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Chronic hyperglycemia and biochemical alterations induce hepatic-renal dysfunction in rats administered ethylacetate fraction of *Plumbago zeylanca*, Linn

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ABSTRACT: *Chronic hyperglycemia is an independent predictor of death in myocardial infarctions and nephropathy and it is a major cause of organ damage. Previous studies have shown that administration of Plumbago zeylanica extract elevated significantly the glucose concentration of the organism even though there have been many claims of the ameliorative effects of the plant. Hence, we investigated the likely dysfunctions of liver and kidney that may results from the induced hyperglycemia. Twenty rats (150-190 g) were grouped into four of five rats per group. Group 1 (control) was administered 2% Tween-20 and groups 2, 3 and 4 were administered 100, 200 and 400 mg/kg b. wt. doses of P. zeylanica ethylacetate (PZE) fraction respectively. Administration of PZE for 28 days significantly increased the plasma glucose level suggesting hyperglycemia. The plasma activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly increased thereby suggesting hepatic dysfunction and the kidney function, monitored by plasma protein, creatinine and urea levels was impaired by PZE suggesting renal dysfunction. However, there was no significant change in the level of lipid profile. PZE elicited hyperglycemic effect, hepatic and renal dysfunctions in a dose-dependent manner. Thus, the inducement of hyperglycemia by PZE may have resulted into organ damage noticed in the liver and kidney. Therefore, PZE should be used as herbal medicine with caution.*

KEYWORDS: *Plumbago zeylanica*, hyperglycemia, hepatic and renal markers, rats

INTRODUCTION

The continuous increase in the incident of diabetes (hyperglycemia) worldwide has been adduced to several factors including commonly prescribed medications [**1**]. Several of these drugs that induce hyperglycemia or new onset diabetes (NOD) are androgen deprivation therapy (ADT) used for prostate cancer treatment and cardiovascular disease [**2**] which creates an insulin resistant that

worsens glucose control [**3**], glucocorticoids, somatostatins analogues (SA), beta-blockers and diuretics, antipsychotics (clozapine, olanzapine and quetiapine), antiretrovirals (protease inhibitors and non-reverse transcriptase inhibitors−NRTIs) and others are (mechanistic target of rapamycin inhibitors –mTORis), post organ transplantation drugs, tyrosine kinase inhibitors and interferon-alpha [**1,4**]. These drugs usually induce the type-2 diabetes with an exception of interferon which induces type-1 diabetes.

Chronic hyperglycemia (CH) is an independent predictor of death in many acute settings such as myocardial infarctions and nephropathy, and it is one of the major causes of organ damage [**5-7**]. According to American Diabetes Association, hyperglycemia exists when the blood glucose level is between 100–126 mg/dL (around 5.6 - around 7 mmol/L) and diabetic when the blood glucose level is greater than7 mmol/L [**7**]. Persistent hyperglycemia in different cell types results in glucose toxicity with severe devastating effects [**7**].

The link between diabetes and liver disease has been established. Liver is an organ which plays a very important role in carbohydrate metabolism as a result of its involvement in glycogenesis, glycogenolysis and gluconeogenesis. The main cause of liver dysfunction include insulin resistance, obesity and fatty liver. It has been reportedly shown that hyperglycemia in type-2 diabetes has a serious connection with non-alcoholic fatty liver disease (NAFLD) which can further results to cirrhosis/fibrosis and hepatocellular carcinoma [**8**]. Diabetic individual also suffer from diabetic nephropathy which is shown by a decline in glomerular filtration and proteinuria. Long duration of glucose exposure can elevate the filtered glucose load and thereby results to glycosuria [**7**].

Plumbago zeylanica, a medicinal plant drug commonly used to treat different ailments, was reported to possess lots of therapeutic and pharmacological properties. The therapeutic and pharmacological properties of the plant include antiplasmodial, anticonvulsant, antioxidant, hepatoprotective, anti-inflammatory, hypolipidaemic, antitumour, anticacinogenic, antifungal, antiviral and antibacterial [**9-12**]. These bioactivities have been variously attributed to a number of isolated and characterized phytochemical compounds from the plant especially plumbagin, the major compound and others like difuranonaphthaquinones, naphthaquinone, lapachol and 2,4-bis(1,1-dimethylethyl) [**13-15**]. In spite of its numerous medicinal uses and pharmacological activities, reports have revealed that administration of crude, ethanolic and 50% ethanolic extracts of roots of *P. zeylanica* can induce hyperglycemia [**16-17**]. Therefore, this research was undertaken to evaluate the effect of ethylacetate fraction of methanolic extract of the root of *P. zeylanica* on glucose estimation, and hepatic and renal dysfunctional parameters in rats.

