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Toxicological evaluations of combined administration of ethanolic stem bark extract of *Enantia chlorantha* and lisinopril in experimental type 2 diabetes

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Abstract

Background: *Enantia chlorantha* is a local medicinal plant commonly use in Nigeria for the treatment of diabetes but without support of scientific data. Large percentage of people suffering from diabetes who uses the plant as antidiabetic agent also combine its administration with standard antihypertensive drugs.

Aim: In the present study, we have investigated the possible toxicological effects of combined administration of *E. chlorantha* bark extract and lisinopril in diabetic model of experimental rats.

Methods: *E. chlorantha* stem bark was extracted by cold maceration of the pulverised stem bark in 70% ethanol. The acute toxicity effect of the plant was then evaluated in rats following oral administration of single dose of the extract. Diabetes was induced by intraperitoneal administration of 40 mg/kg streptozotocin into fructose fed rat. Diabetic rats were then randomly assigned into 6 groups of 7 rats each. One group was kept as the diabetic model while separate treatments were administered to the other six groups. Seven non diabetic rats were kept as the control group and administered normal saline.

Results: The LD_{50} of *E. chlorantha* stem bark was above 5000 mg/kg. Combined administration of lisinopril and *E. chlorantha* showed synergistic effects in the restoration of renal biomarkers (serum creatinine, urea, Na^+ and K^+), cardiac function biomarkers (CK-MB and LDH) and hematological parameters (RBC, WBC, HGB and PCV), while antagonistic effects were however observed with some of the liver biomarkers (AST, ALT, ALP, GGT, total protein and total bilirubin). Rats co-administered lisinopril and *E. chlorantha* also showed fatty liver with cholestasis.

Conclusion: The study concluded that diabetes is associated with kidney and cardiac dysfunction. Combined administration of lisinopril and *E. chlorantha* though may not aggravate these dysfunctions however, it may antagonize the efficacy of the plant in ameliorating liver dysfunction in diabetics.

Keywords: Pharmacodynamics, Drug-herb interaction, Toxicity, Liver function, Diabetes

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Introduction

The use of more than one drug (polypharmacy) is a common practice in the treatment of chronic disorders such as diabetes mellitus, hypertension, single and multiple disorders which occur simultaneously. In such a situation, one drug may interact with other drug (drug-drug interactions) or with herb (herb-drug interaction). Herb-Drug interactions (HDIs) are either pharmacodynamic (PD) or pharmacokinetic (PK) in nature. For the former, this occurs when co-administered herbs and conventional medicines enhance or negate each other's effects as a result of similar or disparate pharmacological activity [7]. Pharmacodynamic interactions on the other hand arise from the ability of the herb to modulate the absorption, distribution, metabolism and/or excretion (ADME) of the drug.

Diabetes mellitus (DM) is a major degenerative disease in the world today afflicting many lives both in the developed and developing countries [25]. Increasing incidence of diabetes in the developing countries, especially in the younger age group, affecting mainly the people in the productive years of their lives is of great concern [18]. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunctions, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [3, 20]. Type 2 diabetes (T2DM) is the most common form and comprises of 90% of people with diabetes around the world [11]. Type 2 diabetes is characterized by the combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells [5, 13]. Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc. These complications have long been assumed to be related to chronic elevation of glucose levels. The management of diabetes mellitus is one of the many disease states where multiple-drug therapy is inevitable and often used to the benefit of the patients.

According to the World Health Organization [35], 40–60% of type 2 diabetics are obese, and have an increased likelihood of developing Coronary Artery Disease (CAD) and other atherosclerotic manifestations including hypertension. Mohan et al. [21] also reported that the prevalence of CAD was 21.4% among diabetics, compare to 9.1% in those with normal glucose tolerance. In such a situation, there may be need for polypharmacy and hence chances for interactions between antidiabetic and antihypertensive drugs, which may be beneficial or harmful. Consequently, there is a need to monitor drug therapy in polypharmacy in order to gain a better therapeutic effect with a lower rate of risk.

