

Neuropharmacological studies on repurposed utilization of pioglitazone in learning and memory: A dose related study

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Abstract: Dose dependent effects of pioglitazone on memory revival and monoamine metabolism were monitored in this study. Pioglitazone is an antidiabetic drug showing potential for memory improvement. Rats were treated with three doses of pioglitazone i.e., 5, 10 and 15mg/kg and effects were monitored in Morris water maze test, Skinner's box, light dark activity box, forced swim test, elevated plus maze and open field apparatus. Results show memory enhancing effects of pioglitazone at all three doses, but these effects were potentiated at the dose of 10mg/kg. Apart from memory enhancing effects, pioglitazone treated rats also exhibited anxiolytic and antidepressant effects. Biogenic amines and metabolites were estimated by reverse phase High Performance Liquid Chromatography with electrochemical detector (HPLC-EC). Effect of low (5mg/kg) dose of pioglitazone was found to be non-significant on dopamine metabolism but significant increase in dopamine metabolism was caused by moderate dose (10mg/kg). Results could be helpful in elucidating the effect of apomorphine at different doses and its implication for extending therapeutics in Parkinson's and related disorders.

Keywords: Pioglitazone, memory, Morris water maze, neurochemistry, repurposing.

INTRODUCTION

Pioglitazone is a thiazolidinedione anti-diabetic drug widely used in a clinical medicine that acts on the PPAR (Peroxisome Proliferator-Activated Receptor)- γ receptors (Giglio *et al.*, 2022). PPARs-ligand activated transcription factor that belongs to nuclear hormone receptor super family consist of three isoforms α , β and γ and in CNS, all of them are expressed by all cell types i.e. astrocyte, microglia and neurons. In healthy individual, PPAR- γ works as a regulator of CNS inflammation, neuroprotective effect on cultured microglia and astrocyte and prevent the neurodegeneration in animal models (Kumaretal., 2021; Prashantha Kumar *et al.*, 2020).

Pioglitazone's primary function is to reduce insulin resistance and it is the only potent insulin sensitizer available at the moment (Wolosowicz *et al.*, 2022). Pioglitazone preserves beta cell function, causes durable reduction in HbA1c and corrects multiple components of metabolic syndrome (Ho *et al.*, 2022). Besides it's another beneficial effect, it has been recently reported to have crucial effects on improvement of cognitive function and memory performance (Alhowail *et al.*, 2022). Its adverse effects are weight gain, edema and fractures but are arguable as outweighed by the multiple beneficial effects along with cost-effective drug for the treatment of diabetes and also these side effects can be diminished by using lower doses (Tomlinson *et al.*, 2022).

Learning and memory are cognitive functions which are

vital for survival of the animal and which cognitive structure is complex. Short-term memory (STM) is a maintenance of memory over a short period of time i.e., in seconds and it is very critical part of cognition process (primary memory). Long-term memory (LTM) is refers to the mechanism of strengthening the acquired memory over time and become resistant to interference (secondary memory). STM includes a conscious maintenance of sensory stimuli for a short period of time and after that absent while LTM includes the reactivation of past experiences. Retrieval depends on the recollection of encoded contextual features of a past event such as, time, people, place, sights, thoughts and emotions. Probe is the spatio-temporal contribution of certain structures and processes and it is important for learning and memory. Learning is a prerequisite for the formation of memory (Liu *et al.*, 2022; Shaham *et al.*, 2022; Singh *et al.*, 2022).

Pioglitazone are the selective agonists of PPAR- γ receptors that is the nuclear receptor protein family which are present in several regions of brain in neuronal and non-neuronal cells and they are involve in transcriptional control of genes regulating various physiological processes such as glucose metabolism, lipid homeostasis, inflammation, cellular differentiation, different metabolic disorders along with the inflammatory responses and immune activity in the central nervous system (Nascimento *et al.*, 2022). Pioglitazone has shown neuroprotective activity in certain diseases such as Alzheimer's disease, Parkinson's disease, oral dyskinesia, vascular dementia and alcohol intoxication. It is reported to improve spatial and non-spatial learning abilities and spatial reference memory (Zamanian *et al.*, 2022). Also

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the memory and learning has a close relationship with different neurotransmitters (Kourosh-Arami *et al.*, 2023; Schmidt *et al.*, 2022). The present experiment was design to monitor the changes in behavioral and neurochemical profile of pioglitazone in a dose-dependent manner. Pioglitazone was injected to rats at three different doses to find the most effective dose of pioglitazone on memory revival. Findings will help in extending therapeutics in Dementia, Alzheimer's, cognitive impairment and related disorders.

MATERIALS AND METHODS

Animals

Locally bred male albino Wistar rats, weighing 180-200g purchased from Dow University of Health Sciences, Karachi, Pakistan were used in the present study. Housing conditions were same as described elsewhere (Ikram *et al.*, 2020). All animal experiments were conducted in accordance with NIH guidelines and approved by the institutional Ethics and Animal Care Committee (approval number: KU-07012023).

Experiment

Twenty-four male Albino Wistar rats were randomly assigned to four groups each containing six rats: (i) Water, (ii) Pioglitazone (5mg/kg), (iii) Pioglitazone (10mg/kg) and (iv) Pioglitazone (15mg/kg) treated rats. Food intake and body weights were measured on day 0 and day 3. On day 0 (training day) rats were trained in Morris water maze and basal activities in Skinner's box, light dark activity box, forced swim test, elevated plus maze and open field were monitored. On day 1, rats were administered with water or pioglitazone. Morris water maze test was performed 1hr, 24hr and 48hr post injection respectively. Activities in novel objection recognition test, Skinner's box, light dark activity box, forced swim test, elevated plus maze and open field were also monitored. Rats were then decapitated, brain samples were collected and stored at -70°C until analysis. Neurochemical analyses were performed by HPLC-EC and molecular analysis by ELISA.