MATERIALS AND METHODS

Plant collection and authentication

Fresh *Plumbago zeylanica* (PZ) roots were collected from Babajakan village in Ayedade Local Government Area, Osun State, Nigeria. The plant material was already identified and authenticated according to Olagunju et al [**18**] with the voucher specimen QC 488 deposited at the Obafemi Awolowo University (IFE) herbarium.

Preparation of extract

The roots were cut off from the stalks of the plant and rinsed thoroughly with running tap water. After the water was allowed to drain off, the roots were air-dried on the laboratory bench for 7 days and then ground to powder with local grinder. About 300 g of the powdered *P. zeylanica* was macerated in 1 L 70% (v/v) for 48 hr. This was filtered with a clean white cotton handkerchief and the residue was re-extracted 3 times while the filtrates were pooled together and evaporated to dryness at 30°C using a rotary evaporator to obtain the methanol extract. The extract was dissolved in 250 mL water:methanol (4:1 v/v) in 1 L separating funnel and partitioned with 125 mL ethylacetate. This was shaken vigorously and ethylacetate layer was collected while the lower aqueous layer was re-partitioned 3 more times. The ethylacetate fractions were pooled together and evaporated to dryness at 30°C with rotary evaporator to obtain the extract of *P. zeylanica* ethylacetate fraction (PZE).

Experimental animals

The animal study was approved by the Animal House and Ethical Committee (AHEC) of Lagos State University College of Medicine. Twenty healthy male and female wistar rats (150-195 g) were obtained from the Animal House of Lagos State University College of Medicine, Ikeja, Lagos, Nigeria. They were kept in metallic cages with saw-dust on its floor to keep the animals warm, and to absorb urine and feaces, under 12-hour light and 12-hour dark cycles with 23-27 °C temperature. The rats were also provided with water and rat feed *ad libitum*. They were allowed to acclimatize for 7 days before administration of PZE.

Experimental design and administration of PZE

After the period of acclimatization, the twenty rats were distributed randomly into four groups (1-4) of five rats per group. Various groups of rats were orally administered PZE already dissolved in 2% (v/v) Tween-20 with cannula as shown below:

Group 1: Administered 1 mL 2% (v/v) Tween-20 (control)

Group 2: Administered 100 mg/kg body weight of PZE

Group 3: Administered 200 mg/kg body weight of PZE

Group 4: Administered 400 mg/kg body weight of PZE

The administration was carried out daily for 28 days, and at the end of this period, the rats were fasted overnight.

Sacrifice of animals and collection of blood samples

Blood samples were collected under chloroform anaesthesia by cardiac puncture into heparin sample bottles, mixed gently and centrifuged at 5000 x g for 10 min and plasma was separated for biochemical analysis.

Evaluation of glucose level, liver and kidney functions

The plasma collected was used to evaluate these parameters. The parameters evaluated include glucose, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, cholesterol and triglyceride concentrations using ERBA Mannheim standard kits (ERBA Diagnostics, Germany) and XL-600 Automated Random Access Clinical Chemistry Analyzer (ERBA Diagnostics, Germany), as described in the instructions provided by the manufacturer.

Statistical analysis

Values were expressed as mean±SEM (standard error of mean). The results were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by separation of groups by Tukey HSD where homogeneity occurred using SPSS statistical analysis software (version 16.0) and Graphpad 5.01 software was used for graph presentation. A p-value less than 0.05 is considered statistically significant.

RESULTS

Blood glucose level in rats

The result of the effect of the administration of PZE on plasma glucose concentration in rats is represented in **Figure 1**. The result showed a significant ($p<0.05$) dose dependent increase of plasma glucose concentration in PZE treated rats compared with the control rats. There was a 166.45% increase of plasma glucose concentration in the highest PZE dose (400 mg/kg b. wt.) rats group compared with the control rats group.

