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Original Article

A Study on the Effect of Pioglitazone on Pharmacokinetic and Antidepressant Activity of Fluoxetine

Arjun Sapkota[1], Ankit Acharya[2]*, Shivakumar Swamy[1], Pramod Dhakal[2], Suyash Adhikari[2]

[1] Department of Pharmacology, Mallige College of Pharmacy, Bangalore-560 090, India
[2] Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, B.G.Nagara-571448, Karnataka, India

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ABSTRACT

It is often observed that diabetic patients are more often suffers from depressive disorders. Depression and diabetes are managed clinically by administering number of drugs for long duration. Hence, polypharmacy are of wide concern in drug-drug interactions which are important cause of adverse drug reactions. The present work studied the possible pharmacokinetic drug interaction between pioglitazone and fluoxetine in healthy albino rats following single and multiple dosage treatment. The effect of pioglitazone on antidepressant activity was studied using three animal models. The serum concentration of fluoxetine was estimated by HPLC and antidepressant activity was studied using despair swim test, tail suspension test and compulsive gnawing test. The concentration of serum fluoxetine was significantly increased after the pioglitazone treatment for 7 days. Maximum concentration was obtained at 4th hour after administration of pioglitazone. The pharmacokinetic parameters like AUC, AUMC, $t_{1/2}$ and Cmax of fluoxetine showed significant changes after pioglitazone treatment for one week in healthy albino rabbits. One week treatment of pioglitazone significantly decreases in immobility time of fluoxetine by despair swim and tail suspension test in rats and mice respectively. The results showed that the interaction between fluoxetine and Pioglitazone may be due to similar metabolic pathway and strong protein binding.

Keywords: Depression, Fluoxetine, Pioglitazone, Drug-drug interaction, Tail suspension test.

INTRODUCTION

Drug-drug interactions may occur when more than one therapeutic agent are administered in a patient to treat a single ailment or multiple ailments [1]. The concomitant use of multiple drugs is often desired to obtain a therapeutic objective or to treat co-existing ailments. Simultaneous use of several therapeutic agents may lead to drug-drug interactions, results in altered patient's response to therapy which may be seen by enhanced or diminished effects of one or both of the drugs or the appearance of a new effect which is not seen with either drug alone [2, 3]. There are several diseases which require lifetime treatment for their management such as hypertension and diabetes. Patients with such diseases are often prescribed with multiple drugs for the treatment of other co-existing diseases, which might be either for a short period of time or lifelong [3, 4]. So, while prescribing medication it is important to determine the incidence and frequency of occurrence of drug interactions, which shows serious implications in hospitalized patients. In addition, it is also important to find out agents that are most likely to produce hazardous interactions. In this present study, an attempted has been made to find out the possibilities of occurrences of interactions between simultaneously used drugs prescribed for treatment of the two diseases namely; diabetes and depression which may co-exist and require chronic treatment [5].

Diabetes mellitus (DM) is a chronic disorder characterized by hyperglycaemia and abnormalities in carbohydrate, fat, and protein metabolism. It results from defects in insulin secretion, insulin sensitivity or both and required lifelong treatment. Diabetes is prevalence for all age-groups worldwide and it was estimated that about 4.4%

***Corresponding Author Address: Mr Ankit Acharya**, Department of Pharmaceutics, S.A.C. College of Pharmacy, B.G. Nagar-571448, Karnataka, India*

population in 2013 was suffered from Diabetes. There are many incident that diabetic patients may also suffered from many other diseases like hypertension, depression, peptic ulcers and fungal infection, which required prolong treatment [2, 6]. Depression is also a common and chronic psychiatric disorder with diverse symptoms and high comorbidity. The underlying pathophysiology of depression remains unclear despite the seriousness and prevalence of this disorder. There are various incidences that several patients are suffering from both diabetes and depression. Among them 50% of patients with diabetes received antidepressant drugs and remaining patients consult with psychiatrist. Since in the both conditions required prolonged medication, there is much more chance of a drug-drug interaction [2, 3, 7].