Morris water maze test

The procedure was essentially similar as described elsewhere (Ikram *et al.*, 2019). The water maze apparatus used in the present study consisted of a tank, 210cm in diameter and filled to a level that was 2cm higher than the platform height. Water temperature was at room temperature, 21±1°C. The platform (10cm×10 cm) was made of clear acrylic and was hidden 2cm below the surface of water in a fixed location. Water was made opaque by adding milk to it. Initially the rats were trained and during the training session each rat was placed into the water facing the wall of the tank and allowed 120 seconds to locate and climb onto the submerged platform. The rat was allowed to stay on the platform for 10 second.

Novel objection recognition test

Novel object recognition test for the novel object recognition task the apparatus used was an open arena of (50 × 50) with 42 cm high walls. In order to saturate it with olfactory stimuli, cleaning of box was not allowed throughout the experiment. The objects to be discriminated were two glasses filled with white cement (used as familiar objects) in order to make them heavy enough so that rats could not be able to move them and a metallic colored object (used as a novel object). The size of the objects was 2.5 times the size of the rat so that the rat could easily sniff it. During the first training session individual rat was permitted to explore the open field arena for 10min, so that the animal was familiarized to the environment. After a delay of 24 hrs second training session was performed. During this session in the open field arena two similar novel objects were placed and allow the animal to explore them for 10 min. After the delay of 24hrs the retention test was performed in which the animal was positioned back into the similar environment the only difference is that one of the familiar objects (used in training session was now replaced by a novel object and each animal was given a maximum of 10 min to accumulate 30 seconds of object exploration (Ikram *et al.*, 2019).

Skinner's box activity

The activity cages used in this experiment, made of transparent Perspex, were cubical boxes of 26×26×26 cm area with saw dust covered floor. Rats were placed individually in these boxes to get familiar with the experimental arena for 15 min. After which the animals were treated with the drug or water and placed again in the activity cage. Activity was monitored for 10 min; starting 15 min after the drug or water administration (Haleem *et al.*, 2022).

Open field activity

A square area (76×76 cm) with walls 42 cm high was used to monitor activity in a novel environment. The floor of apparatus was divided by lines into 25 squares of equal size. Animals were injected with drug or vehicle and placed in the central square of the open field immediately after the injection. Numbers of squares crossed with all four paws were counted for 5 min (Ikram *et al.*, 2020).

Light dark activity box

Specifically designed two Perspex compartments of equal dimensions (26x26x26 cm) were used to monitor the activity. One compartment was transparent and other was black walled with an entry between them. Experiment was conducted in a separate room. To determine light and dark field activity, an animal was taken out from home cage and placed for the first time in the light compartment. Number of entries in light compartment and time spent in the light and dark compartments were monitored for 5 minutes (Ikram *et al.*, 2020).

Forced swim test

Each rat was placed individually into the glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water at 23-25°C. The animals were left in the cylinder for 6 min. The total duration of immobility was recorded by cumulative stopwatches during the last 4 min of the 6-min-long testing period. The rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only the movements necessary to keep its head above the water level (Ikram *et al.*, 2020).

Elevated plus maze

Procedure was same as described earlier (Ikram *et al.*, 2020). Elevated plus-maze is a cross shaped maze that has two open arms and two close arms enclosed by sides, but with an open roof. The entire maze is elevated 50cm above the floor. Rodent's unconditioned aversion to light and open spaces contribute to its effectiveness as a test for anxiety. Test involves placement of animal in the centre of maze and observing the number of entries and time spent in open/fear inducing arm.

STATISTICAL ANALYSIS

Results are given as means±SD. Analysis of the data was performed by one-way ANOVA (SPSS ver 17). Post hoc comparisons were done by Tukey's test. Values of $p < 0.01$ were considered statistically significant.

RESULTS

Fig. 1 Shows dose dependent effects of pioglitazone on (a) food intake and (b) growth rates. Analysis of the data by one-way ANOVA showed significant effects of different doses of pioglitazone on food intake ($df = 3,20$; $F = 85.23$; $p = 0.0001$) but not growth rates ($df = 3,20$; $F = 1.56$; $p = 0.21$). Post hoc analysis by Tukey's test showed decreased ($p < 0.01$) food intake at the dose of 10mg/kg as compared to 0mg/kg pioglitazone administered rats. However no significant difference among groups was found in growth rates.

Fig. 2 shows dose dependent effects of pioglitazone on activities in familiar environment of Skinner's box (fig. 2a) and novel environment of open field (fig. 2b). Analysis of the data on Skinner's box activities (fig. 2a) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df = 3,20$; $F = 102.36$; $p = 0.0001$). Post hoc analysis by Tukey's test showed increased ($p < 0.01$) activities at all three dose as compared to 0mg/kg pioglitazone administered rats. While in rats injected with pioglitazone at the dose of 10mg/kg, cage crossings were increased ($p < 0.01$) as compared to both 5mg/kg pioglitazone administered rats. Fig. 2b shows dose dependent effects of pioglitazone on activity in the novel environment of open field. Analysis of the data by

one-way ANOVA showed significant effects of different doses of pioglitazone ($df = 3,20$; $F = 85.33$; $p = 0.0001$). Post hoc analysis by Tukey's test showed increased ($p < 0.01$) activities at all three dose as compared to 0mg/kg pioglitazone administered rats. While in rats injected with pioglitazone at the dose of 10mg/kg, squares crossed were increased ($p < 0.01$) as compared to both 5mg/kg pioglitazone administered rats.

Fig. 3 shows dose dependent effects of pioglitazone on light dark box activity. Analysis of the data on entries in light compartment (fig. 3a) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df = 3,20$; $F = 96.25$; $p = 0.0001$). Post hoc analysis by Tukey's test showed increased ($p < 0.01$) entries in light compartment at all three doses of pioglitazone as compared to 0mg/kg pioglitazone administered rats. Analysis of the data on time spent in light compartment (fig. 3b) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df = 3,20$; $F = 129.63$; $p = 0.0001$). Post hoc analysis by Tukey's test showed increased ($p < 0.01$) time spent in light compartment at all three doses of pioglitazone as compared to 0mg/kg pioglitazone administered rats. While time spent in light compartment by 10mg/kg administered rats was greater ($p < 0.01$) as compared to respective 5mg/kg pioglitazone administered rats.

