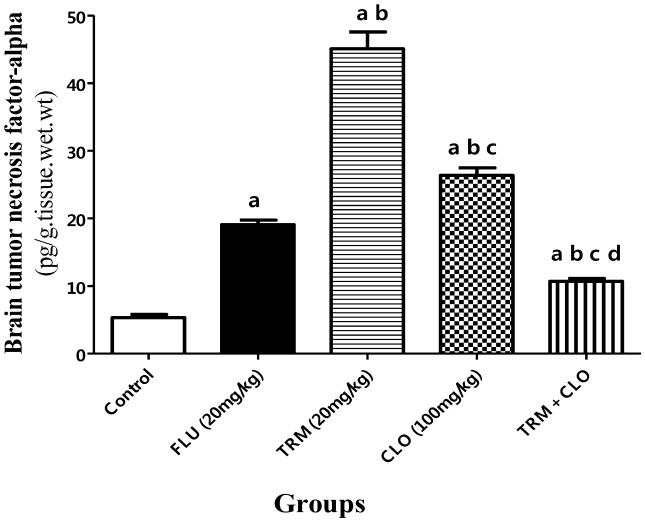

Fig. 3 – Effect of tramadol and/or cilostazol on brain tumor necrosis factor-alpha (TNF-α) content in rats exposed to FST.

Data are represented as mean ± SEM (n = 6). Statistical analysis was performed using ANOVA followed by Tukey's post hoc test. FLU: fluoxetine (20 mg/ kg, p.o.), TRM: tramadol (20 mg/ kg, p.o.), CLO: cilostazol (100 mg/kg, p.o.). Treatments continued for 14 successive days.

a: Significantly different from the control group at $p < 0.05$;
b: Significantly different from fluoxetine group at $p < 0.05$;
c: Significantly different from tramadol group at $p < 0.05$;
d: Significantly different from cilostazol group at $p < 0.05$.

4. Discussion

In the present study, animals were challenged across the tail suspension test and forced swimming test. The mouse tail suspension test is a predictive behavioral test of antidepressant activity (Steru et al., 1985). When mice are suspended by tail, they are subjected to short-term inescapable stress, and they adopt an immobile posture. Increased activity and decreased immobility in TST are strongly correlated with antidepressant effect (Cryan et al., 2005). In the present study, fluoxetine and tramadol caused a clear tendency to decrease immobility time in TST in comparison to the control group. This is similar to the findings of Berrocoso and Mico (2009), who reported that tramadol may decrease immobility time in TST in a dose-related manner. Interestingly, the reduction in immobility time observed by cilostazol with or without tramadol potentiates previous reports about the possible antidepressant effect of cilostazol (Patel et al., 2012).

Moreover, in the FST active behaviors, such as climbing and swimming, may lead to escape from stress, whereas passive behavior, e.g. immobility, conserves energy until a proper chance of escape becomes available (Slattery and Cryan, 2012). Similar to previous studies, fluoxetine induced a significant increase in the swimming behavior in FST (Lucki, 1997). This effect may be attributable not only to the serotonergic effect but also to the potentiated noradrenergic influence of fluoxetine (Page and Abercrombie, 1997; Page et al., 1999). Fluoxetine may enhance the dopamine and norepinephrine levels probably via inhibition of their re-uptake (Wong et al., 1995).

In the present study, tramadol at a dose of 20 mg/kg significantly reduced immobility time and increased swimming time in comparison to the control group in FST. These results are in accordance with previous studies that showed that tramadol may significantly attenuate despair behavior in the FST in the same dose (Jesse et al., 2008; Singh et al., 2004). The antidepressant-like effect of tramadol seems to be mediated by the noradrenergic system rather than the serotonergic or opioidergic system (Rojas-Corrales et al., 1998). Elevated noradrenergic transmission specifically reduces immobility of rats in the FST (Detke et al., 1995). Interestingly, the present results showed that tramadol increased brain dopamine and norepinephrine levels and decreased serotonin level. It was previously reported that tramadol might increase the density of dopaminergic and adrenergic receptors in the rat brain (Faron-Górecka et al., 2004). Activation of dopamine receptors may result in decreased immobility time in FST (Basso et al., 2005). In addition, it was reported that tramadol might increase the release and inhibit the re-uptake of brain serotonin in mice, which may lead eventually to serotonin depletion (Berrocoso et al., 2006). It is noteworthy that increased brain norepinephrine level induces reduction in serotonin level (Valentino et al., 1998).

In the current study, cilostazol either alone or combined with tramadol showed antidepressant activity in TST and FST. Similar behavioral effects were reported by Patel et al. (2012). These behavioral alterations may be attributed to the current ability of cilostazol to elevate brain dopamine and norepinephrine and slightly decrease serotonin contents. Up to our knowledge, this is the first study that reported the modulatory effect of cilostazol

on brain monoamines. Interestingly, it was found that phosphodiesterase inhibitors may enhance noradrenaline and dopamine release and slightly reduce serotonin release from brain slices consequent to the enhancement of cAMP (Schoffelmeer et al., 1985).

