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Evaluation of Biochemical Toxicity and Antioxidant Properties of Pioglitazone on Albino Wistar Rats

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Pioglitazone is one of the thiazolidinedione anti-diabetic drugs which have been used for the treatment of non-insulin dependent diabetes mellitus. This study aims at investigating the biochemical effects and safety of pioglitazone (PIO) at various concentrations in female Wistar rats. A total of 28 rats were randomly divided into four groups of seven animals each. Groups 1-4 were given 0.5 mL kg⁻¹ b.wt., day⁻¹ of distilled water as normal control; 15, 30 and 45 mg kg⁻¹ b.wt., day⁻¹ of PIO, respectively as treatment groups 2, 3 and 4, respectively for 28 days. Using standard biochemical kits and reported chemical procedures, plasma biochemical parameter and organ lipid peroxidation effects were determined in all the groups. There was significant increase (p<0.05) in plasma total protein concentration of group 3 and 4 in comparison with control. There was also significant (p<0.05) reduction in total and LDL cholesterols in PIO-treated groups and concentration of TBARS was reduced in the liver and heart of PIO-treated groups in comparison with normal control. There was no significant alteration in the concentrations and activities of liver and kidney function markers of PIO treated groups in comparison with normal control groups. Pioglitazone at highest concentration of 45 mg kg⁻¹ b.wt., for the duration of 28 days did not elicit any measurable biochemical toxicity on non-diabetic rat model.

Key words: Pioglitazone, hepatic biochemical markers, renal biochemical markers, lipid peroxidation

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INTRODUCTION

Diabetes has been reported as one of the five leading causes of death in the world¹. It is characterized by persistent hyperglycaemia, which is associated with abnormalities in carbohydrate, protein and lipid metabolism caused by failure of insulin secretion and/or increased cellular resistance to insulin. Type 2 diabetes mellitus is a disorder characterized by insulin resistance and a progressive decline in pancreatic β -cell function associated with increasing hyperglycaemia². A chronic hyperglycaemia of diabetes is associated with severe microvascular and macrovascular problems, which includes neuropathy, nephropathy, retinopathy, cardiovascular and peripheral vascular diseases^{3,4}. Diabetes is the most prevalent metabolic syndrome worldwide with an incidence of 2.8% across all age groups in 2000 and projected to increase to 4.4% by 2030⁵. The prevalence of diabetes in Nigeria is about 2.2% of her population, with urban communities having a higher overall prevalence than that of the rural communities^{6,7}. The number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical in-activities.

Thiozolidinediones (TZD), the insulin sensitizers used for the management of diabetes were introduced in 1997. They have been classified as novel group of insulin-sensitizing mediators, capable of improving insulin action with a post-insulin receptor mechanism of action by acting as a synthetic agonist of peroxisome proliferator-activated receptors γ (PPARs- γ) predominantly expressed in the adipocytes and to a lesser extent in the muscle and liver tissues². They alter gene transcription influencing significant changes in carbohydrate and lipid metabolism, resulting in changed amounts of protein synthesis and hence effecting metabolic alterations². Troglitazone, the first agent of TZD to be approved, was effective in controlling glycaemia but was withdrawn from the market in 2000 following reports of fatal hepatotoxicity. The prospect of rosiglitazone is undecided owing to a possible risk of myocardial infarction and cardiovascular-related deaths^{2,8}.

Pioglitazone (PIO) is a prescription drug, the second of the thiazolidinediones (TZD) class with hypoglycemic activity for the management of diabetes. It can be used as monotherapy or in combination with metformin or sulphonylureas for the management of diabetes in obese and non-obese patients who have failed other conservative measures⁹. There is limited information on the sub-chronic toxicity studies of pioglitazone in non-diabetic experimental animal model. Reported clinical cases of pioglitazone-induced side-effects with less severe consequences have been reported^{10,11}. In the few reported cases, most patients improved symptomatically 2-4 weeks following discontinuation of the drug, with normalization of relevant biomarkers¹². Acute toxicity of

pioglitazone has been associated with ventricular hypertrophy with congestion of the liver and kidney in mice¹⁰. As a result of the reported associated toxicity observed with thiozolidinediones, the use of pioglitazone in the management of type 2 diabetes and the paucity of report on sub-chronic toxicity of pioglitazone. The present study was undertaken to assess the toxicological effects of pioglitazone in a 28 day study design.

