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Dose related acute behavioral and neurochemical profile of pioglitazone

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Abstract: Diabetics are twice as likely to have depression. It's normal to have long periods of sadness and anxiety. Pioglitazone has important role in the inflammatory response, which suggests that it might have the associated anti-depressant effects being manifested by its anti-depressant profile which needs further exploration. Monitoring changes in behavioral and neurochemical profile of pioglitazone in a dose-dependent manner was the purpose of this study. Pioglitazone was injected to rats at the doses of 0mg/kg, 2.5mg/kg, 5mg/kg and 10mg/kg. Behavioral activities in open field, Skinner's box and elevated plus maze were monitored 20, 35 and 45 minutes respectively after pioglitazone injection. whole brain samples were collected following decapitation of rats one-hour after injection. Samples were kept at -70ºC till HPLC-EC analysis for neurochemical profile. Results show anxiogenic and sedative effects of pioglitazone at all three doses as indicated by Skinner's box, elevated plus maze activity and open field. Also there was an overall decreased dopamine metabolism and increased serotonin turnover. This suggests that diabetic patients using pioglitazone as a therapeutic option, may experience more potent effects of CNS depressants. Findings may help in extending therapeutics in diabetic patients suffering from anxiety and/or depression.

Keywords: Pioglitazone, depression, anxiety, serotonin, dopamine.

INTRODUCTION

Diabetic management is impaired by depression, which is more frequent among these patients. As per evidences; in comparison to general population, prevalence of depression is greater in diabetic patients. Substantial evidences demonstrate an association of wide range of adverse consequences related to diabetes, despite treatment regimen which is prescribed. These include, greater health expenditures, higher HbA1c levels and fasting glucose and impaied quality of life (Kamrul-Hasan *et al*., 2019; Khan *et al*., 2019; Alajmani *et al*., 2019). Limited information is available regarding the effects of oral anti-hyperglycemic agents on depression in diabetics.

Member of Thiazolidinedione (TZD) group of drugs, pioglitazone is also known as "insulin sensitizer". Potent insulin sensitizer; Pioglitazone, improves nonalcoholic steatohepatitis/fatty liver diseases, treats metabolic syndrome's multiple components, causes durable reduction in HbA1c and preserves pancreatic beta-cell functioning. Despite all these advantages, the side effects must also be regarded such as fractures, fluid retention and weight gain. However, these adverse effects are arguably outweighed by multiple benefits, and also could be diminished with low doses (DeFronzo *et al*., 2019). Nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-gamma) are selectively stimulated by pioglitazone. Regulation of the genes transcription which are insulin-responsive by the activation of these PPAR-gamma receptors is achieved, which in turn controls utilization, production and transport of glucose (Mosure *et al*., 2019; Seok *et al*., 2019).

Population attributable fractions advise that diabetes potentially attributes to more than 9.5 million cases of depression globally. 930,000 to 2.34 million cases of throughout the world could be prevented by a 10-25% reduction in diabetes (Chireh *et al*., 2019; Wang *et al*., 2019). Incident microvascular and macrovascular complications risk is associated with depression in people with diabetes. Both depression and diabetes complications are comorbid. However, the risk of depression in diabetics with complications is not greater than the risk of diabetic complications in depressed individuals (Nouwen *et al*., 2019).

No antidepressant efficacy of pioglitazone in the treatment of bipolar depression in non-diabetic individuals was shown, as monitored in an eight-week randomized trial to test the effectiveness of pioglitazone for pharmacotherapy of depression patients experiencing bipolar episodes (Aftab *et al*., 2019). Pioglitazone was more effective in patients with insulin resist as evaluated in several clinical trials involving depressed patients (Lin

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et al., 2015; Zeinoddini *et al*., 2015; Sepanjnia *et al*., 2012). Pioglitazone is an agonist of receptor activated by peroxisome proliferator. Additional therapeutic applications may arise due to its role in the inflammatory response modulation (Espinoza *et al*., 2019). Antidepressant effects are also exhibited by anti-inflammatory agents (Köhler-Forsberg *et al*., 2019). Associated anti-depressant and anti-anxiety effects are suggested to be there for piolgitazone resulting in its greater anti-depressant and anxiolytic profile which needs further exploration (Beheshti *et al*., 2019).