Enantia chlorantha belongs to annonaceae family. This plant is commonly known as African yellow wood. Among the Yorubas in Nigeria, it is known as Awopa, Osu pupa or dokita igbo [1]. In the traditional medicine, this plant has been used for a long time in many parts of the African continent to treat various ailments of the human body. Many of these uses are supported by some studies. For example, a report by Tsabang et al. [33] indicated that a decoction of 500 g of stem bark in three liters of water for 20 min may be used to treat malaria symptoms, aches, wounds, boils, vomiting, yellow fever, chills, sore, and hepatitis. Although, the combine use of *Enanthia chlorantha* stem bark and antihypertensive drug is a common practice in Nigeria, previous findings from our laboratory showed that the therapeutic efficacy of the plant is not influenced when co-administered with lisinopril (an oral long-acting angiotensin converting enzyme inhibitor). The present study was set up to evaluate the toxicological implications of combine use of the two agents in diabetes condition. This is with a view to evaluate the risk associated with the combine administration of the two agents.

Methodology

Chemicals and drugs

Streptozocin was a product of Sigma- Aldrich, USA. Metformin (glucophage 500 mg) was manufactured by Merck santé, France and lisiofil (lisinopril) was manufactured by Fourrts (India) Laboratories Pvt. Limited. All the reagent kits used for bioassay were sourced from RANDOX Laboratories Ltd., Crumlin, Co. Antrim, UK. All other chemicals used were of analytical grade and sourced from the Biochemistry laboratory, Kwara State University.

Plant collection, identification and crude extract preparation

The stem bark of *E. chlorantha* was obtained from Oja tuntun, Ilorin, Kwara State in June 2018. The plant sample was identified and authenticated at the department of Plant Biology, University of Ilorin, Kwara State, Nigeria. A voucher number UIH/001/1356 was assigned to the plant and a sample specimen was thereafter deposited in the Herbarium. The stem bark was cleaned to remove adhering dirt, air-dried for 2 weeks and ground into powder using an electric blender. Extraction was carried out by cold maceration of 800 g of the coarse powder with 5 L of 70% ethanol for 72 h, with constant shaking. The resultant mixture was filtered using Whatman filter paper (No.1) and the filtrate was concentrated using a rotary evaporator at 40 °C. Aliquot portions of the extract were weighed and the final yield was determined to be 12.5%. The extract was finally reconstituted in distilled water for use in the study.

Experimental protocols

Experimental animals

The sub-chronic toxicity study was carried out on healthy forty-nine (49) male Wistar rats weighing

between 164 and 176 g while the acute toxicity studies was carried out on nine male Wistar rats weighing between 119 and 122 g. The rats were obtained from Can farm Ilorin and housed in cages at the animal house of the Department of Medical Biochemistry and Pharmacology, Kwara State University, Malete, Nigeria. The rats were acclimatized for 14 days and fed with commercial diets and water ad libitum. They were all maintained at 25 ± 2 °C light and dark cycle of 12/12 h respectively.

Acute toxicity test

The acute toxicity test was performed using the OECD guidelines 423 [20, 24]. Standard two-phase approach described by Lorke [17] was used. Nine rats of average weight 120.09 ± 2.51 g were used for this study. The rats were divided at random into three groups of three rats each in the first phase. The rats were first deprived of feed and water for 12 h. Groups I, II and III were orally treated with ethanolic stem bark extract of *E. chlorantha* at 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight respectively. They were then observed for 48 h for signs of toxicity or mortality. In the second phase of the experiment, the remaining three rats were assigned into three groups (IV, V and VI) of one animal each and thereafter administered 1600 mg/kg, 2900 mg/kg and 5000 mg/kg body weight of the extract respectively. The rats were thereafter carefully observed individually after dosing. The observation was done once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days. All the rats were then subjected to detailed gross necropsy by careful examination of the external surface of the body, all orifices and cranial, thoracic and abdominal cavities. Behavioral changes, lethargy, depression, salivation, diarrhea, muscular weakness, sedation and ailment signs were also observed. The LD_{50} was thereafter estimated based on the mortality observed in each group adopting the method of Ajani et al. [2].