In this present study possible interaction between an antidiabetic drug (pioglitazone) and antidepressant drug (fluoxetine) was determined. Pioglitazone is a thiazolidinedione antidiabetic agent and acts by decreasing insulin resistance in the periphery and in the liver resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output [8]. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and prescribed for the treatment of depression. Fluoxetine acts by desensitization of 5-HT_{1A} somatodendritic receptors and $5HT_{1B}$ nerve terminal auto receptors [9]. However, there is no any literature regarding interactions between pioglitazone and fluoxetine has been reported.

The main objective of the present study was to assess the effect of pioglitazone on pharmacokinetic and antidepressant activity of fluoxetine in healthy rat, mice and rabbits and also to suggest the alterations in the dose and frequency of administration of fluoxetine, if necessary.

MATERIAL AND METHODS

Chemical used: Pure sample of Fluoxetine and Pioglitazone was obtained as a gift sample from Time Pharma, Nepal. Tween-80, Surgical spirit, Methanol, Acetonitrile were procured from S.D Fine chemicals, Mumbai, India. All the chemicals used were of analytical grade.

Animal used: Rabbits (2-2.5 kg), rats (150-200 gm), mice (18-22 gm). All animal used were male sex and albino species.

Ethical approval: The study protocol was approved by Institutional Animal Ethics Committee (IAEC), Mallige College of Pharmacy, Bangalore, Reg. no.1432/PO/12 //CPCSEA.

Housing of experimental animals: Rabbits were housed in stainless steel cages with a fenestrated floor to allow faeces to drop through into a pan and were provided with regular rabbit chow. Rats are housed in separate clean cages. The bedding material of the cages rats were removed and replaced thrice a week with fresh materials as often as necessary to keep the animals clean and dry. The animals were provided with distilled water *ad libitum* throughout the experiment. The rats were fed with standard pelleted diet. The animals were acclimatized to standard laboratory conditions of temperature (25 ± 3°) and maintained on 12:12 h natural light: dark cycle. The animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental procedure

Effect of Pioglitazone treatment on pharmacokinetic parameters of Fluoxetine in healthy albino rabbits: Four male albino rabbits were taken and marked suitably. Rabbits were fasted for 18 h before commencing the experiment and the blood was collected (at '0' h) before the administration of fluoxetine. Later all the rabbits received fluoxetine (10 mg/kg) solution orally, the time of administration was noted.

Blood samples were collected thereafter at prefixed time intervals i.e. 0, 2, 4, 8, 16 and 24 h after dosing. Blood samples were collected in tube, kept a side and centrifuge for 15-20 min at 3000 rpm to collect serum. Serum samples were stored at 2-8° for analysis. After blood collection animals were left for a washout period of 15 days with normal diet. The next part of this experiment was conducted on the same group of animals. All the rabbits received pioglitazone (10 mg/kg) orally once a day for one week. On the 7^{th} day, 6 h after administration of the drug, the rabbits were fasted for 18 h. On the 8^{th} day, pioglitazone (5 mg/kg) was administered orally to all the animals; the time of administration was noted. After 60 min of pioglitazone administration, fluoxetine (10 mg/kg) was given orally. Blood samples were collected in a blood collection tube at prefixed time intervals i.e. 0, 2^{nd}, 4^{th}, 8^{th}, 16^{th} and 24^{th} h after fluoxetine dosing, serum was separated from blood and stored at 2-8° for analysis. The serum concentration of fluoxetine was estimated by High Performance Liquid Chromatography method [10, 11].

Effect of pioglitazone treatment on antidepressant activity of fluoxetine in healthy albino rat by despair swim test: Six Male albino rats were brought to the laboratory one day before

the experiment and were housed separately in cages with free access to food and water. Rats are individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm) containing 15 cm of water maintained at 25°.

Rats placed in the cylinder for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2-3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5-6 min immobility reaches a plateau where the rats remain immobile for approximately 80% of the time.

After 15 min in the water the rats are removed and allowed to dry for 1 h, later and the total duration of immobility is measured during a 5 min test. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. In the first part of experiment, animals were administered with fluoxetine (10 mg/kg) in a heated enclosure (32º) before being returned to their home cages. They are again placed in the cylinder 24 h and the total duration of immobility is measured during a 5 min test. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface.