Present study was designed to monitor changes in locomotive, exploratory, and anxiety behavior along with associated neurochemical effects following treatment with pioglitazone. Hypothesis to be tested in this study is whether pioglitazone could produce anti-depressant effects and attenuate the behavioral deficits in rats or not. Results will be helpful to facilitate treatment of depressive disorders prevailing in diabetics.

MATERIALS AND METHODS

Animals

Twenty-four locally bred male Albino Wistar rats weighing 180-220gm purchased from Dow University of Health Sciences animal house were used in the study. All animals were housed individually placed in an environmentally controlled room at room temperature (25±2°C) under a 12:12 hr light/dark cycle. The animals had free access to standard rodent diet and tap water. For acclimatization the animals were kept individually in home cages for 3 days. All experiments were conducted according to the protocol approved by Institutional Animal Ethics Committee (IAEC; Ref No. 01022019).

Drug and doses

Pioglitazone (Glenmark Generics limited, India) was freshly prepared in slightly warm water and was administered orally at the doses of 2.5mg/kg, 5mg/kg and 10mg/kg respectively. Water was given to control animals at the dose of 1.0ml/kg.

Experimental protocol

Twenty four male Albino Wistar rats (8 weeks old) were housed individually in housing cages under standard conditions (temperature: 25±2°C; humidity: 40-50%; under 12hr light dark cycle). Rats were randomly divided into four groups each containing six animals: (i) water-, (ii) pioglitazone (2.5mg/kg)-, (iii) pioglitazone (5mg/kg)-, and (iv) pioglitazone (10mg/kg) injected rats. Rats were orally administered with water (1.0ml/kg) or respective doses of pioglitazone. Behavioral activities in Skinner's box, open field and elevated plus maze were monitored 20, 35, and 45 minutes post injection respectively. Animals were then decapitated one-hour post injection to collect brain samples. Samples were kept at -70ºC until neurochemical analysis by High Performance Liquid Chromatography with Electrochemical detection (HPLC-EC) was performed.

Behavioral procedures

Skinner's Box Activity

Motor-related effects of the drugs were monitored in a Perspex activity cage the "Skinner's box" (A transparent rectangular box with dimension 26x26x26 cm) with saw dust covered floor. 15 minutes before monitoring the activity animal was placed in the box for habituation. The activity was monitored as counts of cage crossings /10 minutes starting 20 minutes post injection (Ikram *et al*., 2018; Ikram and Haleem, 2017; Ikram *et al*., 2007).

Open field activity

The open field apparatus used is a box with square area of 76x76cm with walls 42cm high and the floor divided by lines into 25 equal squares. The animal was placed in the central square of the open field. Activity was recorded as number of square crossed with all four paws for 5min (Ikram and Haleem, 2011; Ikram and Haleem, 2019; Haleem and Ikram, 2013).

Elevated plus maze activity

The elevated plus maze apparatus used as an animal model of anxiety, consisted of four arms in which two were open and two were closed. The arms were of identical length (50cm) and width (10cm). The arms were joined by central area of $5cm^2$. The maze was elevated from the floor at a height of 60cm. Activity was noted as entries and time spent in open arm for 5 minutes (Ikram *et al*., 2014).

Collection of brain samples

Animals were killed 1hr post injection. The skull plates were cut and membrane covering the brain was removed with the help of fine forceps. Using spatula, brain was taken out and washed with ice-cold saline. The collected brains were immediately stored at -70ºC for the determination of dopamine and serotonin metabolism using HPLC-EC (Ikram *et al*., 2019; Ikram *et al*., 2014).

HPLC-EC analysis of dopamine (DA), 5HT and their metabolites

Biogenic amines and their metabolites were extracted with 150µL of perchloric acid (70%) from brain tissue punches (<250µg) using a simple one-step sample preparation method. A 5-µm (particle size) ODS column (4.0mm i.d and 250mm length) was used. Mobile phase comprising methanol (14%), octyl sodium sulphate (0.023%) and EDTA (0.0035%) in 0.1molL-1 Phosphate buffer of pH 2.9 was passed through the column at a constant flow rate (1.0ml min-1) with the help of a water 510 HPLC pump (Waters Corporation, USA). Brain samples were homogenized by using electrical homogenizer and subjected to centrifugation at 6000 rpm

for 20 minutes at 4ºC. Supernatant was separated and injected to HPLC-EC for neurochemical analysis. Electrochemical detection was achieved on a shimadzu LEC 6A detector (Shimadzu, Japan) at an operation potential of +0.8V (Ikram *et al*., 2012; Ikram *et al*., 2011).

