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Original Article

Evaluation of Acute Toxicity of Pioglitazone in Mice

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ABSTRACT

Objectives: The primary objective of the study is to assess the toxic effect of pioglitazone in mice. Pioglitazone belongs to thiazolidinedione group of oral antidiabetic agents. The earlier drug of this group troglitazone has been withdrawn due to its liver toxicity. The other drug rosiglitazone which was used like pioglitazone as insulin sensitizing agent in type 2 diabetes has been banned due to its cardiovascular side effects, recently. So, Pioglitazone was administered in high doses ¼ LD_{50} and ½ LD_{50} in mice to assess the acute toxic effect which also correlate with accidental over dose. **Materials and Methods:** Swiss albino mice of either sex weighing between 20 and 35 gm were selected. 18 mice were taken and divided into 3 groups of 6 each. The mice were kept for overnight fasting and on the following day group I (control) was administered 0.5 ml distilled water as single dose, group II (¼ LD_{50}) 500 mg/kg pioglitazone as single dose and group III (½ LD_{50}) 1000 mg/kg pioglitazone as single dose, orally. All the animals had free access to food and water after drug administration. After 24 hours, mice were sacrificed by cervical dislocation. Heart, liver and kidneys were dissected and subjected to histopathological examination. **Results:** In group I (control), the histopathological examination of heart, liver and kidneys revealed no changes. In group II (¼ LD_{50}), there was ventricular hypertrophy of heart in 4 out of 6 mice. Mild congestion of liver and kidneys was seen in 4 out of 6 and 2 out of 6 mice, respectively. In group III (½ LD_{50}), 2 mice out of 6 have died within 24 hours of pioglitazone administration. The histopathological studies of remaining 4 mice have shown ventricular hypertrophy of heart and congestion of liver and kidneys. **Conclusions:** Acute administration of large doses of pioglitazone has shown ventricular hypertrophy with congestion of liver and kidneys in mice which can happen with accidental overdose of pioglitazone in patients. It is therefore advisable not to prescribe pioglitazone in diabetic patients having congestive heart failure as well as in patients having chronic hypertension, since chronic hypertension leads to ventricular hypertrophy which might get worsened.

Key words: Acute toxicity, diabetes mellitus, pioglitazone, ventricular hypertrophy

INTRODUCTION

Diabetes mellitus is a heterogenous group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in either insulin secretion, insulin action or both.[1]

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The world wide prevalence of diabetes mellitus has risen dramatically over the two decades, from an estimated million cases in 1985 to 177 million in 2000. Based on current trends, more than 360 million individuals will have diabetes by the year 2030.[2] Type 2 diabetes is rising much more rapidly because of increasing obesity and reduced activity.

Criteria for the diagnosis of diabetes mellitus.[3]

- Symptoms of diabetes plus random blood glucose concentration ≥ 11.1 mM (200 mg/dL) or

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- Fasting plasma glucose ≥ 7.0 mM (126 mg/dL) or
- Two-hour plasma glucose ≥ 11.1 mM(200 mg/dL) during an oral glucose tolerance test.
- Hb A_{1c} ≥ 6.5%.

The disease states underlying the diagnosis of diabetes mellitus are now classified in to four categories: Type 1, Insulin dependent diabetes; Type 2, Non-insulin dependent diabetes; Type 3, Other specific types; Type 4, Gestational diabetes mellitus (Expert committee, 2003).[4]

Type 2 diabetes is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production and abnormal fat metabolism.[2]

Insulin is the mainstay for the treatment of virtually all Type 1 diabetes mellitus and many Type 2 diabetes mellitus.[5] Type 2 diabetes mellitus can be managed by diet, exercise, oral anti-diabetic agents and insulin in certain conditions.

Oral anti-diabetic agents consist of insulin secretogogues-sulfanylureas 1st and 2nd generations, meglitinides, biguanides, thiozolidinediones, a-glucosidase inhibitors, incretin-based therapies, and amylin analogs.[4]

Thiozolidinediones were introduced in 1997 as the insulin sensitisers.[5] The first of these agents, troglitazone was associated with the rare development of idiosyncratic liver toxicity, which could progress to hepatic failure and death, and troglitazone was withdrawn from the market in March 2000.[6] Patients using rosiglitazone have experienced a number of serious side effects including cardiovascular events and adverse effects on lipid profile leading to its ban in India in 2010.[7]

In thiozolidinediones group, pioglitazone is more widely used drug. Hence, the present study was undertaken to assess the acute toxicity of pioglitazone which can also occur in the accidental overdose where a very few reports are available of pioglitazone acute toxicity.