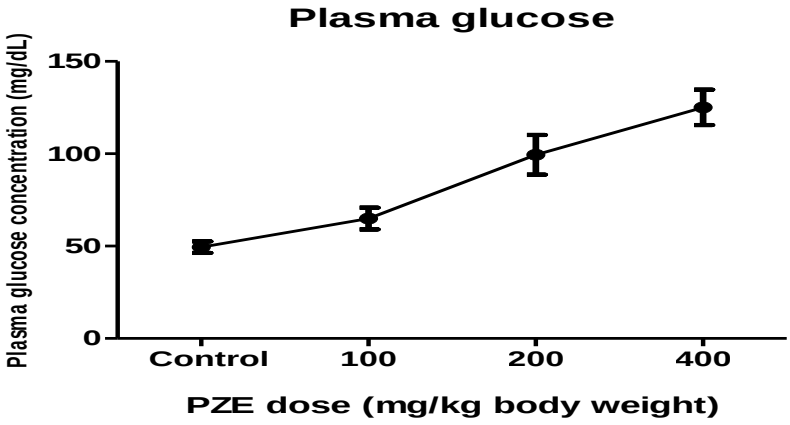

Figure 1: Effect of PZE on plasma glucose concentration in rats. Values are given as mean±SEM for 4 rats in each group. $p<0.05$ is statistically significant

AST and ALT activities in rats

Figures 2 and 3 show the effect of the administration of PZE on AST and ALT activities in rats respectively. There was a significant ($p<0.05$) dose dependent increase in the activities of AST and ALT in PZE treated rats compared with the control rats. The values of the activities (U/L) of AST and ALT in the highest dose (400 mg/kg b. wt.) treated rats were 186.26 and 102.25 compared with the control rats 175.25 and 73.38 respectively.

Protein, creatinine and urea levels in rats

The effect of the administration of PZE on protein, creatinine and urea levels in rats are shown in **Figures 4, 5** and **6** respectively. The results show that protein value was significantly ($p<0.05$) higher in PZE 200 mg/kg b. wt. treated rats than other treated groups compared with the control (Figure 4). The values of plasma creatinine and urea concentrations increased significantly ($p<0.05$) in a dose-dependent manner in PZE administered rat groups compared with the control group.

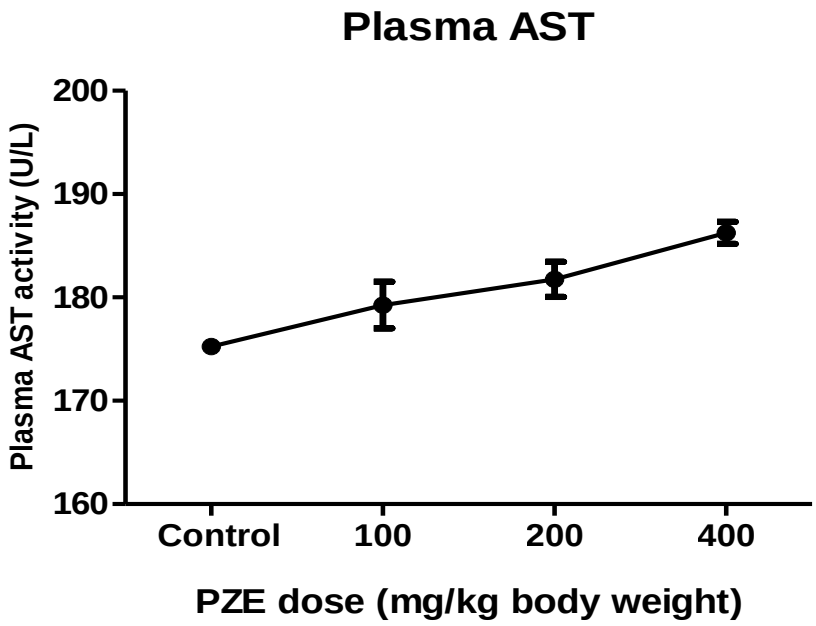

Figure 2: Effect of PZE on plasma AST activity in rat. Values are given as mean±SEM for 4 rats in each group. $p<0.05$ is statistically significant.