STATISTICAL ANALYSIS

The results are presented as means ± SD. Data were analyzed by one-way ANOVA using SPSS ver 19.0. Post-hoc comparisons were done by Tukey's test. Values of $p<0.05$ were considered statistically significant.

RESULTS

Effects of different doses (2.5, 5 and 10mg/kg) of pioglitazone on activities in Skinner's box are shown in fig. 1. Effects of treatments on Skinner's box activity were significant (F= 41.397; df=3,20; p= 0.006) following analysis of data by one-way ANOVA. Post-hoc analysis by Tukey's test showed that all three doses of drug decreased ($p<0.01$) cage crossings as compared to saline injected controls in Skinner's box.

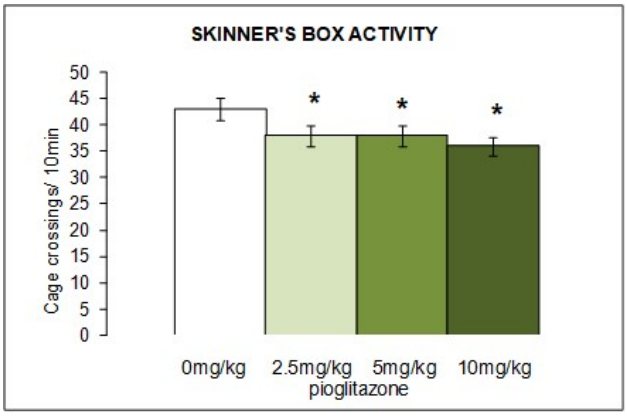

Fig. 1: Effects of different doses (2.5, 5.0 &10.0mg/kg) of pioglitazone on Skinner's box activity. Values are means ±SD (n=6). Significant differences by Tukey's test: *p<0.01 from saline injected controls following one-way ANOVA.

Fig. 2 shows effects of different doses (2.5, 5 and 10mg/kg) of pioglitazone on activities in open field. Analysis of the data by one-way ANOVA showed that effects of treatments on open field activity were significant (F=15.758; df= 3,20; p=0.001) following one-way ANOVA. Post-hoc analysis by Tukey's test showed that the drug decreased ($p<0.01$) activity at all the three doses as compared to saline injected controls in open field.

Fig. 3 shows effects of pioglitazone administration at different doses (2.5, 5 and 10mg/kg) on (a) number of entries and (b) time spent in the open arm of elevated plus maze as monitored 45min post injection for 5 minutes. Effects of treatments on number of entries (F=21.338; df=3,20; p=0.005) and time spent (F= 14.724; df=3,20; p= 0.005) were significant as analyzed by one-way ANOVA. Post hoc analysis by Tukey's test showed that all three doses of drug decreased ($p<0.01$) number of entries as well as time spent in open arm at all the three doses as compared to saline injected controls.

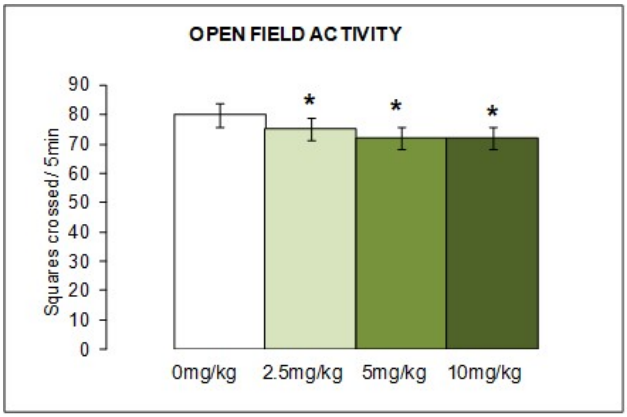

Fig. 2: Effects of different doses (2.5, 5.0 &10.0 mg/kg) of pioglitazone on open field activity. Values are means ± SD (n=6). Significant differences by Tukey's test: *p<0.01 from saline injected controls following one-way ANOVA.