MATERIALS AND METHODS

Materials

Animals

Swiss albino mice for the study were obtained from the animal house of our institute. The study was approved by the institutional animal ethics committee of the institute and the experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Pioglitazone

Pioglitazone, $C_{19}H_{20}N_2O_3S$, has a molecular weight of 392.90. Pioglitazone (Pioz, Usv Limited, Mumbai, India) was procured from the market, dissolved in distilled water and administered to the mice. Its LD_{50} is 2000 mg/kg in mice, given orally.

Methods

Swiss albino mice of either sex weighing between 20 and 35 g were selected for the experiment. A total of 18 mice were taken and divided into 3 groups of 6 each as Control, Test ¼ LD_{50} and Test ½ LD_{50}. The mice in groups were marked and kept for overnight fasting in separate labeled cages. On the following day, the mice were administered as scheduled below.[8]

Group I (contol) - 0.5 ml distilled water, single dose.
Group II (¼ LD_{50}) - 500 mg/kg pioglitazone, single dose.
Group III (½ LD_{50}) - 1000 mg/kg pioglitazone, single dose.

All the animals had free access to food and water after drug administration. After 24 hrs, they were sacrificed by cervical dislocation. Heart, liver and kidneys were dissected and subjected to histopathological examination.

RESULTS

Group I (control) Treated with 0.5 ml distilled water, single dose, oral.

In this group, the histopathological examination of heart, liver and kidneys revealed no changes [Table 1 and Figures 1-3].

Group II (¼ LD_{50}) Treated with 500 mg/kg of pioglitazone, single dose, oral.

There was ventricular hypertrophy of heart in 4 out of 6 mice [Table 2 and Figure 4]. Liver histopathological studies have shown mild congestion in 4 out of 6 mice [Table 2 and Figure 5]. Mild congestion of kidneys was seen in 2 out of 6 mice [Table 2 and Figure 6].

Table 1: Group I (control). Treated with 0.5 ml distilled water, single dose, oral

S. No.	Mouse body weight	Distilled water, single dose	Histopathology		
			Heart	Liver	Kidney
1.	27 g	0.5 ml	Normal	Normal	Normal
2.	32 g	0.5 ml	Normal	Normal	Normal
3.	25 g	0.5 ml	Normal	Normal	Normal
4.	29 g	0.5 ml	Normal	Normal	Normal
5.	30 g	0.5 ml	Normal	Normal	Normal
6.	23 g	0.5 ml	Normal	Normal	Normal
Percentage of toxicity	-	-	0%	0%	0%

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Table 2: Group II (¼ LD_{50}). Treated with 500 mg/kg of pioglitazone, single dose, oral

S. No.	Mouse body weight	Pioglitazone 500 mg/kg, single dose	Histopathology		
			Heart	Liver	Kidney
1.	30 g	15.0 mg	Ventricular hypertrophy	Mild congestion	Mild Congestion
2.	34 g	17.0 mg	Normal	Normal	Normal
3.	25 g	12.5mg	Ventricular hypertrophy	Mild congestion	Normal
4.	32 g	16.0 mg	Ventricular hypertrophy	Mild congestion	Normal
5.	27 g	13.5mg	Normal	Normal	Normal
6.	25 g	12.5mg	Ventricular hypertrophy	Mild congestion	Mild Congestion
Percentage of toxicity		-	66.67%	66.67%	33.33%