MATERIALS AND METHODS

Materials: Pioglitazone hydrochloride, $C_{19}H_{20}N_2O_3S.HCl$, with molecular weight of 392.90 (CAS 112529-15-4) was purchased from Tokyo Chemicals Industry Japan, while all other chemicals were from Sigma-Aldrich (USA) or Randox Laboratories Ltd., UK and were of analytical grade.

Animals: Albino Wistar rats (n = 28, females) weighing (150-200 g) were used for the study. Animals were procured from the Central Animal House of the Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State.

Sub-acute toxicity: All the animals were acclimatized for 14 days under standard husbandry conditions. The animals were housed in standard laboratory cages and maintained under standard laboratory conditions. Rats had free access to food and water *ad libitum* throughout the experimental period, which was replenished daily. Animal handling and experimental procedures were according to international procedures. Ethical approval was obtained from Covenant University Ethics Committee, Ogun state and Nigeria Institute of Medical Research (NIMR) Ethics Committee, Lagos state with project approval number IRB/13/227. Rats were randomized into four groups of seven animals each. They were weighed before the commencement of treatment and weekly throughout the duration of the study. Group 1 represent Normal Control (NC) and received 0.5 mL of distilled water while groups 2-4 received pioglitazone in three different doses of 15, 30 and 45 mg kg^{-1} b.wt., respectively. The animals were dosed daily by gastric intubation. The physical appearance and daily activities of the rats, such as eating patterns and signs of abnormalities were observed and recorded. On the 29th day after treatment, experimental animals were put under light ether anaesthesia and latter sacrificed¹³. Blood samples were collected by cardiac puncture into heparin tubes. The lungs, hearts, intestines, kidneys and livers of rats were also excise, washed in normal saline, weighed and stored at $-20^{\circ}C$. Liver and kidney markers of organ toxicity were evaluated in the experimental animals while lipid peroxidation assessment was carried out in all excised organs.

Biochemical analyses: The blood samples in heparin tubes were centrifuged at 3000 rpm for 10 min to collect the blood plasma. The blood plasma was analysed for parameters such as total protein, albumin, urea, creatinine, triglyceride, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, cholesterol, High Density Lipoprotein-Cholesterol (HDL-C), Low Density Lipoprotein-Cholesterol (LDL-C), total bilirubin and direct bilirubin using Randox test kit (Randox Laboratories Ltd., UK).

Determination of thiobarbituric reactive substances concentration: Blood concentration of thiobarbituric acid reactive substances (TBARS) is an index of lipid peroxidation and oxidative stress. Concentration of thiobarbituric acid reactive substances (TBARS) was determined by the methods of Buege and Aust¹⁴ as described by Ogunlana *et al.*¹⁵. Aliquot of tissue homogenate (0.1 mL) in 0.04 M tri-HCl (pH 8.3) buffer was treated with 2.0 mL of TBA-TCA-HCL, 1:1:1 reagent (thiobarbituric acid (TBA) 0.37%, 0.25 N HCl and 15% (w/v) TCA) and incubated at 95°C for 15 min. The TBARS content was determined using the extinction coefficient of 155 nM⁻¹ cm⁻¹ at 535 nm.

Statistical analysis: All results are reported as means and standard errors of means (Mean±SEM). Statistical evaluations of the data were initially tested for normality and for homogeneity of variances. Comparisons between groups were performed using one way analysis of variance (ANOVA) followed by Dunnett's control comparison tests using R statistical programming language (version 3.1.1). All comparisons were made relative to untreated controls and a difference with a p<0.05 was considered significant. Data were visualized by graph pad prism version 6.