Fig. 4 shows dose dependent effects of pioglitazone on elevated plus maze activity. Analysis of the data on entries in open arm of elevated plus maze (fig. 4a) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df = 3,20$; $F = 105.46$; $p = 0.0001$). Post hoc analysis by Tukey's test showed increased ($p < 0.01$) entries in open arm at all three doses of pioglitazone as compared to 0mg/kg pioglitazone administered rats. Analysis of the data on time spent in open arm of elevated plus maze (fig. 4b) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df = 3,20$; $F = 64.31$; $p = 0.0001$). Post hoc analysis by Tukey's test showed increased ($p < 0.01$) time spent in open arm at all three doses of pioglitazone rats as compared to 0mg/kg pioglitazone. While time spent in open arm by 10mg/kg pioglitazone administered rats was increased ($p < 0.01$) as compared to 5mg/kg pioglitazone administered rats.

Figure 5 shows dose dependent effects of pioglitazone forced swim test. Analysis of the data on number of attempts to jump outside water tank (fig. 5a) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df = 3,20$; $F = 96.12$; $p = 0.0001$). Post hoc analysis by Tukey's test showed increased ($p < 0.01$) attempts at all three doses of pioglitazone rats as compared to 0mg/kg pioglitazone. While attempts were also increased ($p < 0.01$) in 10mg/kg pioglitazone administered rats as compared to 5mg/kg pioglitazone

treated rats. Analysis of the data on struggle time (fig. 5b) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 85.36$; $p= 0.0001$). Post hoc analysis by Tukey's test showed increased ($p<0.01$) struggle time at all three doses of pioglitazone rats as compared to 0mg/kg pioglitazone. While struggle time was also increased ($p<0.01$) in 10mg/kg pioglitazone administered rats as compared to 5mg/kg pioglitazone treated rats.

Fig. 6 shows dose dependent effects of pioglitazone in Morris water maze test. Analysis of the data on memory acquisition (fig. 6a) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 128.13$; $p= 0.0001$). Post hoc analysis by Tukey's test showed decreased ($p<0.01$) time taken to reach platform at all three doses of pioglitazone rats as compared to 0mg/kg pioglitazone. While time taken to reach platform was also decreased ($p<0.01$) in 10mg/kg pioglitazone administered rats as compared to 5mg/kg pioglitazone administered rats. Analysis of the data on memory consolidation (fig. 6b) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 85.66$; $p= 0.0001$). Post hoc analysis by Tukey's test showed decreased ($p<0.01$) time taken to reach platform at all three doses of pioglitazone rats as compared to 0mg/kg pioglitazone.

Analysis of the data on memory retention (fig. 6c) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 104.25$; $p= 0.0001$). Post hoc analysis by Tukey's test showed decreased ($p<0.01$) time taken to reach platform at all three doses of pioglitazone rats as compared to 0mg/kg pioglitazone. While time taken to reach platform was also decreased ($p<0.01$) in 10mg/kg pioglitazone administered rats as compared to 5mg/kg pioglitazone administered rats. Analysis of the data on probe test (fig. 6d) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 104.25$; $p= 0.0001$). Post hoc analysis by Tukey's test showed decreased ($p<0.01$) time taken to reach platform at all three doses of pioglitazone rats as compared to 0mg/kg pioglitazone.

Fig. 7 shows dose dependent effects of pioglitazone in novel object recognition test. Analysis of the data on latency time (fig. 7a) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 56.48$; $p= 0.0001$). Post hoc analysis by Tukey's test showed decreased ($p<0.01$) time taken to approach novel object at all three doses of pioglitazone as compared to 0mg/kg pioglitazone. Time taken to approach novel object at 10mg/kg pioglitazone was decreased ($p<0.01$) as compared to 5mg/kg pioglitazone as well. Analysis of the data on number of attempts (fig. 7b) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 76.21$; $p=$

0.0001). Post hoc analysis by Tukey's test showed increased ($p<0.01$) number of attempts to approach novel object at all three doses of pioglitazone as compared to 0mg/kg pioglitazone. While number of attempts to approach novel object were greater ($p<0.01$) in 10mg/kg pioglitazone treated rats as compared to 5mg/kg pioglitazone treated rats. Analysis of the data on exploration time (fig. 7c) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 94.36$; $p= 0.0001$). Post hoc analysis by Tukey's test showed increased ($p<0.01$) exploration time to explore novel object at all three doses of pioglitazone as compared to 0mg/kg pioglitazone. While exploration time was also greater ($p<0.01$) in 10mg/kg pioglitazone treated rats as compared to 5mg/kg pioglitazone treated rats.

Fig. 8 shows dose dependent effects of pioglitazone on biogenic amines and metabolites in rat whole brain. Analysis of the data on dopamine levels (fig. 8a) by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 74.32$; $p= 0.0001$). Post hoc analysis by Tukey's test showed increased ($p<0.01$) levels of dopamine at 10mg/kg and 15mg/kg pioglitazone as compared to 0mg/kg pioglitazone. Fig. 8b shows dose dependent effects of pioglitazone on levels of DOPAC in rat whole brain. Analysis of the data by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 64.32$; $p= 0.0001$). Post hoc analysis by Tukey's test showed increased ($p<0.01$) levels of DOPAC at 5mg/kg and 10mg/kg pioglitazone as compared to 0mg/kg pioglitazone. While levels of DOPAC were also greater ($p<0.01$) in 10mg/kg pioglitazone treated rats as compared to 5mg/kg pioglitazone treated rats.