In the next part of the experiment, the same group of animals after a gap of 15 days were administered with pioglitazone (10 mg/kg) once a day for one week. On the 8th day, pioglitazone (10 mg/kg) were administered to all the animals, and the time of administration was noted. After 60 min of pioglitazone administration, fluoxetine (10 mg/kg) was administered, the test was repeated and the total duration of immobility for duration of 5 min was measured at 0, 2nd, 4th, 8th, 16th and 24th h after fluoxetine administration [12].

Effect of pioglitazone treatment on antidepressant activity of fluoxetine in healthy albino mice by tail suspension test: This experiment was carried out to find out the effect of pioglitazone (15 mg/kg) treatment on the antidepressant activity of fluoxetine (10 mg/kg) by using tail suspension test in healthy albino mice.

In the first part of experiment, animals were administered with fluoxetine (10 mg/kg). The time of the drug administration was noted for all the animals. The animals were subjected to tail suspension test and the duration of immobility was measured for duration of 6 min at 0, 2nd, 4th, 8th, 16th and 24th h after drug administration. In the next part of the experiment, the same groups of animals after a gap of 15 days were administered with pioglitazone (15 mg/kg) for one week, once a day. On the 7th day, 6 h after administration of drug, the rats were fasted for 18 h. On the 8th day, pioglitazone (15mg/kg) was administered and after 1 h fluoxetine (10 mg/kg) was administered, the test was repeated and the total duration of immobility for duration of 5 min was measured [13, 14].

Compulsive gnawing in mice: Male mice having a body weight between 18-20 gm were injected with 10mg/kg apomorphine S.C. 30 min, prior to apomorphine injection the animals were treated with the test drug or the vehicle. Immediately after apomorphine injection, 6 mice were placed into a cage with wired lid. The bottom of the cage was covered with corrugated paper, the corrugation facing upwards. The mice started biting into paper causing fine holes or tearing the paper. The number of bites into the corrugated paper was evaluated by placing template upon paper. The template had 10 rectangle windows divided into 10 areas of the same size. In a total of 100 areas the number of bites was checked. In this way percentage of damaged paper was calculated. Percent gnawing of the test compound was compared with that of standard antidepressant drug imipramine, considering its value as 100% [15]. The results obtained are tabulated in table 3.

Statistical Evaluation: The data of methods are expressed as mean ± SEM for each treatment group. The data obtained from each response measures were subjected to student‘t’ test using parametric statistics, Graph Pad Prism trial version 6.01. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Serum concentration of fluoxetine before and after pioglitazone treatment in healthy albino rabbits: As shown in table 1, the serum concentration of fluoxetine at 2 h was 81.35 ng/ml and the peak concentration was at 4th h i.e. 96.10 ng/ml. It started declining at 8th h. The serum concentration of fluoxetine at 2nd, 4th, 8th, 16th and 24th h was increased after pioglitazone treatment. The peak concentration was observed at 4th h i.e. 112.2 ng/ml and started declining at 8th, 16th, 24th h. The pharmacokinetic parameters are tabulated in table 2. It revealed that AUC and AUMC of fluoxetine was changed after pioglitazone treatment. The C_{max}, AUC and AUMC of fluoxetine are increased due to pioglitazone treatment. These results revealed the absorption of fluoxetine was increased by pioglitazone treatment.

Effect of pioglitazone treatment on antidepressant activity of fluoxetine by despair swim test in healthy albino rats: As shown in figure 1, fluoxetine exhibited immobility time of 80 sec at the initial state i.e. 0 h followed by 66, 39,49, 55 and 60 sec at 2nd, 4th, 8th, 16th, and 24th h respectively. The maximum effect is shown in 4th h i.e. 39 seconds after fluoxetine treatment only. Simultaneously effect was decreased after 4th h i.e. 49, 55 and 60 second at 8th, 16th and 24th h respectively. These results confirm their antidepressant activity tested in this animal model. After a week administration of Pioglitazone alone and with fluoxetine after wash out period, data showed difference in immobility time. Pioglitazone treatment for one week increased the immobility time in healthy albino rats significantly at 2nd, 4th, 8th, 16th and 24th h. The immobility time is increased 2nd, 4th, 8th, 16th, and 24th h. But significant increase was shown in 4th, 8th, and 16th and 24th h. The least immobility time was seen in 4th h i.e. 39 sec.