RESULTS

Table 1 shows the biochemical effect of pioglitazone on liver function markers of rats. There was a significant

increase (p<0.05) in serum Total Proteins (TP) in the 30 and 45 mg kg⁻¹ of pioglitazone treatment groups. However, no significant change (p>0.05) was observed in other liver function biochemical markers assayed in this study in all treatment groups when compared with the control. Table 2 shows the biochemical effect of pioglitazone on kidney function markers of rats. There was no observed significant change (p>0.05) in the kidney function biochemical parameters assayed in this study in all treatment groups when compared with the control. Figure 1 shows the effects of pioglitazone on lipid profile of experimental rats. There was a significant decrease (p<0.05) in total plasma cholesterols and low density lipoprotein-cholesterols was observed in pioglitazone treatment groups when compared with the control. There was no significant (p>0.05) change in the plasma levels of triglycerides and high density lipoprotein-cholesterols when compared with the control. Figure 2 shows the effects of pioglitazone on concentration of thiobarbituric acid reactive substances (TBARS) in major organs of experimental rats. There was a significant decrease (p<0.05) in the concentrations of TBARS in the liver and heart of rats in pioglitazone treated groups when compared with the control. However, no significant (p>0.05) changes were observed in the TBARS concentration of the kidneys, intestine and lungs in pioglitazone treated groups when compared to the control.

DISCUSSION

This study demonstrates that pioglitazone, an antidiabetic drug at moderately high doses of 45 mg kg⁻¹ b.wt., of rats did not exhibit a significant biochemical toxicity in the major tested organs of healthy non-diabetic rats with drug exposure for a period of 28 days. Pioglitazone, a selective agonist of nuclear peroxisome proliferator activated receptor γ (PPAR- γ) regulates the transcription of genes encoding proteins involved in carbohydrate and lipid metabolisms. It increases glucose uptake in muscles and adipose tissue and decreases hepatic

Table 1: Biochemical effect of pioglitazone on liver function markers of rats after 28 days of oral administration

Groups	AST (U L ⁻¹)	ALT (U L ⁻¹)	ALP (U L ⁻¹)	Alb (mg dL ⁻¹)	DB (mg dL ⁻¹)	TB (mg dL ⁻¹)	TP (g dL ⁻¹)
Control	3.387±1.59	6.040±3.34	13.200±2.93	3.192±0.10	0.286±0.08	0.382±0.09	8.363±0.36
15 mg kg ⁻¹	5.727±3.27	4.962±5.37	10.010±1.39	3.490±0.17	0.617±0.25	0.687±0.16	10.300±0.75
30 mg kg ⁻¹	5.428±2.06	2.427±1.50	10.950±1.71	3.626±0.12	0.370±0.09	0.470±0.09	11.570±0.51*
45 mg kg ⁻¹	5.953±1.98	3.213±3.09	8.635±3.64	3.797±0.19	0.315±0.09	0.657±0.10	13.640±0.55*

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatases, Alb: Albumin, DB: Direct bilirubin, TB: Total bilirubin, TP: Total protein. Results expressed as Mean±SEM of 7 replicates study. *Values are significantly different at p<0.05 compared with control group

Table 2: Biochemical effect of pioglitazone on kidney function markers of rats after 28 days of oral administration

Groups	Urea (mg dL ⁻¹)	Creatinine (mg dL ⁻¹)	Na ⁺ (mmol L ⁻¹)	K ⁺ (mmol L ⁻¹)	Cl ⁻ (mmol L ⁻¹)	HCO ₃ ⁻ (mmol L ⁻¹)
Control	60.84±3.55	0.820±0.07	144.5±1.71	9.250±1.31	117.5±2.10	21.50±5.14
15 mg kg ⁻¹	56.80±3.18	0.820±0.05	145.8±3.82	10.500±1.04	119.2±1.18	22.00±5.45
30 mg kg ⁻¹	47.48±5.19	0.858±0.03	143.7±2.88	10.830±1.08	119.5±6.14	20.33±5.64
45 mg kg ⁻¹	48.05±1.97	0.910±0.10	147.0±2.54	9.667±0.61	124.3±2.56	17.50±3.77

Na⁺: Sodium ions, K⁺: Potassium ions, Cl⁻: Chlorine ions, HCO₃⁻: Bicarbonate ions. Results expressed as Mean±SEM of 7 replicates study