Fig. 8c shows dose dependent effects of pioglitazone on levels of HVA in rat whole brain. Analysis of the data by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 84.32$; $p= 0.0001$). Post hoc analysis by Tukey's test showed increased ($p<0.01$) levels of HVA at 10mg/kg pioglitazone as compared to 0mg/kg and 5mg/kg pioglitazone treated rats. Fig. 8d shows dose dependent effects of pioglitazone on levels of 5HT in rat whole brain. Analysis of the data by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 84.32$; $p= 0.0001$). Post hoc analysis by Tukey's test showed increased ($p<0.01$) levels of 5HT at 5mg/kg pioglitazone as compared to 0mg/kg pioglitazone. While levels of 5HT were lower ($p<0.01$) in 10mg/kg and 15mg/kg pioglitazone treated rats as compared to 5mg/kg pioglitazone treated rats.

Fig. 8e shows dose dependent effects of pioglitazone on levels of 5HIAA in rat whole brain. Analysis of the data by one-way ANOVA showed significant effects of different doses of pioglitazone ($df= 3,20$; $F= 84.32$; $p= 0.0001$).

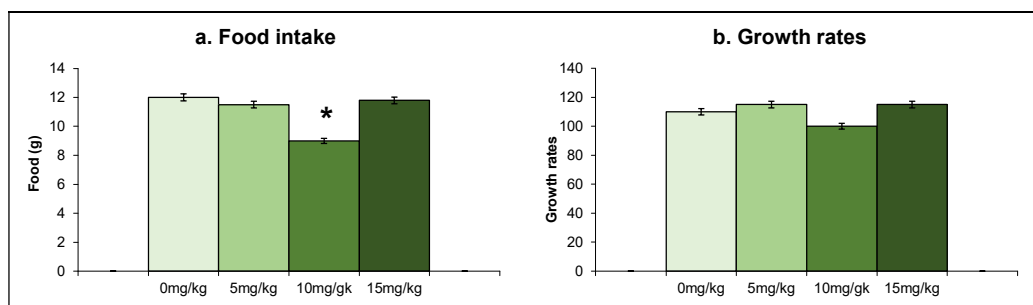


Fig. 1: Dose dependent effects of pioglitazone on (a) food intake and (b) growth rates. Values are means $\pm$ SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to 0mg/kg pioglitazone treated rats, following one-way ANOVA.

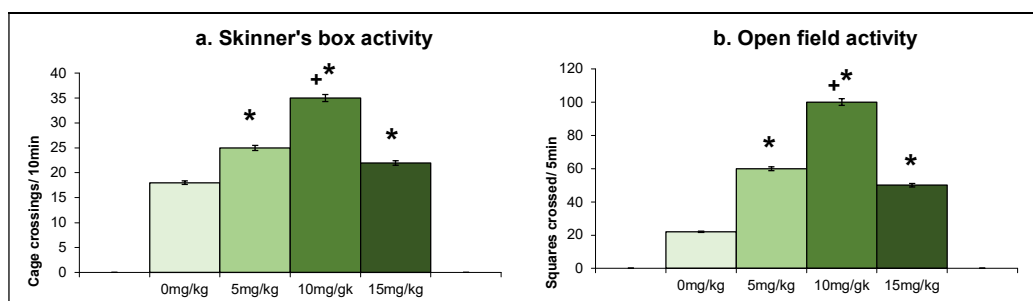


Fig. 2: Dose dependent effects of pioglitazone on Skinner's box activities. Values are means $\pm$ SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to 0mg/kg pioglitazone treated rats; +p<0.01 as compared to respective 5mg/kg pioglitazone treated rats, following one-way ANOVA.

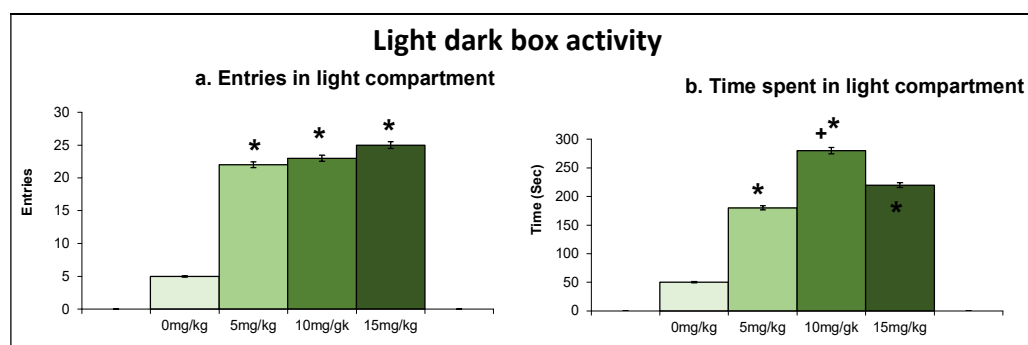


Fig. 3: Dose dependent effects of pioglitazone on light dark box activity. Values are means $\pm$ SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to 0mg/kg pioglitazone treated rats; +p<0.01 as compared to respective 5mg/kg pioglitazone treated rats, following one-way ANOVA.

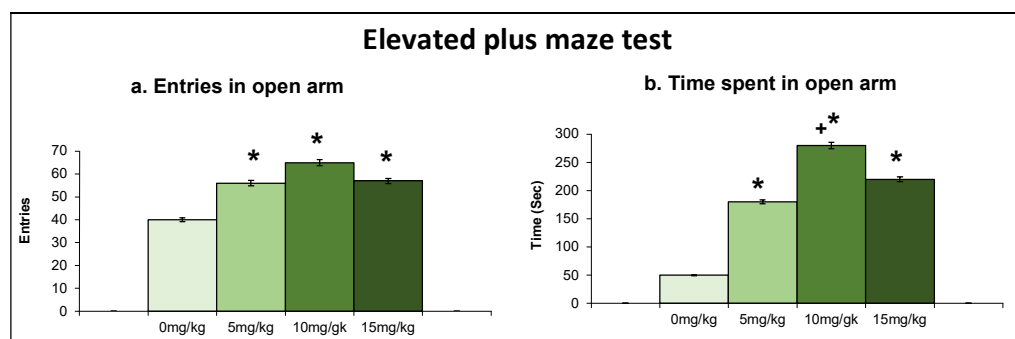


Fig. 4: Dose dependent effects of pioglitazone on elevated plus maze activity. Values are means $\pm$ SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to 0mg/kg pioglitazone treated rats; +p<0.01 as compared to respective 5mg/kg pioglitazone treated rats, following one-way ANOVA