Fig. 4 shows effects of pioglitazone administration at different doses (2.5, 5 and 10mg/kg) on biogenic amines and metabolise in rat whole brain. Effects of treatments on dopamine levels were significant (F= 13.87; df=3,20; p= 0.001) as analyzed by one-way ANOVA. Post hoc

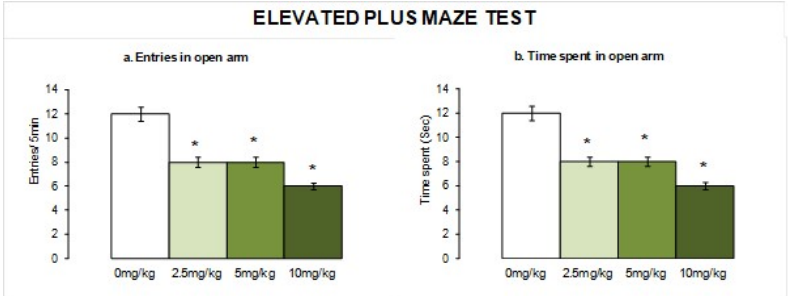

Fig. 3: Effects of different doses (2.5, 5.0 &10.0mg/kg) of pioglitazone on (a) number of entries and (b) time spent in open arm. Values are means ±SD (n=6). Significant differences by Tukey's test: *p<0.01 from saline injected controls following one-way ANOVA.

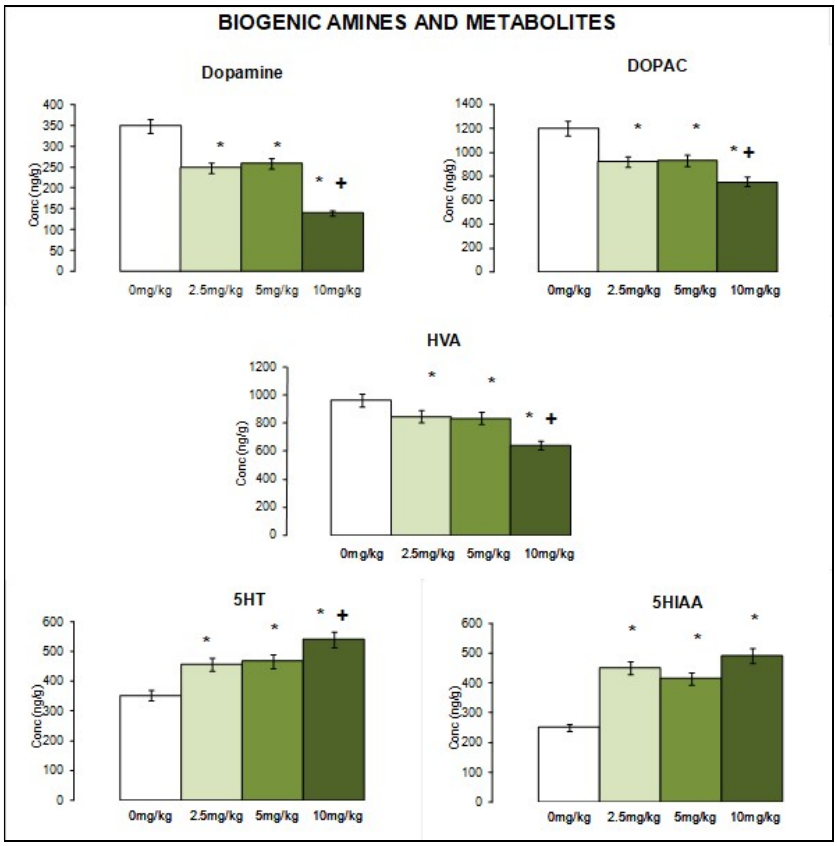

Fig. 4: Effects of different doses (2.5, 5.0 &10.0mg/kg) of pioglitazone on biogenic amines and metabolites in rat whole brain. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 from saline injected controls; +p<0.01 from 2.5mg/kg pioglitazone injected rats following one-way ANOVA.

analysis by Tukey's test showed that pioglitazone decreased (p<0.01) dopamine levels at all the three doses as compared to saline injected controls. While levels of dopamine in 10mg/kg pioglitazone injected rats were also lower (p<0.01) than 2.5mg/kg pioglitazone injected rats. Analysis of the data on DOPAC levels as analyzed by one-way ANOVA showed that effects of treatments were significant (F=14.76; df=3,20; p=0.001). Post hoc analysis by Tukey's test showed that pioglitazone decreased (p<0.01) DOPAC levels at all the three doses as compared to saline injected controls. While levels of DOPAC in 10mg/kg pioglitazone injected rats were also lower (p<0.01) than 2.5mg/kg pioglitazone injected rats. Effects on HVA levels were significant (F= 16.54; df= 3,20; p=0.001) as analyzed by one-way ANOVA. Post hoc analysis by Tukey's test showed that pioglitazone decreased (p<0.01) HVA levels at all the three doses as compared to saline injected controls. While levels of HVA in 10mg/kg pioglitazone injected rats were also lower (p<0.01) than 2.5mg/kg pioglitazone injected rats.