Effect of pioglitazone treatment on anti-depressant activity of fluoxetine by tail suspension test in healthy albino mice: The results of tail suspension test are shown in figure 2, indicates that fluoxetine exhibited immobility time of 114 sec at the initial state i.e. 0 h followed by 94, 49,70, 85 and 120 sec at 2nd, 4th, 8th, 16th, and 24th h respectively. The maximum effect is shown in 4th hour i.e. 49 sec after fluoxetine treatment only. Simultaneously effect was decreased after 4th h i.e. 70, 85, 120 sec at 8th, 16th and 24th h respectively. Immobility time show significant changes during 4th, 8th and 16th h, but at 0 and at 24th h no significant change occurred. The effect of fluoxetine was maximum at 4th h.

After a week administration of Pioglitazone alone and with fluoxetine after wash out period day data showed different in immobility time. This treatment for one week increased the immobility time in healthy albino mice significantly. But significant increase is shown in 4th, 8th and 16th h, i.e. 82, 94, 131 respectively. However at 0, 2nd and 24th h did not showed significant difference. The least immobility time was seen in 4th h i.e. 60 sec.

DISCUSSION

In this present study possible interaction between fluoxetine and pioglitazone were determined in healthy rats, where fluoxetine and pioglitazone were used in depression and diabetes respectively. Since both the drugs are administered for longer duration and metabolized by the common enzymes CYP3A4, there was significant drug interaction which may be harmful to the patient. Hence the present study has been taken up to evaluate the influence of pioglitazone on the pharmacokinetic and antidepressant activity of fluoxetine in healthy rabbits, rats and mice. The healthy animal model served to quickly to identify the interactions. The possibility of interaction between these two drugs might be due to the alteration in the absorption site, replacement at protein binding site (distribution), metabolism site and elimination site. It was found that both the drugs are metabolized by common enzymes CYP3A4 and both of them binds to the common protein i.e. albumin.

Single administration of fluoxetine in rabbits showed its maximum serum concentration i.e. 96.10 ± 1.075 ng/ml at 4 h. Other pharmacokinetic parameters like AUC, AUMC, Cmax and MRT showed value at 1964.001 (ng/ml/h), 57083.92 (ng/ml/h), 96.10 ng/ml and 29.065 h respectively. But after co-administration of fluoxetine and Pioglitazone, serum concentration increased to 112.2 ±1.181 ng/ml and $t_{1/2}$ increased from 19.257 to 28.19 h. Other pharmacokinetic parameters have also been increased. Similarly, Tmax remained same i.e. 4th h in both the cases. This might be due to displacement of fluoxetine from the protein binding site, so the concentration of drug has been increased or other possible mechanism might be metabolism of both the drugs by same metabolic enzyme i.e. CYP 3A4.

In force swim test, immobility time of experimental animal was reduced after administration of fluoxetine. This result confirms the antidepressant activity of fluoxetine in experimental animals. It may be due to drugs stimulating the serotonergic system, such as selective serotonin reuptake inhibitors, preferentially stimulate active swimming in the water tank, drugs primarily blocking noradrenaline uptake preferentially increase climbing behavior. Swimming behavior was increased when given in combination with pioglitazone than alone. Evidence regarding the effect of pioglitazone combined with fluoxetine on animal models of depression is controversial.
In test swim test, the immobility time was increased in all hours with combination therapy rather than fluoxetine alone. It may be due to the decreased concentration of fluoxetine in mice after both drug therapies.

CONCLUSION

The present study suggested that there is an interaction when pioglitazone is co-administered with fluoxetine. The interaction between pioglitazone and fluoxetine appears to be both pharmacokinetic and pharmacodynamic interaction. The possible interactions at pharmacokinetic and

Pharmacodynamic level may be due to presence of common metabolizing enzyme CYP3A4 and interaction between agonist and antagonist at drug receptor respectively. So, the interfering effects of pioglitazone and fluoxetine must be considered if patient is consuming pioglitazone and fluoxetine together.