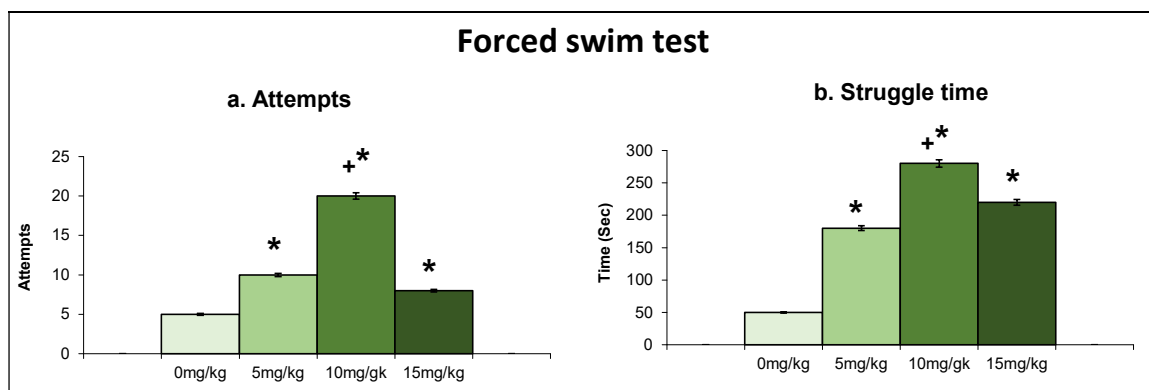


Fig. 5: Dose dependent effects of pioglitazone on forced swim test. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to 0mg/kg pioglitazone treated rats; +p<0.01 as compared to respective 5mg/kg pioglitazone treated rats, following one-way ANOVA.

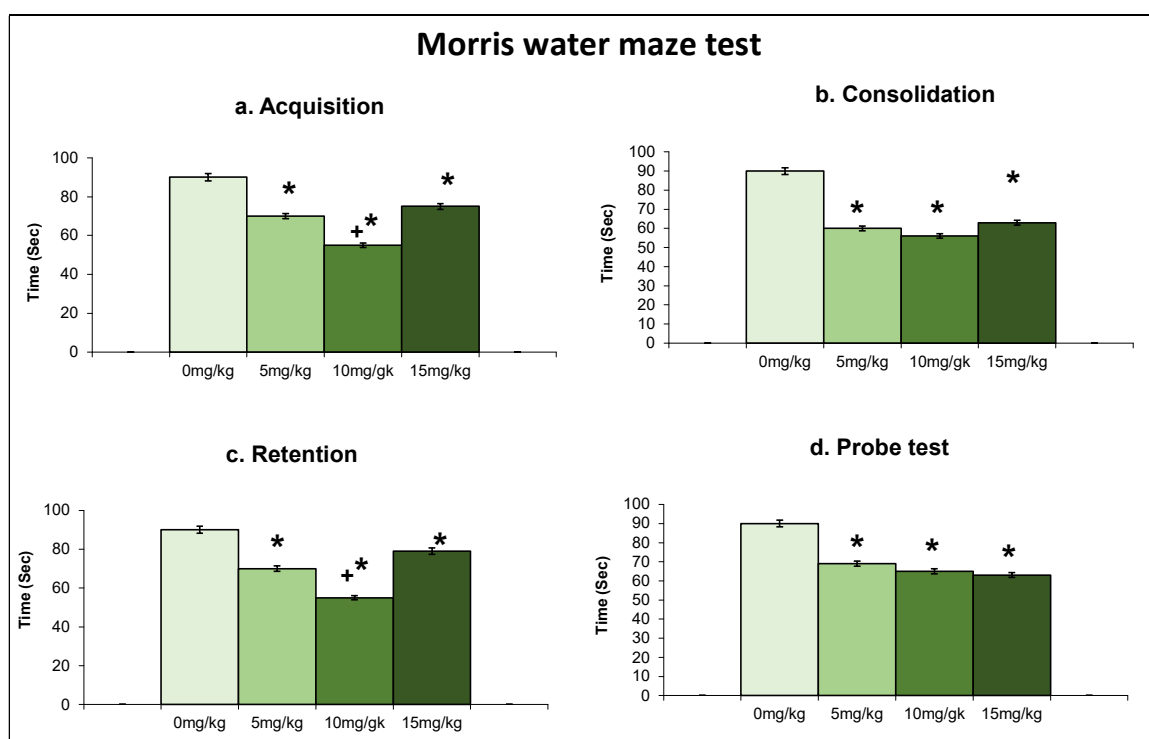


Fig. 6: Dose dependent effects of pioglitazone on Morris water maze test. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to 0mg/kg pioglitazone treated rats; +p<0.01 as compared to respective 5mg/kg pioglitazone treated rats, following one-way ANOVA.

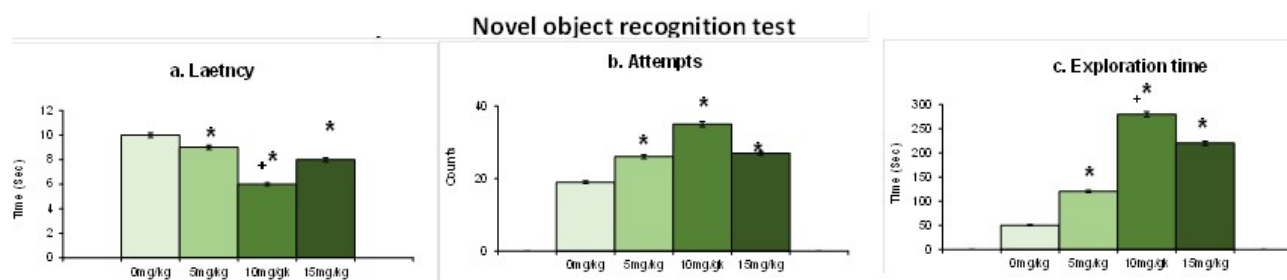


Fig. 7: Dose dependent effects of pioglitazone on novel object recognition test. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to 0mg/kg pioglitazone treated rats; +p<0.01 as compared to respective 5mg/kg pioglitazone treated rats, following one-way ANOVA.