$$LD_{50} \geq \text{Maximum-dose Y/number of rats per group}$$

$$\text{Where Y} = \text{Sum of mean death}$$

Induction of diabetes

Type 2 diabetes was induced following the method of Wilson and Islam [34]. The rats were first fed 15% fructose solution (w/v) for 4 weeks, after which they were fasted overnight and thereafter administered streptozotocin (40 mg/kg i.p) freshly prepared in 0.1 M sodium citrate buffer. The diabetic state was confirmed 72 h after streptozotocin injection. All rats having fasting blood glucose levels greater than 200 mg/dl were considered diabetic.

Sub-acute oral toxicity study

Animal grouping/administration Forty- two (42) diabetic rats were randomly assigned to 6 groups consisting of 7 rats each. The rats were labelled and treated as follow; Diabetic model group (DM); Diabetic treated groups (administered *E. chlorantha* [T1], Glucophage [T2], *E. chlorantha* + Glucophage [T3], Lisinopril [T4], and lisinopril + Glucophage [T5]). Another 7 rats (non-diabetic) were kept as the control group (NC). The extract was administered at 200 mg/kg dose while glucophage and lisinopril were administered at 7.14 mg/kg and 0.14 mg/kg dose respectively. All administrations were carried out orally as a single dose daily by gavage for 4 weeks. The rats were housed in rat cages in the animal facility center at the department of Medical Biochemistry and Pharmacology, Kwara State University, Malete, Nigeria. The rats were maintained in accordance with the principles of laboratory animal care [22] guidelines. The experiment protocol was designed according to the Department Animal Ethics Committee guidelines. Twenty- four hours after the last administration and under mild diethylether anesthesia, the animals were sacrificed and blood was obtained from the jugular vein. Blood sample was transferred into plain centrifuge tube and allowed to clot at room temperature. It was then centrifuged within 1 h of collection at 4000x g for 10 min to obtain the serum.

Assay procedure

Biochemical and Haematological assay

Liver, renal and cardiac function parameters were evaluated according to manufacturer's instructions on the assay kits. Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were assayed for by Reitman and Frankel [29] method. Alkaline Phosphatase (ALP) and Lactate Dehydrogenase (LDH) were assayed according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie [36]. γ-glutamyl transferase (GGT) was assayed for by Reitman and Frankel [29] method while total protein was assayed for by Peters [26] method. Total bilirubin was assayed for by Roma-Bikai et al. [30] method and creatine kinase (CK-MB) was assayed for using enzyme immunoassay technique [6]. Creatinine was assayed for by Tietz et al. [32] method. The plasma concentrations of sodium (Na^+) and potassium ions (K^+) were determined by Flame Photometry using Coming 410C Flame Photometer. Urea was assayed by Fawcett and Scott (1960) method. Automated hematological analyzer Sysmex, KX-21 (Japan) was used to analyzed haematological parameters.

Histopathological evaluation

The rats were quickly dissected and the liver and pancreas isolated, blotted with clean tissue paper and cleaned of fat. These were then used for histological

examination. The method of Thapa and Anuj [31] was adapted for the histopathological examination of the harvested liver sections. Microscopic features of the cells of the treated rats were compared with both normal and the model groups.

Data analysis

All statistical analysis was performed with SPSS software version 16.0. Data are expressed as the means standard error of mean (S.E.M). Student's t-test and analysis of variance (ANOVA) were employed in comparing means of continuous variables as appropriate. Differences were considered statistically significant if $p < 0.05$.

Results

Acute oral toxicity study

Ethanolic extract of *E. chlorantha* bark did not show any toxic or adverse effect nor mortality at 5000 mg/kg dose. There was no observable clinical adverse effect of substance related toxicity on the behavioral responses of the tested rats during the 14 days monitoring period (Table 1). Physical observations also indicated that all the rats behaved essentially normal with no signs of changes in the skin, fur, eyes, mucous membrane, behavioural patterns and tremors. Salivation and diarrhea were not evident following single oral dosing in the tested rats.

