Pharmacological Reports 69 (2017) 358–364

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

# Telmisartan attenuates diabetes induced depression in rats

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ARTICLE INFO

*Article history:*
Received 8 September 2016
Received in revised form 9 November 2016
Accepted 8 December 2016
Available online 9 December 2016

ABSTRACT

*Background:* Role of brain renin angiotensin system (RAS) is well understood and various clinical studies have proposed neuroprotective effects of ARB's. It is also assumed that diabetic depression is associated with activation of brain RAS, HPA axis dysregulation and brain inflammatory events. Therefore, the present study was designed to investigate the antidepressant effect of low dose telmisartan (TMS) in diabetes induced depression (DID) in rats.

*Methods:* Diabetes was induced by injecting streptozotocin. After 21 days of treatment the rats were subjected to forced swim test (FST). The rats, with increased immobility time, were considered depressed and were treated with vehicle or TMS (0.05 mg/kg, *po*) or metformin (200 mg/kg, *po*) or fluoxetine (20 mg/kg, *po*). A separate group was also maintained to study the combination of metformin and TMS. At the end of 21 days of treatments, FST, open field test (OFT) and elevated plus maze (EPM) paradigm were performed. Blood was drawn to estimate serum cortisol, nitric oxide (NO), interleukin-6 (IL-6) and interleukin-1β (IL-1β).

*Results:* Persistent hyperglycemia resulted in depression and anxiety in rats as observed by increased immobility, reduced latency for immobility, reduced open arm entries and time spent. The depressed rats showed a significant rise in serum cortisol, NO, IL-6 and IL-1β ($p < 0.001$). TMS antagonized depression and anxiety. It also significantly attenuated serum cortisol, NO, IL-6 and IL-1β ($p < 0.001$).

*Conclusions:* Low dose TMS and its combination with metformin normalizes depressive mood, reduces pro-inflammatory mediators and ameliorates the HPA axis function; thereby providing beneficial effects in DID.



## Introduction

Multiple studies have shown that depression is more common in patients with Type-I and Type-II diabetes than in normal people [1]. Around the globe, depression in diabetes exists regardless of culture or country. Twenty six percent of diabetic patients worldwide are reported to suffer from depression [2]. The diabetic depressed patients have an overall reduced quality of life, decreased adherence to treatment, poor metabolic control, higher morbidity and mortality rates [2–4]. In a recent study role of RAS has been recognized in various neurological disorders [5]. Further, the localization and characterization of angiotensin II (Ang II) receptors in the brain have been established using autoradiography [6]. Also it has been firmly established that Ang II is synthesized in the brain independently of peripheral sources [5]. An overactive RAS has been reported to contribute for diabetes induced end organ damage and complications [7]. In addition, the morbidity and stress experienced by the diabetic patients is due to activation of brain RAS as well as brain inflammatory events that may lead to depression [8,9]. It is also reported that angiotensin receptor blocker (ARB) treatment reduces comorbidity associated with hypertensive depressed patient and also reduced the dose of antidepressants [10]. ARB's have also been reported to produce beneficial effects on mood and HPA axis regulation in diabetic patients [11].

The consequences of diabetes-associated inflammatory alterations on psychoneuroimmune process have been recognized recently [12]. Studies have shown that binding of Ang II to its receptor mediates proinflammatory molecule production and free radical generation that contribute to tissue damage and inflammation [12,13]. Major depression has been verified to involve activation of inflammatory response due to increased production of IL-1β, IL-6 [14] and tumor necrosis factor-α (TNF-α) [15]. The antihypertensive drug, candesartan showed protective effect in LPS induced inflammatory factors affecting the brain, by inhibiting IL-6, IL-1β and TNF-α [5]. Telmisartan (TMS) is the most popular, clinically used antihypertensive as it is a potent, long lasting, non-peptide antagonist, selectively inhibiting $AT_1$ receptor stimulation

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http://dx.doi.org/10.1016/j.pharep.2016.12.004
1734-1140/

by angiotensin II. Interestingly, among all the ARB's it is the most lipophilic agent, hence it readily crosses the blood brain barrier facilitating the $AT_1$ receptor blockade [16]. Moreover, TMS treatment has demonstrated direct neuroprotection in *in vitro* studies [17]. ARB's have been reported to exhibit neuroprotective role due to its property of inhibiting proinflammatory and inflammatory factors in the brain [17–19]. TMS is also reported to exhibit PPAR-γ agonist activity. Fourteen days treatment with TMS improved motor coordination, cognition in LPS induced neuroinflammation model [19]. The scarcity of data on telmisartan with respect to diabetes induced depression led us to test the effect of low dose TMS in DID in rats.