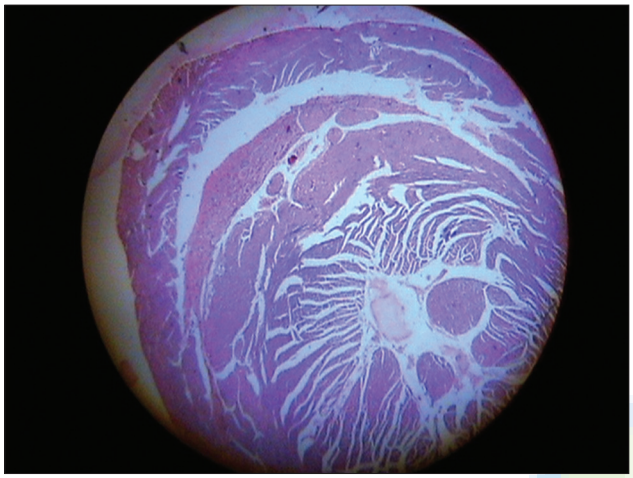


Figure 1: Group I(Conrol) Heart-Normal

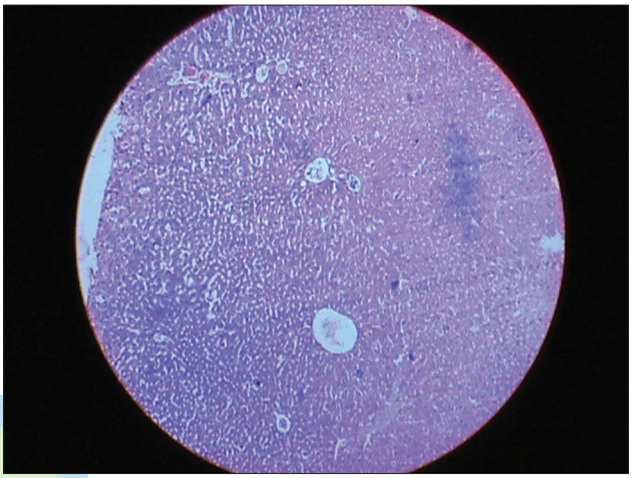


Figure 2: Gorup I Liver-Normal

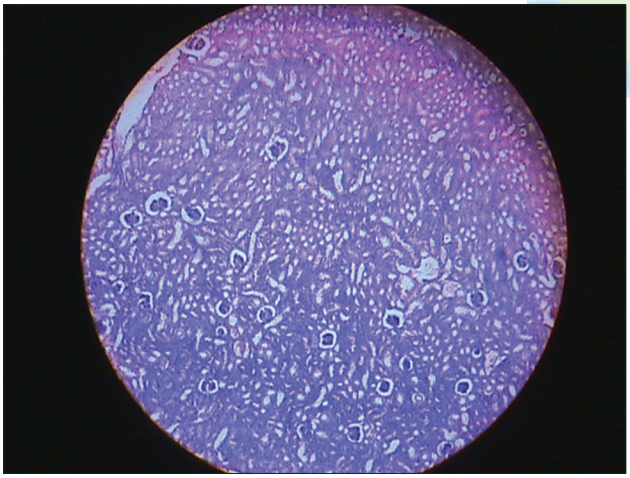


Figure 3: Group I (Control) Kidney-Normal

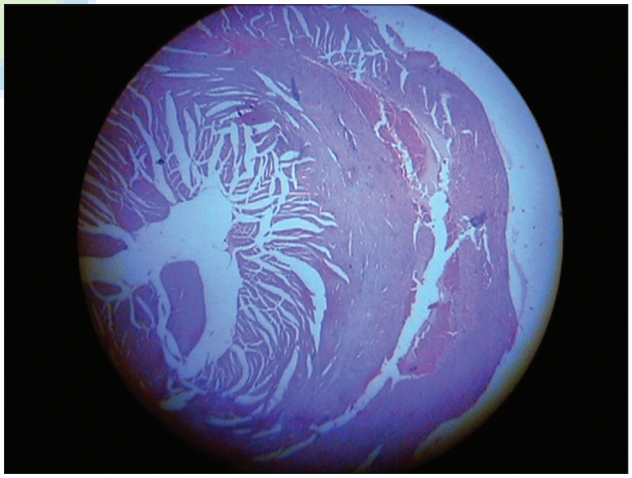


Figure 4: Group II(1/4 LD50) Heart-Ventricular Hypertrophy

Group III (½ LD_{50}) Treated with 1000 mg/kg of pioglitazone, single dose, oral.

Two mice out of 6 have died within 24 hours of pioglitazone administration.

The histopathological studies of remaining 4 mice have shown ventricular hypertrophy of heart and congestion of liver and kidneys [Table 3 and Figures 7-9].

DISCUSSION

The study was done to assess the acute toxicity of pioglitazone in mice. Pioglitazone, a thiazolidinedione, is a selective agonist of nuclear peroxisome proliferator activated receptor γ (PPAR-γ). This nuclear receptor regulates the transcription of genes encoding proteins involved in carbohydrate and lipid metabolism. It increases glucose uptake in muscles and adipose tissue. It also

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Table 3: Group III (½ LD_{50}). Treated with 1000 mg/kg pioglitazone, single dose, oral

S. No.	Mouse body weight	Pioglitazone 1000 mg/kg, single dose	Histopathology		
			Heart	Liver	Kidney
1.	29 g	29 mg	Ventricular hypertrophy	Congestion	Congestion
2.	22 g	22 mg	Ventricular hypertrophy	Congestion	Congestion
3.	30 g	30 mg	Ventricular hypertrophy	Congestion	Congestion
4.	27 g	27 mg	Ventricular hypertrophy	Congestion	Congestion
5.	25 g	25 mg	Died		
6.	28 g	28 mg	Died		
Percentage of toxicity			≥ 66.67%	≥ 66.67%	≥ 66.67%