Analysis of the data on 5HT levels as analyzed by one-way ANOVA showed that effects of treatments were significant (F=23.87; df=3,20; p=0.001). Post hoc analysis by Tukey's test showed that pioglitazone increased (p<0.01) 5HT levels at all the three doses as compared to saline injected controls. While levels of 5HT in 10mg/kg pioglitazone injected rats were also greater (p<0.01) than 2.5mg/kg pioglitazone injected rats. Effects on 5HIAA levels were significant (F= 18.76; df= 3,20; p= 0.001) as analyzed by one-way ANOVA. Post hoc analysis by Tukey's test showed that pioglitazone increased (p<0.01) 5HIAA levels at all the three doses as compared to saline injected controls.

DISCUSSION

To evaluate the potential of pioglitazone; a thiazolidinedione compound, to produce anxiety like behavior in rodents, present study was designed. Gain in weight, breathlessness and ankle swelling are manifested due to the retention of fluid by administration of thiazolidinedione compounds (Nanjan *et al*., 2018). However, there is no documentation of changes in behavioral activities or alterations in central nervous system (CNS). Spontaneous activity in open field is one of the most important components of locomotion in rodents. Movement from one location to another is referred to as locomotor activity. Several behavioral and physiological functions are involved in locomotor activity and exploration (Zahid *et al*., 2018). Effects of various doses of pioglitazone on activities in open field and

Skinner's box revealed decreased locomotor activity. Theses results suggests that pioglitazone produces impaired motor co-ordination in these rats. Screening test for putative anxiolytic / anxiogenic compounds as well as testing of neurobiological anxiety could be achieved by using elevated plus maze as a rodent model of anxiety (Zhang *et al*., 2020). The rodent's aversion of open and elevated spaces is depicted in this model. Rodents confine their movements to enclosed spaces or to the edges of a bounded space which involves avoidance of open areas due to aversion. Restriction of movements to the open arm is translated as increased entries in enclosed arm (Mei *et al*., 2020).

Less anxious animals will spend decreased time in the open arms than anxious animals. Therefore anxiety index to be used is time spent in the open arms (Hiew *et al*., 2020). At all the three doses, pioglitazone significantly decreased time spent in open arms. As compared to low and moderate doses (2.5 and 5.0mg/kg) pioglitazone decreased entries in open arm, while at higher dose (10.0mg/kg) entries in open arm were more. It is in accordance with the previous report that increase in the time spent in open arm of the elevated plus maze is observed by anxiolytic drugs (Arora *et al*., 2020). In the present study we observed decreased time spent in open arms along with decreased number of entries in the open arm of elevated plus maze. At all three doses of pioglitazone, dopamine metabolism was also decreased in the rat whole brain (fig. 4). It has been reported by others that striatal dopamine levels as well as motor behaviours were elevated more efficiently by the higher doses of pioglitazone (30mg/kg) when injected acutely, as monitored in a rat model of Parkinson's disease (Schneider *et al*., 2020). We also monitored an overall increased serotonin metabolism in the rat whole brain (fig. 4) at all three doses of pioglitazone. When acutely administered at higher doses (40mg/kg), serotonin-induced scratching was also decreased by pioglitazone, suggesting its anti-scratching effects in mice. To some extent NO pathway as well as activation of PPAR-γ receptor initiate anti-scratching outcome of acute pioglitazone as reported by others (Elkholy *et al*., 2020). Results indicate that pioglitazone at does (2.5-10mg/kg) has quite opposing effects on serotonin and dopamine metabolism as compared to higher doses (30-40mg/kg).

CONCLUSION

Pioglitazone at the doses 2.5-10mg/kg, can cause anxiogenic and sedative effects during acute course of treatment. In diabetic patients using pioglitazone as a therapeutic option, antidepressants may produce more potent effects as pioglitazone can further enhance the CNS depressant effects of these drugs. Findings may help in extending therapeutics in diabetic patients suffering from anxiety and/or depression.

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