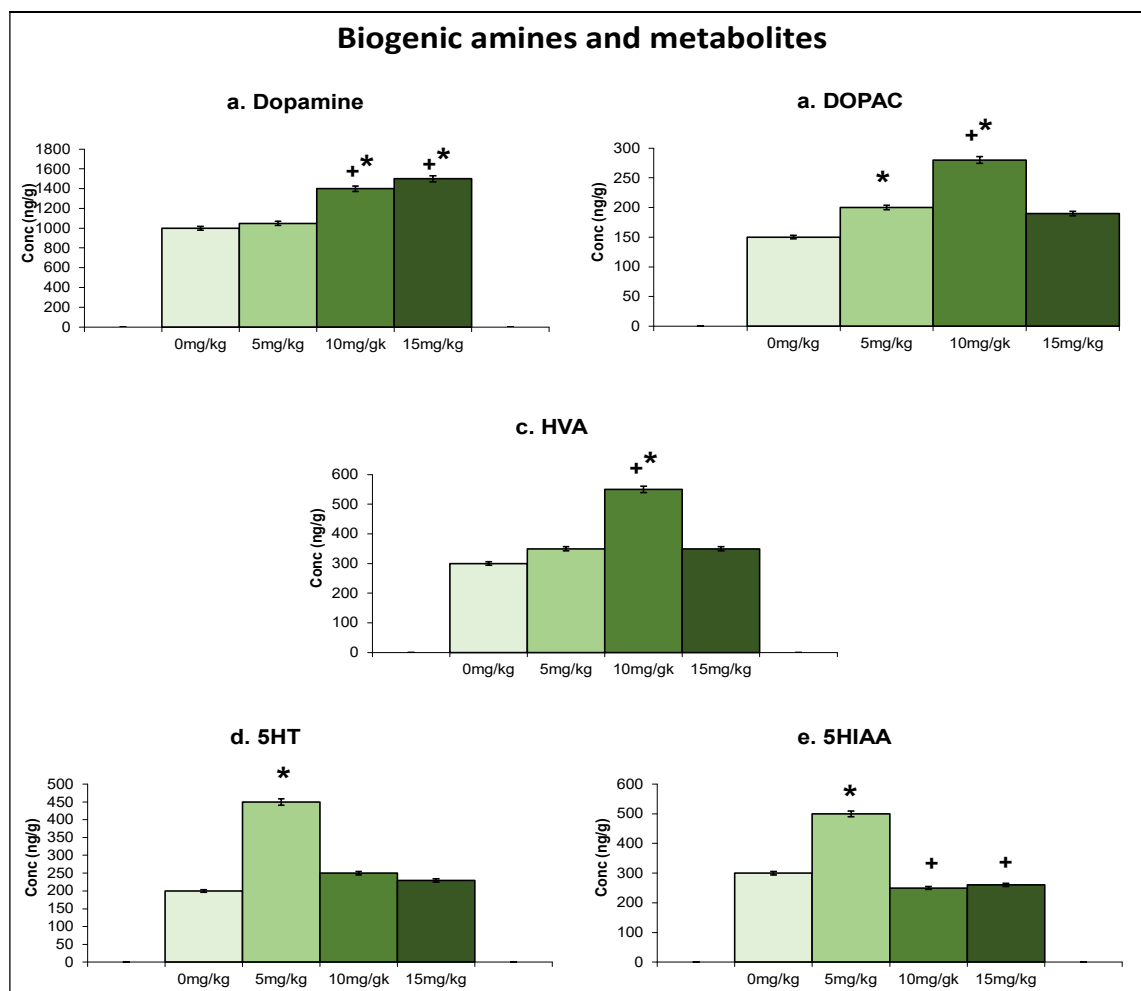


Fig. 8: Dose dependent effects of pioglitazone on levels of biogenic amines and metabolites in rat whole brain. Values are means $\pm$ SD (n=6). Significant differences by Tukey's test: * $p<0.01$ as compared to 0mg/kg pioglitazone treated rats; + $p<0.01$ as compared to respective 5mg/kg pioglitazone treated rats, following one-way ANOVA.

Post hoc analysis by Tukey's test showed increased ($p<0.01$) levels of 5HIAA at 5mg/kg pioglitazone as compared to 0mg/kg pioglitazone. While levels of 5HIAA were decreased ($p<0.01$) in 15mg/kg pioglitazone treated rats as compared to 0mg/kg pioglitazone treated rats. Levels of 5HIAA in 10mg/kg and 15mg/kg pioglitazone treated rats were also decreased ($p<0.01$) as compared to respective 5mg/kg pioglitazone treated rats.

DISCUSSION

In the present study pioglitazone was administered orally at three different doses i.e., 5, 10 and 15mg/kg, out of which a decrease in food intake of 10mg/kg pioglitazone administered rats was observed as compared to 0mg/kg administered rats. Others have reported increased food intake following 25mg/kg pioglitazone administration (Quaresma *et al.*, 2016). Though pioglitazone administration is associated with weight gain, present results show no significant effects of pioglitazone on growth rates at any dose. This might be due to differences

in hypoglycemic efficacy at different doses of the drug (Zhang *et al.*, 2021).

Results of this experiment showed an increase in activity by pioglitazone at the dose of 10mg/kg as monitored in familiar environment of Skinner's box as well as in the novel environment of open field. Open field is a sensitive method for measuring gross and fine locomotor activity. Results showed significant increase in open field activity in rats administered with 10mg/kg. This shows hyperlocomotive effects of pioglitazone as anxiolytic compounds and screening of novel drug targets also involves use of open field and increased activity in open field test is used to study neurobiological basis of anxiolytic effects of such compounds (Kraeuter *et al.*, 2019).