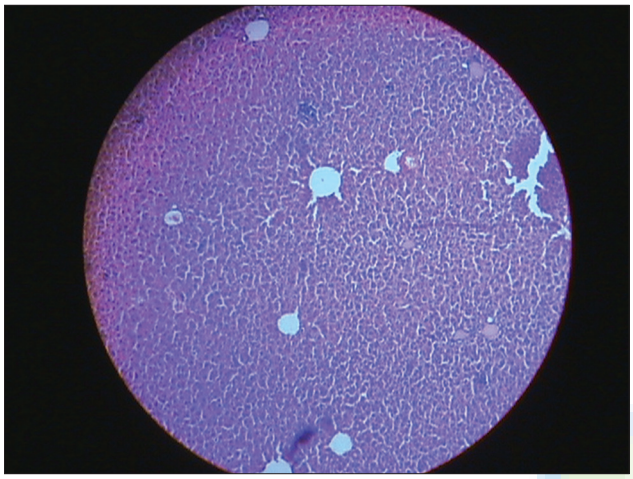


Figure 5: Group II (1/4 LD50)Liver-Mild congestion

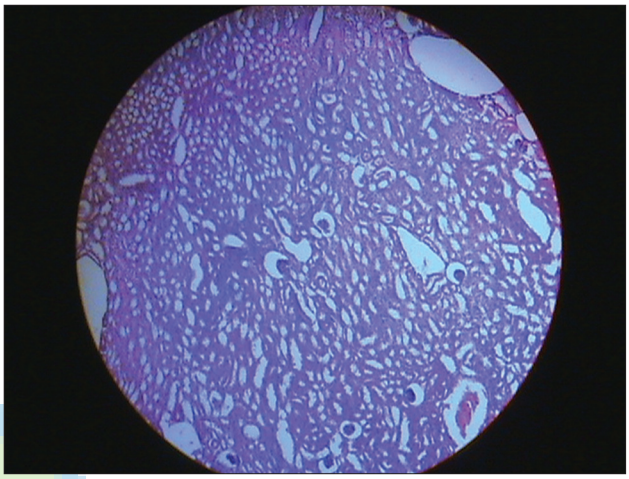


Figure 6: Group II (1/4 LD50) Kidney-Mild congestion

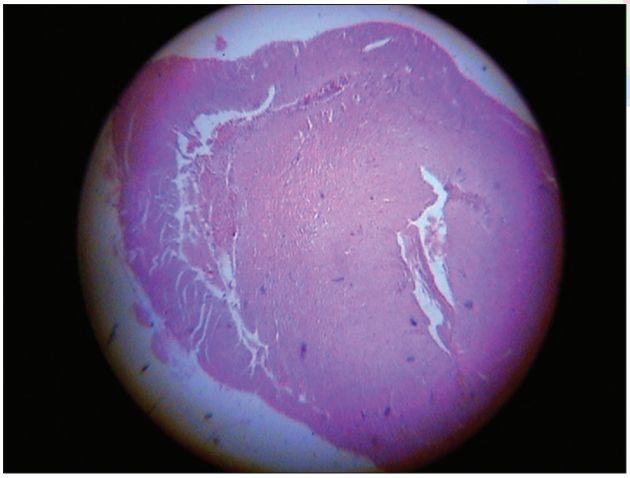


Figure 7: Group III(1/2 LD50) Heart-Ventricular Hypertrophy

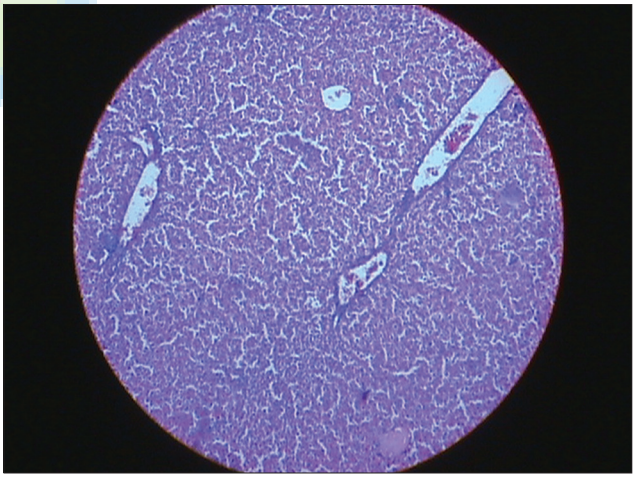


Figure 8: Group III (1/2 LD50) Liver-congestion

increases $GLUT_4$ and decreases hepatic gluconeogenesis and reduces blood glucose level.