## Material and methods

### *Drug and chemicals*

Streptozotocin (STZ) was purchased from Selleck Chemicals LLc, USA, O-Thalaldialdehyde was purchased from Thomas baker Ltd. Metformin (Granules India Ltd. India), Fluoxetine (Cadila, India), and TMS (Medreich Ltd, Hyderabad, India) were obtained as gift samples. Interleukin 6 and 1β kits were procured from Quantikine (R&D systems, USA). All other chemicals and reagents used were of analytical grade.

### *Experimental animals*

Wistar rats (200–250 g) of either sex were obtained from CPCSEA approved local animal supplier. Animals were maintained under standard laboratory conditions at temperature 23 ± 2 °C with relative humidity 55 ± 10% on a 12 h light-dark cycles. Animals had free access to food and water *ad libitum*. The animals were moved to the experimental area 1 h prior to the experiment. All the experiments were performed according to the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethical Committee (IAEC- 1139/a/07) of our institute. The IAEC approval no. of the protocol is IAEC-2012-31

### *Pilot studies for dose selection of TMS*

A pilot study for the dose selection of TMS was carried out using FST in non diabetic rats. The apparatus consisted of vertical plastic cylinder (height: 40 cm, diameter: 30 cm) containing 25 cm of water at 23–25 °C. Five groups of animals viz., normal (without any treatment), vehicle control (0.05% CMC, *po*) and three groups of TMS with different doses (0.05, 0.1, 1 mg/kg, *po*) (n = 6) were subjected to two water exposure sessions. In the first session, rats were subjected to water for 10 min (pretest session). After 24 h, the day was considered as day 0, rats were subjected to water for 5 min (test session). The rats were then treated with the TMS at various doses, i.e. 0.05, 0.1, 1 mg/kg *po* for 21 days. On day 7, day 14, day 21, the rats were administered the respective doses of TMS 30 min prior to the test session. The duration of immobility of each animal was recorded by the video tracking system (VJ Instruments, India). Immobility was considered when the animal ceased struggling and remained floating motionless in the water and making minimal movements necessary to keep its head above water.

### *Induction of diabetes*

STZ was prepared in cold citrate buffer (pH 4.5, 0.1 M). A different set of rats (n = 40) were fasted overnight and administered STZ (30 mg/kg, *ip*). Rats with blood glucose levels more than 250 mg/dL were considered as diabetic [20]. These rats were left untreated for the induction of depression.

### *Induction of diabetic depression*

After 21 days of administration of STZ, FST was performed for evaluation of induction of depression [20].

### *Treatment schedule*

The first group was vehicle control: 0.05% CMC, 10 ml/kg *po* was given. Diabetic depressed rats were divided into 5 groups (n = 6). STZ control group: treated with vehicle (0.05% CMC only), MET (200) group: treated with vehicle (0.05% CMC) + MET (200 mg/kg, *po*), FLX (20) group: treated with vehicle (0.05% CMC) + FLX (20 mg/kg, *po*), TMS (0.05) group: treated with vehicle (0.05% CMC) + TMS (0.05 mg/kg, *po*), TMS (0.05) + MET (200) group: treated with TMS (0.05 mg/kg, *po*) and MET(200 mg/kg, *po*). All the animals were treated with respective drugs for 21 days. On day 21, EPM and OFT were performed while FST followed by locomotor activity was done on 22nd day of respective treatments. Animals were anesthetized [Ketamine HCl (80 mg/kg) + Xylazine (20 mg/kg) *ip*]; blood was withdrawn through retro orbital puncture for biochemical estimations.