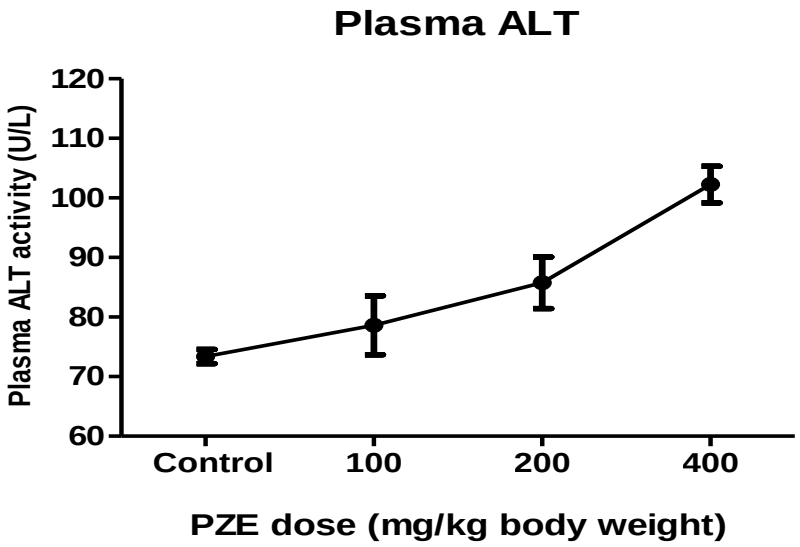

Figure 3: Effect of PZE on plasma ALT activity in rat. Values are given as mean±SEM for 4 rats in each group. $p<0.05$ is statistically significant.

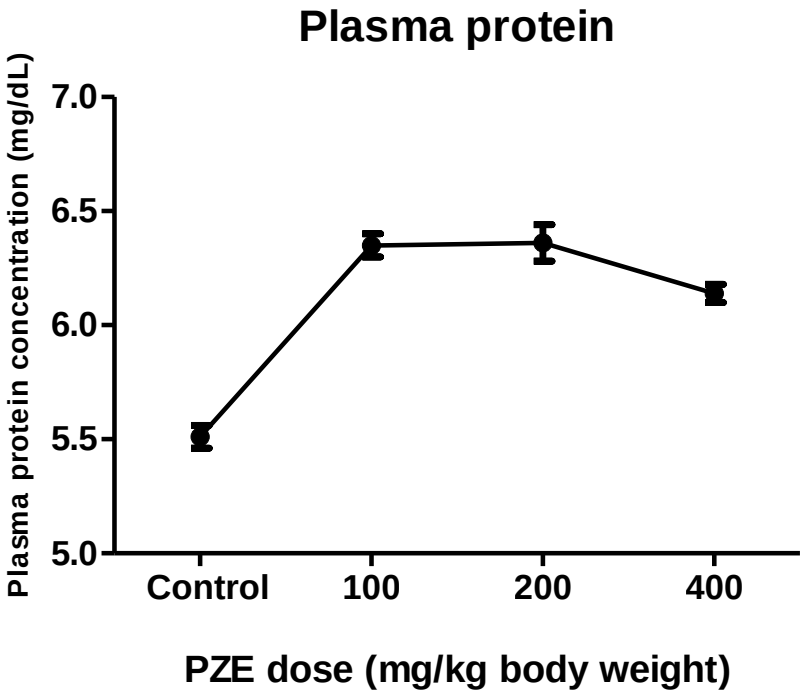

Figure 4: Effect of PZE on plasma protein concentration in rat. Values are given as mean±SEM for 4 rats in each group. $p<0.05$ is statistically significant.

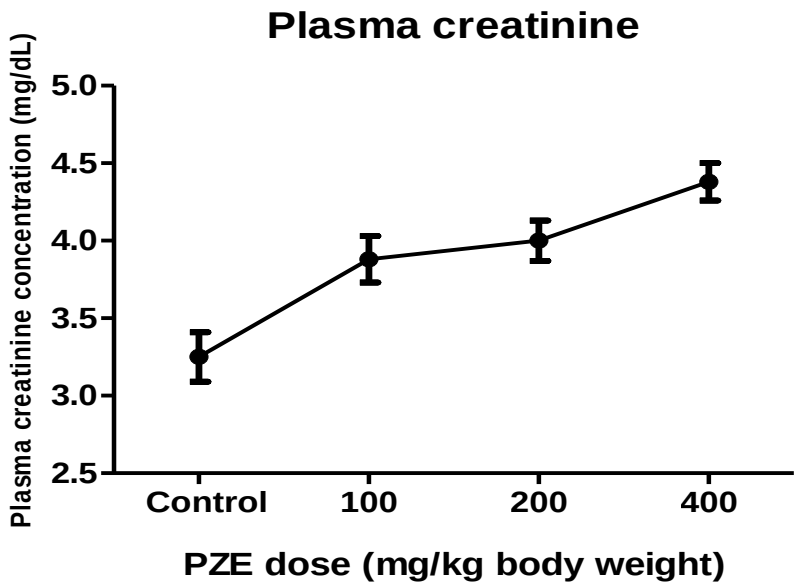

Figure 5: Effect of PZE on plasma creatinine in rat. Values are given as mean±SEM for 4 rats in each group. $p<0.05$ is statistically significant.