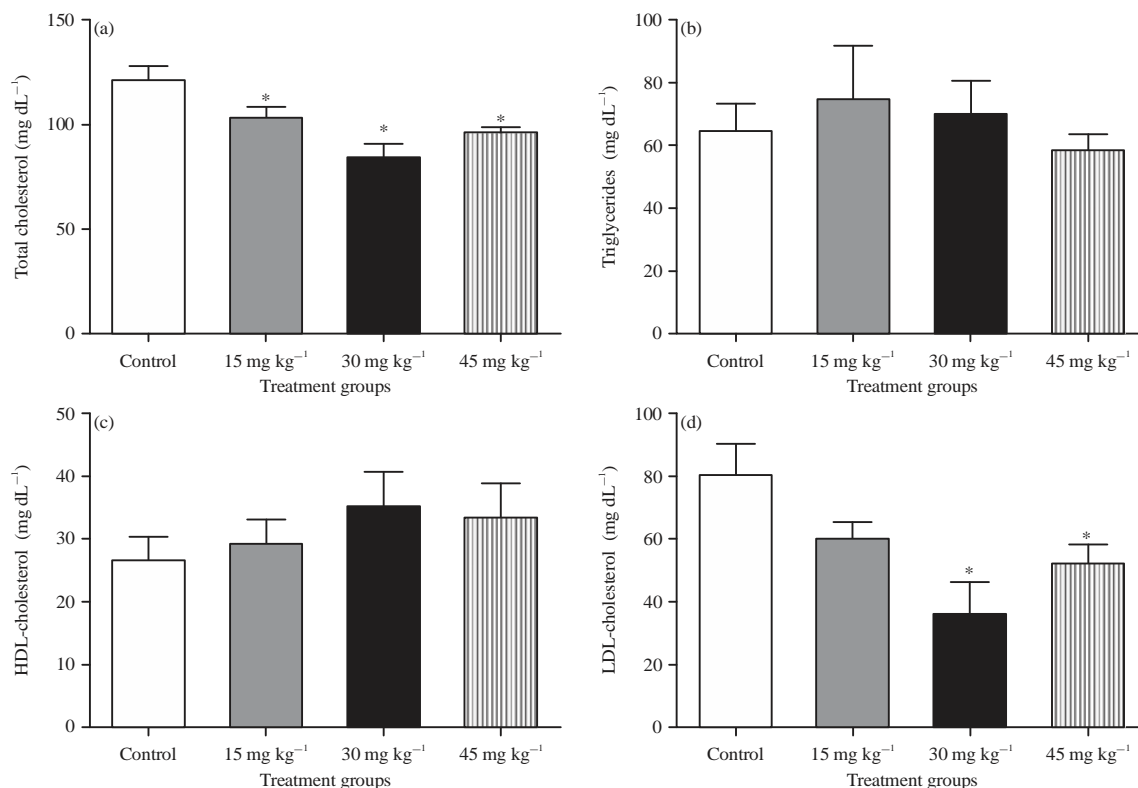


Fig. 1(a-d): Effects of pioglitazone on lipid profile of Wistar albino rats after 28 days of oral administration. Results expressed as Mean±SEM of 7 replicates study, concentrations of plasma (a) Total cholesterol, (b) Triglycerides, (c) HDL-cholesterol and (d) LDL-cholesterol. *Values are significantly different at $p < 0.05$ compared with control group

gluconeogenesis as well as blood glucose level. Pioglitazone enhances insulin action on liver, adipose tissue and skeletal muscles and thus improving glycaemic control in persons with type 2 diabetes¹⁰. All biochemical tests carried out on the blood and tissue homogenate of experimental rats are indices for the assessment of health status for human. In the current study kidney markers were used to assess the functionality and cellular integrity of the kidney. Plasma concentrations of urea, creatinine, sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-) were not significantly affected by pioglitazone administration. However, in streptozotocin (STZ) induced diabetic rats, after undergoing renal ischemia/reperfusion (I/R) injury and PIO treatment for 15 days, the animals exhibited significantly higher serum creatinine, urea and uric acid levels as compared to I/R control group alone while treatment with glimepiride significantly reduced serum uric acid when compared to control group¹⁶. The renoprotective activity of pioglitazone against cisplatin-induced acute renal failure in experimental animals has previously been reported¹⁷⁻¹⁹, these corroborate the findings in this study. The concentration of liver function markers are indices for the assessment of hepatocellular functionality and integrity. The insignificant changes of liver function markers in this experimental rat model after high