## Behavioral parameters

### *Elevated plus maze (EPM)*

The elevated plus maze consists of two opposing open arms (10 × 50 cm) and two opposing closed arms (50 × 20 × 50 cm), together forming the shape of a cross and connected by a central square (10 × 10). During test session each animal was gently placed on the central square, facing an enclosed arm, and allowed to freely explore the maze for 5 min. The session was recorded by a video tracking system. Subsequent behavioral analyses were carried out by an experienced observer, blind to the treatment condition, through Maze Master (VJ instruments, India) software. After each trial, the elevated plus-maze apparatus was wiped clean. The parameters such as open and closed arm entries, time spent in open and closed arm were measured.

### *Open field test (OFT)*

At the end of the study, all the animals were subjected to the OFT, it comprised of a circular arena with wall placed on a wooden platform (wall height 20 cm; diameter 64 cm) with 36 houses/squares [21]. At the beginning of the test, animals were placed gently in the center of the arena and allowed to explore the area. The exploration in the open field, i.e. ambulation (the number of squares crossed with all paws), rearing (raised front paws from the floor and either placing them on lateral walls or placed in front of the body) and grooming (paw strokes over the face or licking) were recorded by video tracking system (VJ instruments, India) and scored by a blind observer.

### *Forced swim test (FST)*

Animals were subjected to testing session. Onset of immobility (latency) and total immobility time (duration) was recorded using FST as described above with little modification (no pretest was carried out).

### *Locomotor activity*

Locomotion was measured post treatment in all groups in Photo-actometer (INCO, India). The rat was placed in an activity chamber with a closed lid, and was allowed to explore and acclimatize for first 2 min of 6 min test session. The total

locomotion was recorded for 6 min and readings were observed for last 4 min.

## Biochemical parameters

*Effect on blood glucose level*

The blood glucose level was estimated using GOD/POD method. Glucose is oxidised to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide reacts with phenol and 4-aminoantipyrine thereby forming red coloured quinoneimine dye complex. The intensity of colour formed is directly proportional to the glucose in the sample. The blood glucose measurement was carried out prior and post to the drug treatments using autoanalyzer (RETINA, Delta Lab, India) [22].

*Nitric oxide estimation*

Nitric oxide concentration was estimated by using the Griess reagent method, nitric oxide concentration was estimated spectrophotometrically (Jasco V-730, Japan) at 546 nm. The concentration of nitrite was determined from standard curves constructed with serial concentrations of Sodium nitrite [23].

*Cortisol estimation*

Distilled water (0.5 ml) was added to 0.5 ml of serum or brain homogenate in a glass tube. This was followed by the addition of 7.5 ml of methylene chloride (MC). The tube was bunged and shaken slowly for 20 min to extract the steroid. The phases were allowed to settle and the supernatant aqueous layer was pipetted out and discarded. The MC extract so collected was then added to the fluorescent reagent (prepared by mixing 7:3, concentrated sulfuric acid: ethanol) in a clean dry glass stopper tube and was shaken vigorously for 20 s. The MC layer was pipetted out and discarded and the acid extracts were transferred to the cuvettes 13 min after mixing the MC extracts. The same procedure carried out for all test solutions. The fluorescence was read at 540 nm with excitation wavelength at 430 nm using Spectrofluorometer (Varian, USA). Calculations were done by extrapolating the unknown on a standard cortisol curve.

*IL-6 and IL-1β estimation*

Estimation of IL-6 and IL-1β was carried out in the serum as per the instructions and procedure given in IL-6 and IL-1β Quantikine ELISA kit (R&D Systems, USA) using an ELISA reader (Readwell touch, Robonik India Pvt Ltd, India).

## Statistical analysis

Data for each parameter was analyzed by one way ANOVA followed by Dunnett's *post hoc* test, except for data of FST pilot study (immobility time) was analysed using two way ANOVA followed by Bonferroni's post test, using graph pad, prism software, version 5, USA.