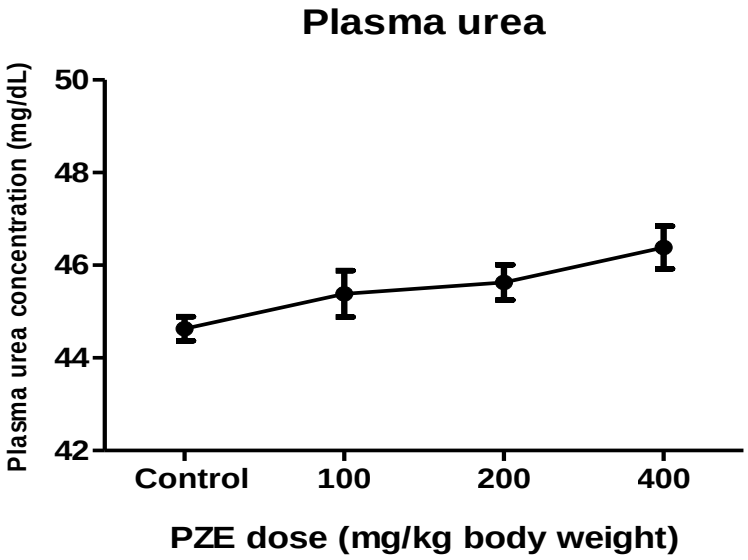

Figure 6: Effect of PZE on plasma urea concentration in rat. Values are given as mean±SEM for 4 rats in each group. $p<0.05$ is statistically significant.

Cholesterol and triglyceride levels in rats

The results of the effect of administration of PZE on cholesterol and triglyceride concentrations are depicted in **Figures 7** and **8** respectively. While the level of cholesterol showed an insignificantly ($p>0.05$) increased in PZE treated rat groups compared with the control group, there was no significant ($p>0.05$) change in the level of triglyceride in PZE treated groups compared with the control group.

DISCUSSION

The present study has demonstrated that administration of PZE significantly altered the concentration of glucose and liver-kidney function in rats. The results of the current study showed that the values of glycemic, hepatic and renal variables were significantly increased in PZE treated rats compared with control.

Glucose, a simple form of carbohydrate, is the primary fuel for cells. A high level of blood glucose for longer periods indicates hyperglycemia [**7**]. Chronic super-physiological glucose concentration negatively affects a large number of organs and tissues [**19**]. Blood sugar levels are raised in diabetic patients, increasing the risk of other complications like retinopathy, nephropathy, and neuropathy [**20, 21**]. In this study, administration of PZE in rats caused a very significant increase in the level of glucose (>160%) which supports the previous findings by Oyedapo and Amos [**17**]. Induction of chronic hyperglycemia by PZE may at the same time lead to cellular damage of the liver and kidney organs. In hyperglycemia induced cellular damage, it is important for the plasma glucose to be transported first across the cell membrane via facilitative glucose transporters. To maintain a relatively constant intracellular glucose level, majority of cells decrease the rate of intracellular glucose transport when faced with a hyperglycemia condition. However, some cell types cannot regulate this process effectively and are more vulnerable to elevated plasma glucose levels therefore susceptible to hyperglycemic damage as excess glucose enters the cell [**22, 23**]. This might likely be the case in this situation.

Liver plays an important role in carbohydrate metabolism. Serum AST, ALT and ALP activities are known hepatic marker enzymes used as indicators for the diagnosis of hepatic injury because they are related to the hepatic cell functions [**24**]. It was proposed by Nannipieri et al., that the level of serum activity of enzymes associated with hepatic dysfunction and occasioned by tissue injuries could be used as indices for diagnosis and prediction of incidence of diabetes mellitus (hyperglycemia) [**25**]. The alteration of activities of AST and ALT may be due to cellular damage and dysfunction. Increase in the activities of these marker enzymes, in dose dependent manner, in PZE treated rats revealed the possibility of tissue damage and impairment of functional integrity of hepatic membrane that could increase the membrane permeability thereby leading to the release of cytoplasmic contents into circulation.