It is well-known that psychological stress may alter the balance between oxidant and antioxidant factors leading to the accumulation of free radicals and subsequent lipid peroxidation, DNA damage, and cell death (El Morsy et al., 2015; Matsumoto et al., 1999). Brain is particularly sensitive to free radical insults since it contains high concentrations of polyunsaturated fatty acid and deficient levels of antioxidant compounds (Ahmed et al., 2014; Jain et al., 1991). High concentrations of nitric oxide may interact with superoxide anion and produce peroxynitrite radical (Ahmed, 2014, 2015). The latter may induce nitrosative stress and cause neuronal membrane damage (Terada et al., 1991). In the present study, tramadol enhanced brain NO content in a similar fashion to the findings of Ahmed and Kurkar (2014). A plausible explanation may be attributed to the ability of tramadol to enhance the release of norepinephrine in the brain of rats. The latter was associated with enhancement of nitric oxide synthase (NOS) activity via α_1-adrenoreceptors stimulation (Canteros et al., 1996; Grange-Messent et al., 2004). Similarly, an increase in brain NO content was observed by cilostazol, which was previously attributed to activation of eNOS via cAMP/protein kinase A dependent mechanism (Oyama et al., 2011). Interestingly, co-administration of cilostazol to tramadol-treated rats induced a significant reduction in brain NO content as compared to either of the drugs alone, which may be attributed to the inhibition of norepinephrine release. Interestingly, inhibition of inducible nitric oxide synthase (iNOS) and subsequent inhibition of nitric oxide content was associated with antidepressant-like effects in mice (Montezuma et al., 2012).

In the present study, tramadol induced a significant increase in brain MDA and GSH contents. Tramadol is a synthetic opioid that may induce cellular toxicity by increasing lipid peroxidation (Popovic et al., 2009). Enhanced GSH level following tramadol administration was previously reported and attributed to enhancement of cellular antioxidant defenses as a compensatory mechanism following the initial tramadol-induced oxidative stress (Bilir et al., 2007). Likewise, cilostazol increased brain MDA and GSH contents. Enhanced lipid peroxidation was correlated with decreased serotonin level (Ahmed and El-Awdan, 2015; Chang et al., 2009). However, the antioxidant capacity of cilostazol and its ability to scavenge hydroxyl radicals and suppress production of the intracellular reactive oxygen species was previously demonstrated (Önem et al., 2012). The administration of both tramadol and cilostazol to rats in this study produced significantly lower MDA levels in the brain of rats as compared to either of the drugs alone. A reasonable explanation may involve higher induction of serotonin release by the drug combination than any of the individual drugs. Elevated brain serotonin level was reported to be associated with enhanced antioxidant status (Min et al., 2015).

Enhanced brain SOD activity was previously observed in stressed rats, probably as an adaptive response to depression-induced increase in lipid peroxidation (Shaheen et al., 1996). In the current study, administration of either tramadol or cilostazol to rats inhibited brain SOD activity as compared to the control stressed group. This finding may be attributed to tramadol-induced elevation in brain GSH content (Ahmed and Kurkar, 2014), and to the direct peroxide radicals scavenging activity of cilostazol (Kurtoglu et al., 2014). In addition, cilostazol may induce up-regulation of Nrf2/HO-1 pathway (Park et al., 2010). Nrf2 controls cellular redox status by induction of transcription of SOD and other antioxidant enzymes (Satoh et al., 2006).

The association of stress-related depression with the activation of inflammatory signaling pathways has been previously reported (Bierhaus et al., 2003). Nitric oxide and pro-inflammatory cytokines such as TNF-α are known to be important mediators in inflammation and brain injury (Meda et al., 1995). The present study showed that tramadol induced a significant increase in brain TNF-α content in rats, in a similar manner to previous reports (Andrade et al., 2011; Bianchi et al., 2007). This effect of tramadol may be attributed to decreased level of serotonin since an inverse relationship has been demonstrated between TNF-α and serotonin levels (Kubera et al., 2005). On the other hand, the current enhancement in brain TNF-α level by cilostazol may be attributed to enhanced oxidative stress-induced TNF-α production (Larrick and Wright, 1990). Interestingly, co-administration of tramadol and cilostazol to rats in the present study significantly reduced brain TNF-α level in rats as compared to either of them. This effect may be subsequent to enhanced serotonin release by this drug combination, since binding of 5-HT to 5-HT_2 receptors was associated with inhibition of TNF-α production (Arzt et al., 1991).

5. Conclusion

Concomitant administration of both tramadol and cilostazol to rats showed a promising antidepressant activity in experimental animals. Moreover, this drug combination showed ameliorative neurochemical and biochemical effects in rats exposed to FST. Future studies are recommended to elucidate the mechanisms underlying the antidepressant effects of tramadol and/or cilostazol. Clinical studies are greatly recommended to investigate the efficacy of both tramadol and cilostazol combination in human cases of pain-associated depression such as post-operative management, myocardial infarction, and carotid artery stenting.

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