doses of PIO, might be a function of the hepatoprotective activity of pioglitazone. Hepatoprotective activity of PIO has been reported in high fat diet-fed rats for 16 weeks^{20,21}. Observed increase in total plasma protein at moderately high concentration of pioglitazone in healthy rats does not correlate with increase in plasma albumin concentration. It is proposed that pioglitazone did not significantly distraught the osmotic albumin concentration in healthy experimental rat model. The hepatoprotective activity of pioglitazone in rat model of diet-induced alcoholic fatty liver disease and acetaminophen-induced hepatotoxicity has been reported^{22,23}. However, acute high doses of pioglitazone have been associated with ventricular hypertrophy with congestion in liver and kidneys which can also happen with accidental overdose of pioglitazone in patients¹⁰.

It has been established that type 2 diabetes has an increased risk of all the manifestations of atherosclerotic vascular disease and it is highly associated with cardiovascular disease, a major contributing factor to this risk is the abnormal lipid profile, dyslipidaemia, which is characterized by low HDL-C, raised triglyceride and predominance of small dense LDL-C particles²⁴. Antidiabetes with the ability of attenuating dyslipidaemia is a better monotherapy or in combination with other antidiabetic drugs. Significant reduction in

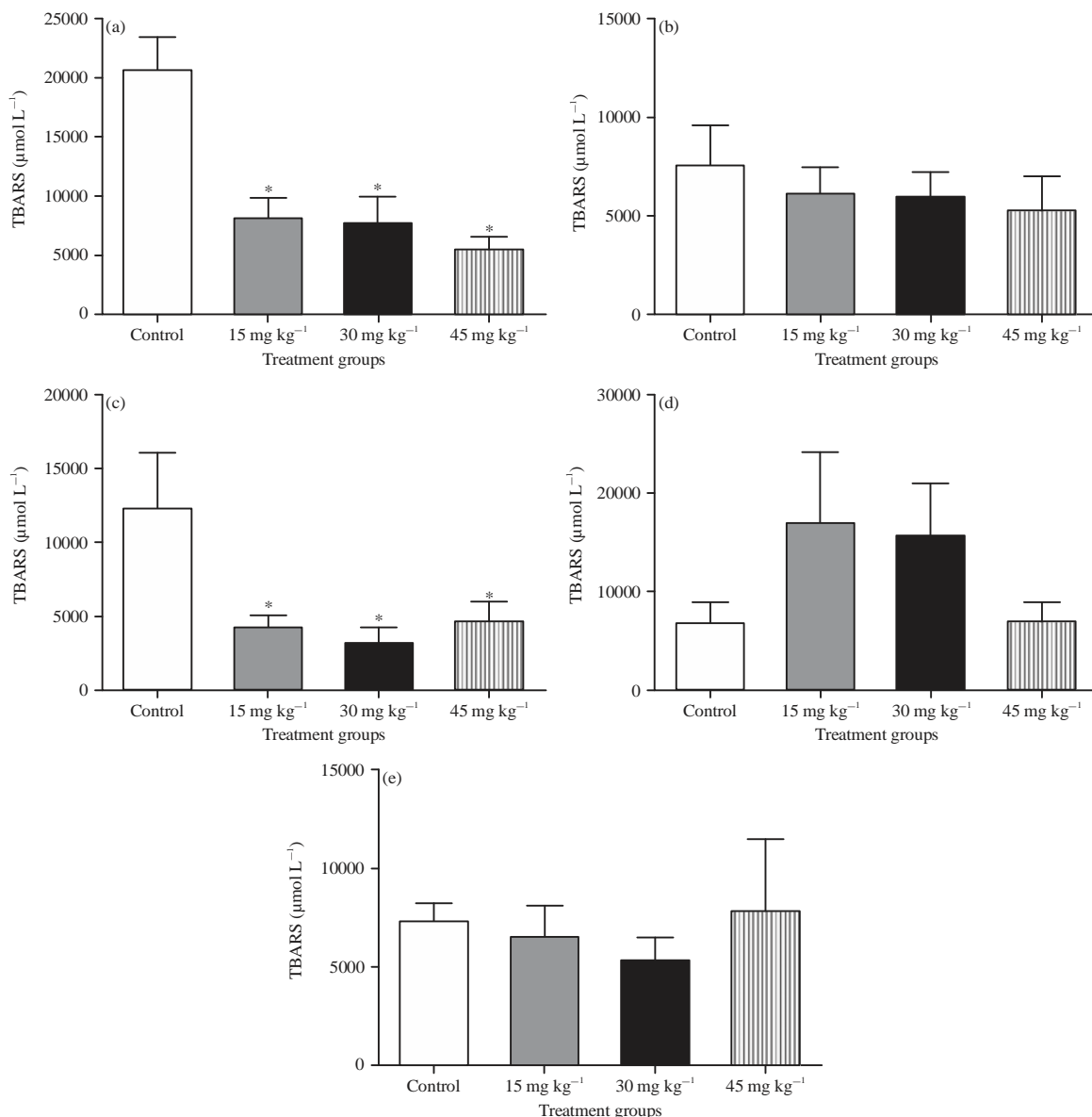


Fig. 2(a-e): Effects of pioglitazone on concentration of thiobarbituric acid reactive substances (TBARS) in major organs of experimental rats after 28 days of oral administration. Concentrations of TBARS in the (a) Liver, (b) Kidney, (c) Heart, (d) Intestine and (e) Lungs. Results expressed as Mean±SEM of 7 replicates study. *Values are significantly different at $p < 0.05$ compared with control group

concentrations of LDL-cholesterol and total plasma cholesterol observed in this experiment further potentiate the ameliorative effect of pioglitazone on lipid metabolism. The lipid ameliorative effect of PIO in this study was supported by the reports of Rosenblatt *et al.*²⁵ and Goldberg *et al.*²⁶. Etiopathogenesis of several chronic diseases has been attributed to increase in generation of free radicals, which is a product of normal cellular metabolism. Lipid is one of the biological targets of oxidative stress. Lipid oxidation gives rise to a number of secondary products which are mainly aldehydes with ability to aggravate oxidative damage and toxicological

response. Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation²⁷. Concentration of MDA is directly correlated with levels of thiobarbituric acid reactive substances (TBARS). Significant reduction in the concentrations of TBARS observed in liver and heart of experimental rats after pioglitazone exposure further potentiates the protective effects of pioglitazone at these concentrations in rat model. This is the first time a comparative assessment on TBARS concentration in different organs of experimental animal model is been carried out. Decrease in TBARS concentrations after

pioglitazone treatment has been reported in humans^{28,29}. Pioglitazone-treated non-alcoholic steatohepatitis (NASH) rats has been reported to show significant reduction in malondialdehyde levels when compared with that of NASH induced control group³⁰. These previous studies further substantiate the ameliorative effects of PIO in both human and animal models.

CONCLUSION

The study concludes that pioglitazone has no biochemical toxicological effect in a sub-chronic 28 days study at concentrations tested in non-diabetic animal model. However, it is suggested that more researches on the effects of PIO should be undertaken in diabetes using different experimental models.

SIGNIFICANCE STATEMENTS

Pioglitazone (PIO) is a prescription drug which acts as insulin sensitizer. It is used for the treatment of type 2 diabetes and other related medical conditions such as hepatic steatosis. The safety of pioglitazone is controversial despite its high usage. There have been limited reports of its associated weight gain, increase risk of congestive heart failure, liver disorder and bladder cancer in patients undergoing treatment with pioglitazone. Despite its continued usage, it is therefore imperative to further study the toxicological effects of pioglitazone and establish its biochemical and antioxidant effects.

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