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Table 1: Serum concentration of fluoxetine before and after pioglitazone treatment in healthy albino rabbits

Sl. No	Time (h)	Serum concentration of Drug in ng/ml	
		Fluoxetine (10 mg/kg)	Fluoxetine (10 mg/kg) + Pioglitazone (5 mg/kg)
1	0	0	0
2	2	81.35± 1.192	96.20 ± 1.619***
3	4	96.10 ± 1.075	112.2 ± 1.181***
4	8	50.19 ± 1.701	61.44 ± 1.313**
5	16	39.35± 1.247	49.12± 1.258 **
6	24	28.22 ± 0.882	41.46± 1.232**

Where, number of rabbit per group (N) =4, values are expressed as Mean ± SEM, *p is <0.05, **P is <0.01 and ***p is <0.001.

Table 2: Data showing the pharmacokinetic parameters of fluoxetine before and after Pioglitazone treatment in healthy albino rabbits

Pharmacokinetic parameters	Fluoxetine (10 mg/k.g, *p.o.*)	Fluoxetine (10 mg/kg, *p.o.*) + Pioglitazone (5 mg/kg, *p.o.*)
AUC0-t (ng/ml/hr)	1964.001	3142.948
AUMC0-t (ng/ml/hr)	57083.92	1316.5
t1/2 (hr)	19.25	28.19
Cmax (ng/ml/hr)	96.10	112.2
Tmax (hr)	4	4
MRT (hr)	29.065	41.872

Where, AUC_{0-t} is Area under curve, $AUMC_{0-t}$ is area under first order moment curve, $t_{1/2}$ is terminal half -life, C_{max} is concentration maximum, T_{max} is time of concentration maximum, MRT is Mean residential time and p.o is per oral treatment.

Table 3: Data showing the number of bites within 1 hour interval time of Fluoxetine before and after Pioglitazone treatment in healthy albino mice using compulsive gnawing test

Time (hr)	Percentage of damage corrugated paper	
	Drug treatment	
	Apomorphine (10mg/kg) +Fluoxetine (10mg/kg)	Apomorphine (10mg/kg) +Fluoxetine (10mg/kg) +Pioglitazone (15mg/kg)
1	30	37