Elevations of concentrations of blood creatinine and urea are recognized diagnostic markers for renal dysfunction. Elevated serum urea level has been associated with kidney diseases such as glomerulonephritis, urinary tract obstruction, and excessive protein catabolism [**26, 27**].

Administration of PZE in rats resulted in significant elevation of the levels of creatinine and urea. These results may be due to high degree of involvement of the renal tubular cells being susceptible to damage which could have resulted to the renal dysfunction.

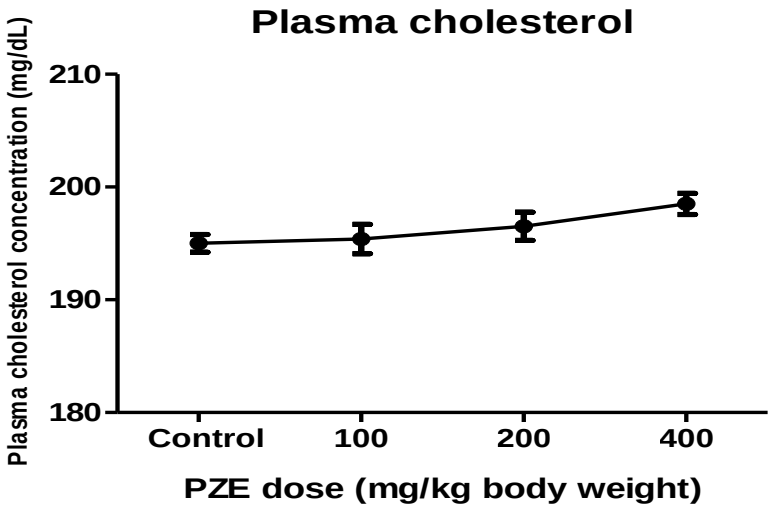

Figure 7: Effect of PZE on plasma cholesterol concentration in rat. Values are given as mean±SEM for 4 rats in each group. $p<0.05$ is statistically significant.

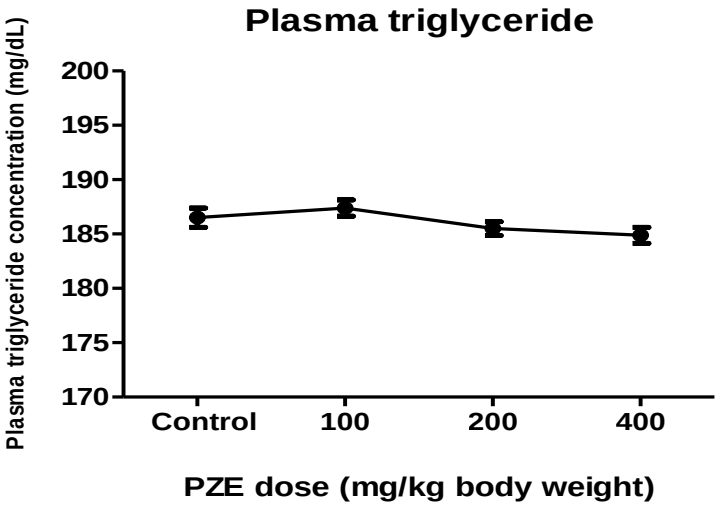

Figure 8: Effect of PZE on plasma triglyceride concentration in rat. Values are given as mean±SEM for 4 rats in each group. $p<0.05$ is statistically significant.

There is no significant change observed in the concentrations of cholesterol and triglyceride in PZE administered rats compared with the control. Hence, the result of the lipid level could not have any negative effect on the hepatic-renal function.

CONCLUSION

From the results of this study, our findings provide evidence that the administration of ethylacetate fraction of 70% ethanol extract of *P. zeylanica* in rats led to hyperglycemia and alterations of liver-kidney function parameters. The occurrence of these can result in liver and kidney tissues being damaged or necrotic indicating hepatic and renal dysfunctions. Therefore, the use of the plant as a therapeutic agent against diseases or ailments should be with caution.

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