## Results

*Selection of dose:* The low doses of TMS (0.05, 0.1, 1 mg/kg) have shown significant reduction in immobility time as compared to control group. The dose of 0.05 mg/kg has shown significant reduction in the immobility time compared to other doses (Fig. 1(a)).

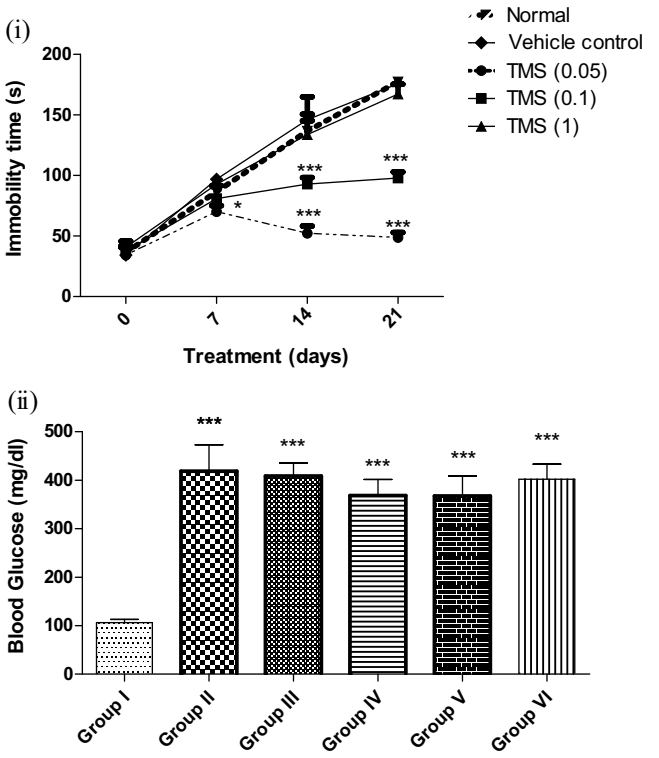


**Fig. 1.** Effect of various doses of telmisartan on (a) Immobility time. (b) Induction of diabetes in rats. Data is expressed as mean ± SEM (n = 6). Statistical significances were determined using two way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test for immobility time (a) and one-way ANOVA followed by Dunnetts *post hoc* test for blood glucose measurement (b). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to vehicle control. TMS: telmisartan. The figure in parenthesis indicates the dose in mg/kg *po.*

*Induction of diabetes depression*

STZ at a dose of 30 mg/kg *ip* produced significant hyperglycemia as compared to normal group (Fig. 1(b)). Persistent hyperglycaemia for 21 days developed depression-like symptoms. All the treatment groups showed decreased latency to be immobile and prolonged duration of immobility in the FST in comparison with the normal group, suggesting the induction of DID in rats (Table 1). Moreover the locomotor activity was not affected in all groups.

*Effect of TMS on FST, locomotor activity in DID rats*

Persistent hyperglycemia reduced the latency to be immobile and increased immobility duration in the STZ control group. There was a significant increase in latency to become immobile in FLX (20) ($p < 0.001$), TMS (0.05) ($p < 0.001$) and TMS + MET (0.05 + 200) ($p < 0.001$) treated groups. All the treated groups exhibited significant reduction in total immobility time as compared to the STZ control ($p < 0.001$, $p < 0.01$). MET failed to increase latency time and reduce immobility period. Locomotor activity still was not affected after chronic treatment of drugs (Table 2).

*Effect of TMS on OFT, elevated plus maze in DID in rats*

DID caused decrease in ambulation, rearing and grooming activities ($p < 0.001$) as compared to vehicle control rats. The groups treated with FLX (20), TMS (0.05), TMS + MET (0.05 + 200) has shown a significant increase in ambulation ($p < 0.001$), rearing ($p < 0.001$, $p < 0.05$, $p < 0.001$) as well as grooming ($p < 0.05$,

**Table 1**
Effect of hyperglycemia on forced swim test and locomotor activity: induction of diabetes induced depression in rats.