Increased activity in light dark activity box and elevated plus maze further validate these anxiolytic effects of pioglitazone. Light dark box activity shows determines effects of drug on anxiety. An increase in time spent in

light compartment shows anxiolytic properties of the drug and vice versa (Pirino *et al.*, 2022). Results from the present study show dose dependent increase in number of entries in light compartment at all the three doses. Elevated plus maze is widely used test to assess anxiety responses of rats and the result for this particular test shows the rats administered with 10mg/kg time took least time to enter open arm as well as time spent in the same. In rodents, measuring anxiety-like behaviors involves elevated plus maze test. Conditions such as posttraumatic stress disorder and other related disorders could be studied in detail using this animal model. If the animal is spending more time in the enclosed arms of the maze, an aversion to open spaces, would be inferred as anxiogenic property of tested drug (Kraeuter *et al.*, 2019). Forced swim test results from this study showed increment in struggle time at all the three doses or pioglitazone with more potentiation at 10mg/kg. This suggests anti-depressant effect of pioglitazone with increasing dose more specifically at 10mg/kg dose. Forced swim test is extensively used to evaluate depression-like states. In an inescapable situation, coping strategies could be evaluated in forced swim test setup (Armario *et al.*, 2021; Yankelevitch-Yahav *et al.*, 2015). Others have reported significantly reduced immobility time at the dose of 20mg/kg. NMDA receptors are reported to be involved in mediating antidepressant effects of pioglitazone. Pioglitazone is reported to have agonistic properties towards these NMDA receptors and administration of NMDA antagonists reverses antidepressant effects of pioglitazone as observed in forced swim test (Salehi-Sadaghiani *et al.*, 2012).

Morris water maze test and novel object recognition test showed that pioglitazone enhances learning and memory, preferably at the dose of 10mg/kg. It has been suggested in literature that peroxisome proliferator-activated receptor gamma (PPAR γ) agonist pioglitazone promotes spatial learning and preservation of synaptic density. Therefore, these memory enhancing effects of pioglitazone are attributed to its neuroprotective effects (Blume *et al.*, 2022). Other studies also have reported that pioglitazone can decrease inflammation as it has a neuroprotective impact and activates peroxisome proliferator-activated receptor-gamma. A considerable body of literature suggests memory enhancing, neuroprotective, antioxidant and anti-inflammatory impact of pioglitazone (Zamanian *et al.*, 2022; Zeng *et al.*, 2022; Ogura *et al.*, 2022).

Neurochemical analysis elucidated the pioglitazone dose dependently increased dopamine metabolism, with potentiated effects at the dose of 10mg/kg. The analysis for the dose dependent effect of pioglitazone on 5HT metabolism showed increased metabolism only at the dose of 5mg/kg but not at other doses of pioglitazone. Others have reported prevention of dopaminergic neurons

degeneration by pioglitazone (De Iuliis *et al.*, 2022). Most peripheral serotonin (5-HT) is synthesized in enterochromaffin cells and most circulating 5-HT is stored in platelets. Numerous clinical trials have focused on increasing 5-HT activation in the central nervous system, including those involving anti-obesity drugs currently in the market. Recent studies have revealed that both the peripheral and central serotonergic systems play a vital role in diabetes and its complications (Cai *et al.*, 2022).

CONCLUSION

Pioglitazone administration showed increasing learning and memory activity in a dose dependent manner with more potentiated effects monitored at the dose of 10mg/kg. Significant effects of this dose were also monitored on dopamine and 5HT metabolism. Results have shown that moderate (10mg/kg) dose of pioglitazone is the optimum dose, which could increase the cognitive performance. Therefore, this dose of pioglitazone could be used for the treatment of learning and memory disorders like Alzheimer's disease, ADHD etc.

REFERENCES

- Alhowail A, Alsikhan R, Alsaud M, Aldubayan M and Rabbani SI (2022). Protective effects of pioglitazone on cognitive impairment and the underlying mechanisms: a review of literature. *Drug Des. Devel. Ther.*, **16**(1): 2919-2931.
- Armario A (2021). The forced swim test: Historical, conceptual and methodological considerations and its relationship with individual behavioral traits. *Neurosci. Biobehav. Rev.*, **128**(1): 74-86.
- Blume T, Deussing M, Biechele G, Peters F, Zott B, Schmidt C, Franzmeier N, Wind K, Eckenweber F, Sacher C, Shi Y, Ochs K, Kleinberger G, Xiang X, Focke C, Lindner S, Gildehaus FJ, Beyer L, von Ungern-Sternberg B, Bartenstein P, Baumann K, Adelsberger H, Rominger A, Cumming P, Willem M, Dorostkar MM, Herms J and Brendel M (2022). Chronic PPAR γ stimulation shifts amyloidosis to higher fibrillarity but improves cognition. *Front. Aging Neurosci.*, **14**(1): 854031.
- Cai Y, Li X, Zhou H and Zhou J (2022). The serotonergic system dysfunction in diabetes mellitus. *Front. Cell Neurosci.*, **16**(1): 899069.
- De Iuliis A, Montinaro E, Fatati G, Plebani M and Colosimo C (2022). Diabetes mellitus and Parkinson's disease: dangerous liaisons between insulin and dopamine. *Neural Regen. Res.*, **17**(3): 523-533.
- Giglio RV, Papanas N, Rizvi AA, Ciaccio M, Patti AM, Ilias I, Pantea Stoian A, Sahebkar A, Janez A and Rizzo M (2022). An update on the current and emerging use of thiazolidinediones for type 2 diabetes. *Medicina (Kaunas)*, **58**(10): 1475.