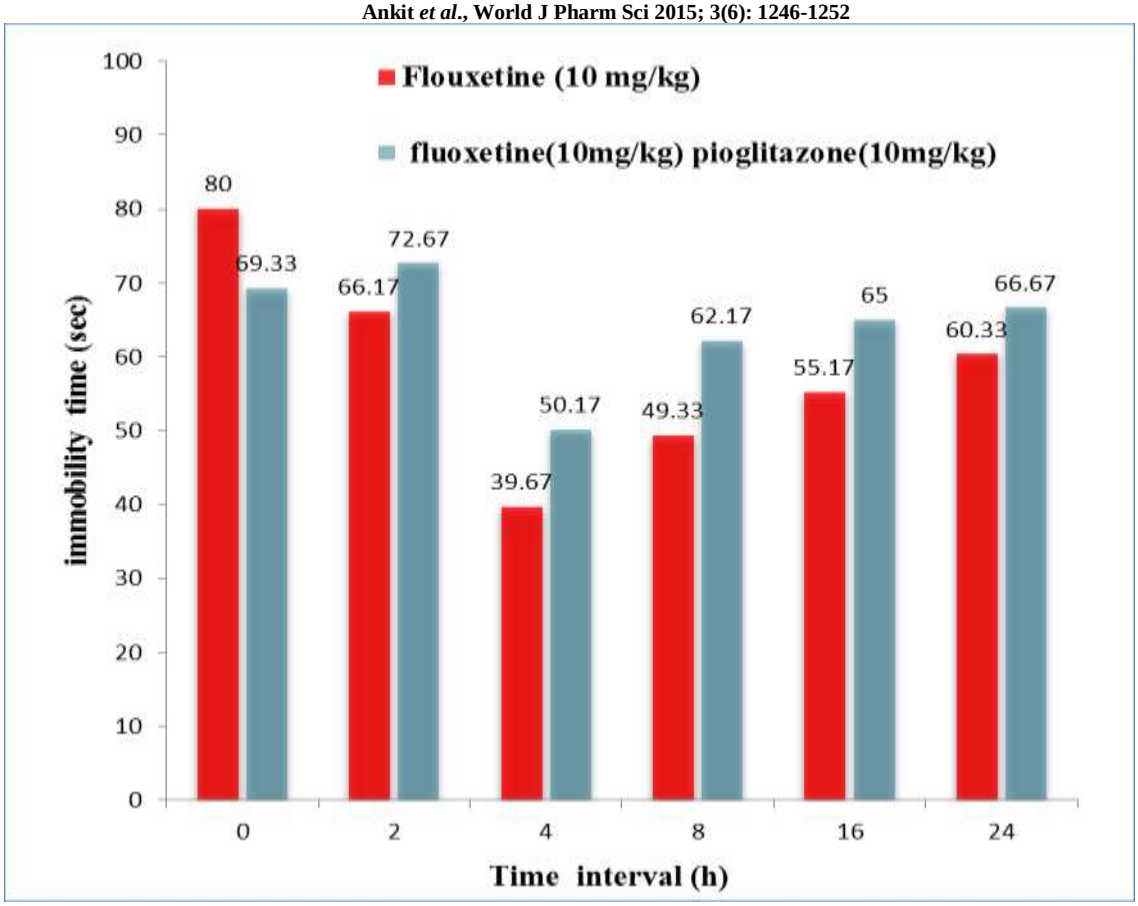

Fig. 1: Graphical representation of a pioglitazone treatment on immobility time of fluoxetine in despair swim test

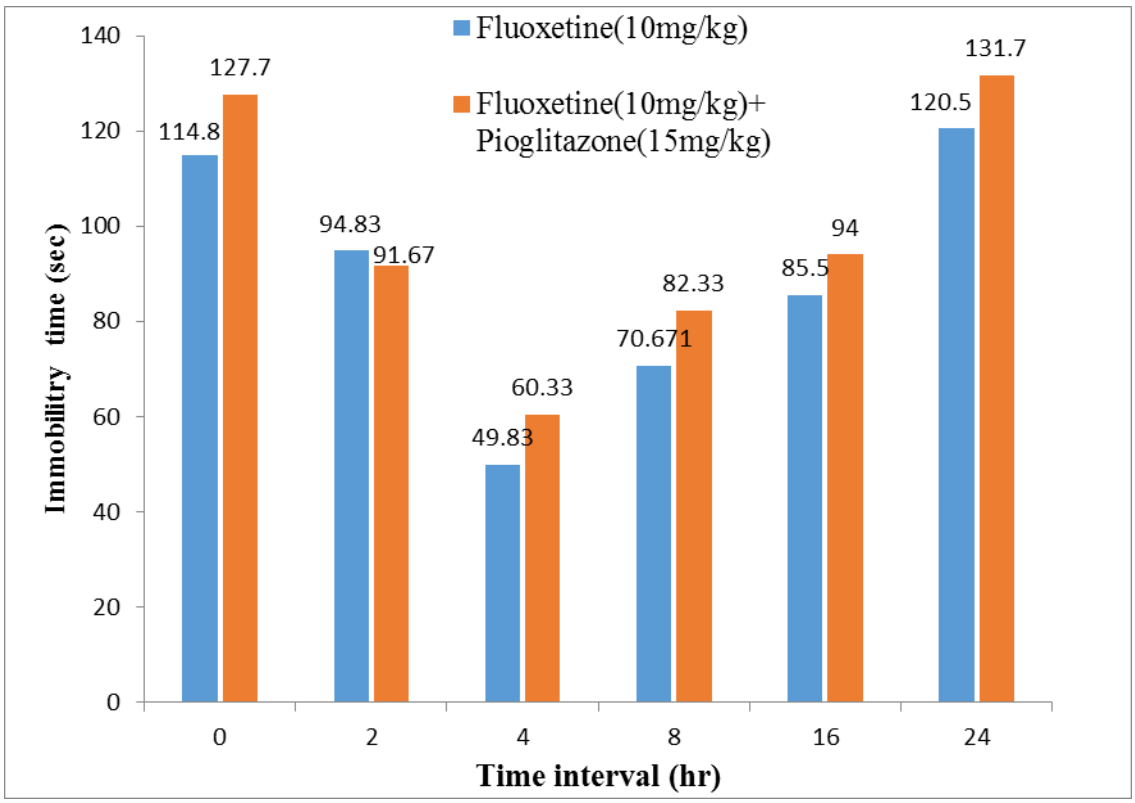

Fig. 2. Graphical representation of a pioglitazone treatment on immobility time of fluoxetine in tail suspension test

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