| Groups | Locomotor Activity (No./unit time) | Forced Swim Test | |
|---|---|---|---|
| | | Latency to immobility (s) | Duration of immobility (s) |
| Group I | 199.3 ± 5.40 | 57.33 ± 4.38 | 48.17 ± 5.07 |
| Group II | 185 ± 20.17 | 11.83 ± 2.38 ### | 167.2 ± 5.73 ### |
| Group III | 197.83 ± 15.18 | 11.33 ± 1.54 ### | 169.3 ± 3.8 ### |
| Group IV | 197.83 ± 21.81 | 10 ± 2.1 ### | 165.3 ± 7.89 ### |
| Group V | 196.33 ± 26.28 | 8.50 ± 2.07 ### | 165.2 ± 4.26 ### |
| Group VI | 183.5 ± 15.91 | 7.33 ± 1.2 ### | 169.5 ± 5.92 ### |

Data is expressed as mean ± SEM (n = 6). Statistical significances were determined using one way analysis of variance (ANOVA) followed by Dunnetts *post hoc* test, ###$p < 0.001$ as compared to group-I. Groups II–VI were diabetic untreated rats.

**Table 2**
Effect of 21 day treatments of test drugs on forced swim test, locomotor activity in DID rats.

| Groups | Locomotor activity | Forced swim test | |
|---|---|---|---|
| | | Latency(s) | Immobility(s) |
| Vehicle control | 242.2 ± 12.74 | 68 ± 11.57 | 54.33 ± 6.63 |
| STZ control | 260.8 ± 11.69 | 6.5 ± 1.43### | 177.3 ± 1.85### |
| MET (200) | 250.3 ± 30.26 | 12.83 ± 3.74 | 150.3 ± 7.03 |
| FLX (20) | 273.2 ± 16.52 | 93.67 ± 7.07*** | 41.5 ± 8.06*** |
| TMS (0.05) | 264.5 ± 7.80 | 56.5 ± 4.59*** | 72.5 ± 18.4*** |
| TMS (0.05) + MET (200) | 261.3 ± 11.88 | 43.33 ± 6.27*** | 73.83 ± 13.3*** |

Data is expressed as mean ± SEM (n = 6). Statistical significances were determined using one way ANOVA followed by dunnetts post hoc test, ###$p < 0.001$ as compared to normal, **$p < 0.01$ ***$p < 0.001$ as compared to STZ control. MET- metformin, FLX- fluoxetine, TMS- telmisartan; the figure in parenthesis indicates the dose in mg/kg *po*.

$p < 0.01$, $p < 0.05$) as compared to STZ control group. The MET group had shown no significant improvement.

The total number of% entries as well as% time spent in open arm was less in STZ control group as compared to vehicle control ($p < 0.05$). All treatments significantly increased% open arm entries as compared to STZ control group ($p < 0.001$) (Table 3). Only TMS (0.05) and TMS + MET (0.05 + 200) group showed reduction in% time spent in open arm.

*Effect of TMS on biochemical parameters in DID in rats*

*Blood glucose level*

STZ caused significant rise in the blood glucose as compared to the vehicle control rats ($p < 0.001$). Repeated treatment with MET (200) ($p < 0.001$), TMS and MET combination (0.05 + 200) ($p < 0.01$) for 21 days significantly lowered the blood glucose level as compared to STZ control. Other treatment groups could not show a significant reduction in blood glucose in diabetic rats (Fig. 2).

*Nitric oxide level, serum cortisol, IL-6 and IL-1β level*

Persistent hyperglycemia raised the plasma nitric oxide as compared to vehicle control ($p < 0.001$). Twenty one day treatment with MET (200), FLX (20), TMS (0.5), TMS + MET (0.05 + 200) attenuated nitric oxide concentration ($p < 0.001$) as compared to the control.

Diabetic control rats showed elevated serum cortisol ($p < 0.001$) TMS (0.05) and TMS + MET (0.05 + 200) treatment significantly reduced it ($p < 0.001$) as compared to STZ control. MET and FLX had no effect on serum cortisol. The STZ control group showed an elevation in serum IL-6 and IL-1β and concentrations as compared to vehicle control ($p < 0.001$). All treatment groups have shown significant reduction in IL-6 level. IL-1β was significantly reduced by FLX (20) ($p < 0.001$), TMS (0.05) ($p < 0.001$), TMS + MET (0.05 + 200) ($p < 0.01$) ($p < 0.05$) (Fig. 2).