- Haleem DJ, Salman T, Nawaz S and Ikram H (2022). Co-treatment with low doses of buspirone prevents rewarding effects of methylphenidate and upregulates expression of 5-HT1A receptor mRNA in the nucleus accumbens. *Behav. Brain Res.*, **418**(1): 113660.
- Ho CC, Yang YS, Huang CN, Lo SC, Wang YH and Kornelius E (2022). The efficacy of pioglitazone for renal protection in diabetic kidney disease. *PLoS One*, **17**(2): e0264129.
- Ikram H, Ahmed S and Haleem DJ (2020). Restraint-induced behavioral deficits are attenuated or impaired by pre- or post-injection of apomorphine: A context-based study. *Pak. J. Pharm. Sci.*, **33**(3): 961-968.
- Ikram H and Haleem DJ (2019). Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's dementia. *Pak. J. Pharm. Sci.*, **32**(2): 555-562.
- Kourosh-Arami M, Komaki A and Zarrindast MR (2023). Dopamine as a potential target for learning and memory: contribution to related neurological disorders. *CNS Neurol. Disord. Drug Targets*, **22**(4): 35440323.
- Kraeuter AK, Guest PC and Sarney Z (2019). The open field test for measuring locomotor activity and anxiety-like behavior. *Methods Mol. Biol.*, **1916**(1): 99-103.
- Kumar AP, Kumar BRP, Jeyarani V, Dhanabal SP and Justin A (2021). Glitazones, PPAR- γ and Neuroprotection. *Mini Rev. Med. Chem.*, **21**(12): 1457-1464.
- Liu B, Li X, Theeuwes J and Wang B (2022). Long-term memory retrieval bypasses working memory. *Neuroimage*, **261**(1): 119513.
- Nascimento MT, Cordeiro RSO, Abreu C, Santos CP, Peixoto F, Duarte GA, Cardoso T, de Oliveira CI, Carvalho EM and Carvalho LP (2022). Pioglitazone, a peroxisome proliferator-activated receptor- γ agonist, downregulates the inflammatory response in cutaneous leishmaniasis patients without interfering in leishmania braziliensis killing by monocytes. *Front Cell Infect. Microbiol.*, **12**(1): 884237.
- Ogura J and Yamaguchi H (2022). The effectiveness of antidiabetic drugs in treating dementia: A peek into pharmacological and pharmacokinetic properties. *Int. J. Mol. Sci.*, **23**(12): 6542.
- Pirino BE, Martin CR, Carpenter BA, Curtis GR, Curran-Alfaro CM, Samels SB, Barker JM, Karkhanis AN and Barson JR (2022). Sex-related differences in pattern of ethanol drinking under the intermittent-access model and its impact on exploratory and anxiety-like behavior in Long-Evans rats. *Alcohol Clin. Exp. Res.*, **46**(7): 1282-1293.
- Prashantha Kumar BR, Kumar AP, Jose JA, Prabitha P, Yuvaraj S, Chipurupalli S, Jeyarani V, Manisha C, Banerjee S, Jeyabalan JB, Mohankumar SK, Dhanabal SP and Justin A (2020). Minutes of PPAR- γ agonism and neuroprotection. *Neurochem. Int.*, **140**(1): 104814.
- Quaresma PG, Reencober N, Zannotto TM, Santos AC, Weissmann L, de Matos AH, Lopes-Cendes I, Folli F, Saad MJ and Prada PO (2016). Pioglitazone treatment increases food intake and decreases energy expenditure partially via hypothalamic adiponectin/adipoR1/AMPK pathway. *Int. J. Obes. (Lond)*, **40**(1): 138-146.
- Salehi-Sadaghiani M, Javadi-Paydar M, Gharedaghi MH, Zandieh A, Heydarpour P, Yousefzadeh-Fard Y and Dehpour AR (2012). NMDA receptor involvement in antidepressant-like effect of pioglitazone in the forced swimming test in mice. *Psychopharmacology, (Berl)*. **222**(1): 345-55.
- Shaham N, Chandra J, Kreiman G and Sompolinsky H (2022). Stochastic consolidation of lifelong memory. *Sci. Rep.*, **12**(1): 13107.
- Singh A, Dargar SK, Gupta A, Kumar A, Srivastava AK, Srivastava M, Kumar Tiwari P and Ullah MA (2022). Evolving long short-term memory network-based text classification. *Comput. Intell. Neurosci.*, **2022**(1): 4725639.
- Tomlinson B, Chan P and Lam CWK (2022). An overview of alogliptin + pioglitazone for the treatment of type 2 diabetes. *Expert Opin. Pharmacother.*, **23**(1): 29-42.
- Wolosowicz M, Prokopiuk S and Kaminski TW (2022). Recent advances in the treatment of insulin resistance targeting molecular and metabolic pathways: Fighting a losing battle? *Medicina (Kaunas)*, **58**(1): 472.
- Yankelevitch-Yahav R, Franko M, Huly A and Doron R (2015). The forced swim test as a model of depressive-like behavior. *J. Vis. Exp.*, **97**(1): 52587.
- Zamanian MY, Taheri N, Oplencia MJC, Bokov DO, Abdullaev SY, Gholamrezaipoor M, Heidari M and Bazmandegan G (2022). Neuroprotective and Anti-inflammatory Effects of Pioglitazone on Traumatic Brain Injury. *Mediators Inflamm.*, **2022**(1): 9860855.
- Zamanian MY, Taheri N, Oplencia MJC, Bokov DO, Abdullaev SY, Gholamrezaipoor M, Heidari M and Bazmandegan G (2022). Neuroprotective and Anti-inflammatory Effects of Pioglitazone on Traumatic Brain Injury. *Mediators Inflamm.*, **2022**: 9860855.
- Zeng H, Dumitrescu AV, Wadkins D, Elwood BW, Gramlich OW and Kuehn MH (2022). Systemic treatment with pioglitazone reverses vision loss in preclinical glaucoma models. *Biomolecules*, **12**(2): 281.
- Zhang F, Tang L, Li J, Yan Z, Li J and Tong N (2021). Comparison between pioglitazone/metformin combination therapy and sitagliptin/metformin combination therapy on the efficacy in Chinese type 2 diabetic adults insufficiently controlled with metformin: Study protocol of an open-label, multicenter, non-inferiority parallel-group randomized controlled trial. *Diabetes Metab. Syndr. Obes.*, **14**: 1243-1252.