Pioglitazone enhances insulin action on liver, adipose tissue and skeletal muscles and confer improvement in glycemic control in persons with type 2 diabetes. It is approved as a monotherapy and in combination with metformin, sulfonylureas, and insulin for the treatment of type 2 diabetes.

It is a well known fact that type 2 diabetes has an increased risk of all the manifestations of atherosclerotic vascular disease. The adverse effect of pioglitazone is edema, weight gain, reduction in hematocrit and worsening of congestive heart failure, macular edema, usually occur in association with more general fluid retention.[9] There is also an increase in the risk of bone fractures in women.[4]

Recent evidence suggests that rosiglitazone, but not

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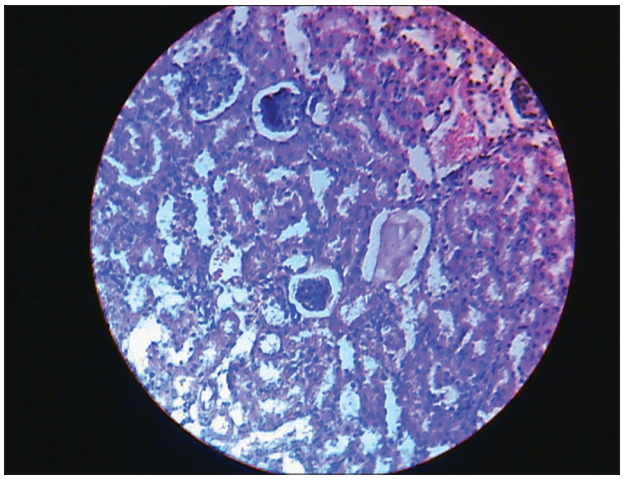


Figure 9: Group III (1/2 LD50) Kidney-congestion

pioglitazone, increases the risk of cardiovascular events (myocardial infarction, stroke). But in post marketing experience with pioglitazone, cases of congestive heart failure have been reported in patients both with and without previously suffering from heart disease.[10]

In the experiment done in mice, acute administration of large doses ¼ LD_{50} and ½ LD_{50} of pioglitazone resulted in ventricular hypertrophy with congestion in liver and kidneys which can also happen with accidental overdose of pioglitazone in patients. It is therefore advisable not to prescribe pioglitazone in diabetic patients associated with congestive heart failure as well as chronic hypertension, as chronic hypertension leads to ventricular hypertrophy which might worsen due to chronic administration of pioglitazone. Further study has to be done as how to reduce ventricular hypertrophy.

REFERENCES

1. Mizrachi E, Bernel-Mizrachi C. Diabetes mellitus and related disorders. In: Cooper DH, Krainik AJ, Lubner SJ, Reno HEL, editors. Washington manual of medical therapeutics. 32nd ed. Wolters Kluwer/Lippincott Williums and Wilkins2007; Philadelphia, PA; p. 600-23.
2. Fauci AS, Braunwald E, Casper DL, Hauser SL, Lango DL, Jameson JL, *et al*., editors. Harrison's Principles of Internal Medicine. 17th ed. The McGraw Hill Companies; 2008.New York, NY: p. 2275-304.
3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33:S62-9.
4. Martha S. Nolte. Pancreatic hormones and Antidiabetic drugs. In: Katzung BG, Masters SB, Trevor AJ, editors. Basic and clinical pharmacology. 11th ed. New Delhi: Tata McGraw Hill Education Private Limited; 2009. p. 727-51.
5. Brunton LL, Lazo JS, Parker KL, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th ed. McGraw Medical Publishing Division United States of America; 2006. p. 1613-45.
6. Watkins PB, Whitcomb RW. Hepatic dysfunction associated with troglitazone. N Engl J Med 1998;338:916-7.
7. http://www.igovernment.in/site/india-bans-diabetes-drug-rosiglitazone-38763.
8. Ghosh MN. Fundamentals of Experimental Pharmacology. 3rd ed. Kolkata: Hilton and Company; 2005. p. 190-7.
9. Kahn CR, Weir GC, King GL, Jacobson AM, Moses AC, Smith RJ, editors. Joslin's Diabetes mellitus. 14th ed. Lippincott Williams and Wilkins a Walters Kluwer Company; Boston, MA: 2006. p. 700-3.
10. PDR Staff and Physicians. Physician's Desk Reference. 63rd ed. Thomson PDR; Montvale, NJ 2009. p. 3073

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