## Discussion

Depression is twice as common in patients with diabetes as in the general population and its prevalence appears to increase with the number of diabetic complications [24]. Diabetes associated depression may occur due to changes in the quality of life imposed by treatment, or may be a consequence of the biochemical changes accompanying the disease [25]. Also the alterations in the function of HPA axis, immune system, reduced brain monoamine level, neuronal loss, altered neuronal synaptic plasticity and increased oxidative stress plays a key role in the pathogenesis of diabetes induced depression [25]. The inflammatory hypothesis of depression proposes a bidirectional relationship between inflammation and depression [26]. ARB's have been proposed as a novel integrated approach beneficial in neuroinflammatory disorders [5,27]. Besides, clinical studies carried by Nasr et al. [10], clearly demonstrated that hypertensive patients (44%) treated with β-blocker were additionally supported with antidepressants as compared to those on ARB's.

Twenty one day treatment with TMS (0.05 mg/kg) displayed antidepressant effect as evidenced by reduced immobility time in pilot study. The higher doses (0.1 and 1 mg/kg, *po*) of TMS exhibited

**Table 3**
Effect of 21 day repeated dose of telmisartan and other treatments on open field parameters and elevated plus maze in diabetes induced depression in rats.

| Groups | Open Field Test | | | Elevated Plus Maze | |
|---|---|---|---|---|---|
| | Ambulation | Rearing | Grooming | % entries in open arm | % time spent in open arm |
| Vehicle control | 40.83 ± 2.75 | 20.50 ± 1.11 | 10 ± 1.06 | 49.21 | 11.44 |
| STZ control | 13.33 ± 4.39### | 3.83 ± 0.7### | 2.5 ± 0.67### | 17.76# | 4.22 |
| MET (200) | 8 ± 1.06 | 6.66 ± 1.08*** | 3.66 ± 1.20 | 43.18 | 18.22* |
| FLX (20) | 85 ± 3.89*** | 19.17 ± 0.7*** | 8.33 ± 0.88*** | 115.27*** | 45.55*** |
| TMS (0.05) | 57.5 ± 4.08*** | 13.67 ± 1.28* | 9.33 ± 0.76*** | 119.33*** | 46.66*** |
| TMS (0.05) + MET (200) | 85.5 ± 2.68*** | 8.5 ± 1.33 | 7.5 ± 0.76** | 73.80*** | 42.22*** |

Data was expressed as mean ± SEM (n = 6). Statistical significances were determined using one way analysis of variance (ANOVA) followed by dunnetts post hoc test, ###$p < 0.001$ as compared to normal, **$p < 0.01$ ***$p < 0.001$ as compared to STZ control. MET- metformin, FLX- fluoxetine, TMS- telmisartan, the figure in parenthesis indicates the dose in mg/kg *po*.

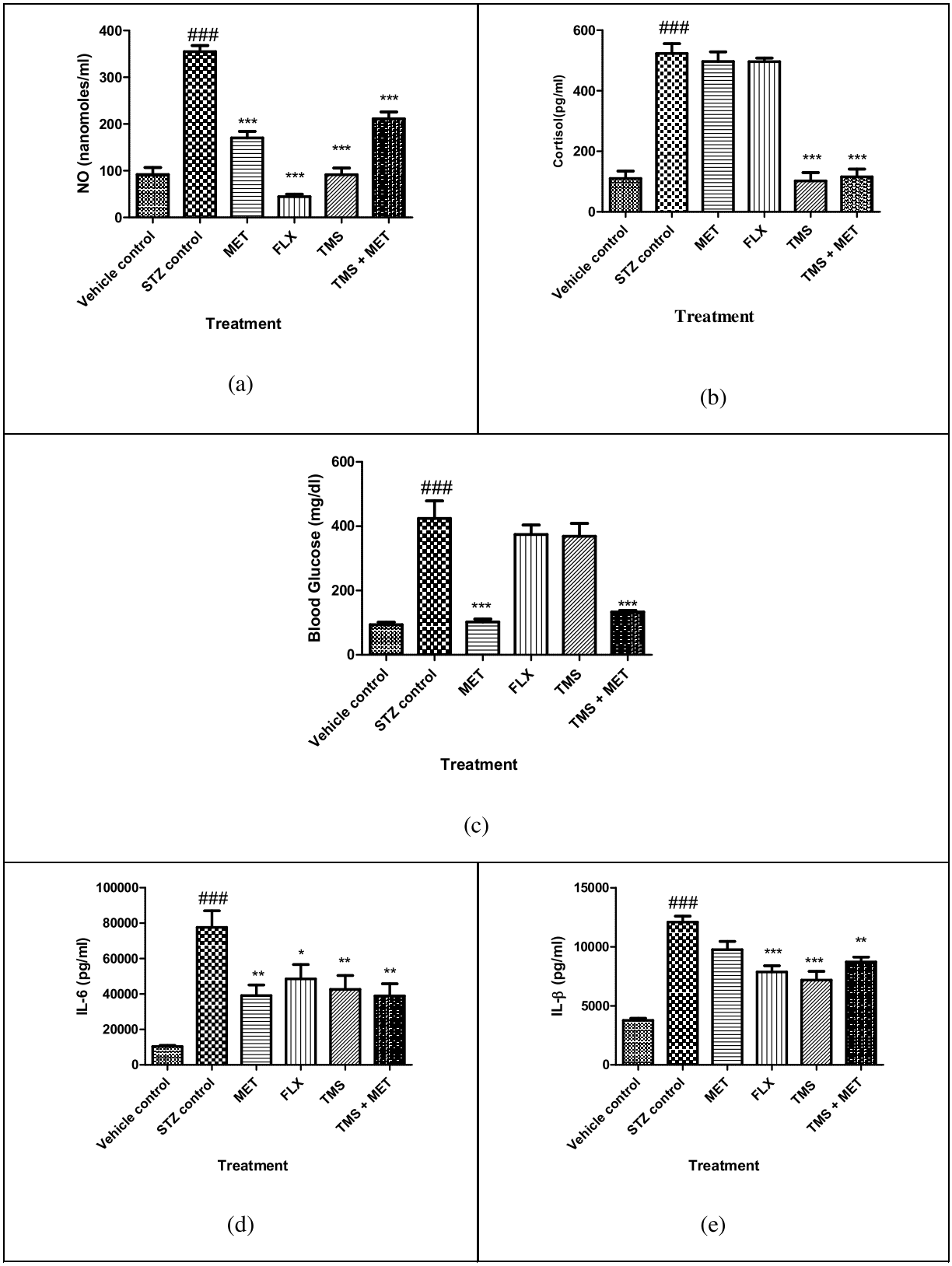


**Fig. 2.** Effect of telmisartan and other treatments on (a) nitric oxide, (b) serum cortisol (c) blood glucose (d) IL-6 (e) IL-1β level. Data is expressed as mean ± SEM (n = 6). Statistical significances were determined using one way ANOVA followed by Dunnett's *post hoc* test. ###$p < 0.001$ as compared with normal, *$p < 0.05$, *$p < 0.01$, ***$p < 0.001$ as compared to STZ control. TMS: telmisartan, MET: metformin, FLX: fluoxetine. The figure in parenthesis indicates the dose in mg/kg *po*.

increased immobility time which corroborate with the previous findings [28]. The antidepressant efficacy ofTMS (0.05 mg/kg) was further assessed in a rat model of diabetes induced depression. STZ induced diabetes is a common animal model of type I diabetes, characterized by an increase in plasma glucose level. As previously reported, STZ induced diabetic animal exhibits depression on day

14 [29]. Our study demonstrates 21 days of hyperglycemia induced depression like symptoms as observed by different behavioral paradigms. The reduced exploratory behaviour of diabetic rats using EPM showed anxiety associated with diabetes induced depression. Treatment with TMS, fluoxetine as well as combination of TMS with metformin increased open arm entries and time spent indicating their anxiolytic potential. Other ARB's and fluoxetine has been reported to reduce anxiety [30,31]. Metformin for obvious reasons failed to have any effect. TMS alone improved ambulation, rearing and grooming, synergistic effects were observed with metformin. The possible hypothesis of TMS improving the OFT parameters may be due to modulation of brain RAS, whereas with metformin is due to its antihyperglycemic potential. The results obtained in the present study supports the findings of Vijayapandi et al. [28]. These findings are also in line with the observations found in elevated plus maze. Consistent hyperglycemia resulted in behaviour despair as observed by increased immobility time and reduced latency to be immobile in FST. As locomotion was not affected, we can predict increased immobility is not due to physical condition of the rats. FST, though does not induce in rats a symptomatology similar to clinical depression, is a suitable and established tool for evaluation of antidepressant drugs. Majority of antidepressants reduce immobility time and their effectiveness can be well correlated with their clinical potency [32]. Imipramine, a tricyclic antidepressant has shown to decrease the duration of immobility in FST [33]. In present study, fluoxetine, TMS and combination of TMS with metformin prolonged latency and reduced immobility time demonstrating their antidepressant efficacy. Fluoxetine is a potent antidepressant which acts by increasing the brain serotonin concentration. The antidepressant like effects of angiotensin receptor blockers have been reported clinically [34]. The mechanism suggested for TMS as an antidepressant is by brain $AT_1$ receptor blockade.

Diabetes exhibits high oxidative stress due to constant and chronic hyperglycemia, which thereby depletes the activity of the antioxidative defense system and thus promotes denovo free radical generation [35]. Long term exposure of endothelial (HUVEC) cells to glucose has shown to increase stimulation of iNOS induced NO [36]. The high concentration of NOs leads to various pathological effects [37]. In current study, high NO level in control group indicated oxidative stress, which was attenuated by metformin, fluoxetine and TMS. These observations are parallel with the previous findings [38–41]. Diabetes induced depression is considered as a neuroinflammatory disorder [42,43], characterized by increased production of IL-6 and IL-1β [14]. Similarly, activation of inflammatory response also grounds HPA axis hyperactivity leading to elevated cortisol level [44], this is primarily believed to be a reflection of cytokine induced disruption of negative feedback *via* glucocorticoid receptors at the level of both the hypothalamus and anterior pituitary [45]. In the present study, increased level of IL-6 and IL-1β in control group indicates involvement of proinflammatory mediators in DID. Treatment with TMS, fluoxetine, metformin and combination reduced the level of these pro-inflammatory mediators demonstrating the role of TMS in reducing inflammation in the brain. Candesartan, an analogue of TMS is also reported to reduce LPS induced inflammatory cytokines in the brain cells [17]. Role of PPAR-γ in reducing neuro-inflammation by TMS has been well documented [19]. Fluoxetine has been previously reported to inhibit NF-κB, which is an important transcription factor for the production of proinflammatory mediators like NO, IL-6, IL-1β and TNF-α [46].

Failure to adapt stressful stimuli results in depression, which also amplify $AT_1$ receptor expression in HPA axis, thereby elevating cortisol level. The present study indicates that TMS, not fluoxetine ameliorates the level of cortisol in accordance with our previous findings [47]. Current findings also support the clinical observations reported by Pavlatou et al. and Nasr et. al [10,11]. We propose that TMS might be a novel and promising candidate for the treatment of diabetes induced depression.

## Conclusion

Based on the observations, it can be concluded that low dose TMS and its combination with metformin normalizes depressive mood, reduces proinflammatory mediators and ameliorates the HPA axis function; thereby provide beneficial effects in diabetes induced depression.

## Conflict of interest

Authors declare that there is no conflict of interest.

## Funding

None declared.

## Acknowledgement

The authors would like to acknowledge, Dr M.N.Navale, Founder, Sinhgad Technical Education Society, Pune and Principal, Sinhgad Institute of Pharmacy for providing the research facilities.

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