Sub-acute oral toxicity study

Liver function test

The result showed a significant increase in serum activities of all the enzymes (AST, ALT, ALP, GGT) evaluated in the untreated diabetic rats (model group) when compared with the control group (Table 2). Treatment with the extract restored the enzyme activities to the control values. The enzyme activities obtained in the rats treated with the extract alone were significantly lower than that of the diabetic rats treated with glucophage alone and were also lower than that of diabetic rats co-administered glucophage and lisinopril and that of the rats co-treated with the extract and lisinopril. The Table also showed that the enzyme activity obtained in the diabetic rats treated with lisinopril alone was higher than that of the control group (NC) but it was however significantly lower than that of the diabetic model group. Similar results were obtained with total protein and bilirubin levels.

Table 1 Result of acute toxicity study

Group	Dose (mg/kg)	Sign of toxicity (ST/NB)*	Mortality (D/S)*
Group I	10	0/3	0/3
Group II	100	0/3	0/3
Group III	1000	0/3	0/3
Group IV	1600	0/1	0/1
Group V	2900	0/1	0/1
Group VI	5000	0/1	0/1

*: *ST* sign of toxicity, *NB* normal behavior, *D* died, *S* survived
Values are expressed as number of animals

Kidney function test

Table 3 is the result of the effects of treatments on some serum biomarkers of kidney functions (urea, creatinine, Na^+ and K^+). The result showed a significant increase in serum urea, creatinine, Na^+ and K^+ level in rats administered streptozotocin without treatment (model group) when compared with the control group. Treatment with the extract restored the serum levels of all the biomarkers to the control values. When compared among the treatment groups, the serum urea in the group administered glucophage although lower than that of the model group, was however higher than the value obtained in the control group. The observed serum urea level of the extract treated rats was however not significantly different from all other treatment groups. No significant difference ($p > 0.05$) was observed among the treatment groups for all other biomarkers of kidney functions monitored in the study.

Cardiac function

The result showed a significant increase in serum CK and LDH activities in rats administered streptozotocin without treatment (model group) when compared with the control group (Table 4). Treatment with the extract reduced the serum creatinine kinase activity below the observed activity in the model group. The observed activity was however higher than that of the control (NC) value. The result also showed that the lactate dehydrogenase activity of the extract treated rat was not significantly different from that of the model group. Combined administration of the extract and lisinopril however brought the lactate dehydrogenase activity to the pre-treatment value though the observed value was not significantly different from that of the model group.

Haematological evaluation

Table 5 is the result of the effects of treatments on total erythrocyte, leucocyte, haemoglobin and PCV levels. The result showed a significant decrease in total erythrocyte, haemoglobin and PCV levels in rats administered streptozotocin without treatment (model group) when compared with the control group. Treatment with the extract restored the values of all these parameters. Similar results were obtained with all other treatment groups. The total erythrocyte, haemoglobin and leucocyte level obtained in the rats treated with the extract alone was not significantly different from that of all other treatment groups. The Table also showed that the PCV obtained for the rats co-administered the extract and

Table 2 Effect of combined administration of lisinopril and ethanolic extract of *E. chlorantha* on serum biomarkers of liver function

Group	Treatment	AST (U/l)	ALT (U/l)	ALP (U/l)	GGT (U/l)	Total protein	Total Bilirubin
NC	Distilled water only	30.36 ± 2.45^{a}	29.69 ± 0.83^{a}	1.04 ± 0.76^{a}	0.25 ± 0.84^{a}	0.79 ± 0.82^{d}	0.93 ± 0.31^{a}
DM	STZ-fructose+ distilled water	77.68 ± 4.67^{e}	85.80 ± 1.04^{d}	1.54 ± 0.54^{d}	1.14 ± 0.60^{d}	1.50 ± 0.47^{a}	2.46 ± 0.53^{c}
T1	Extract only	37.10 ± 2.68^{ab}	42.60 ± 4.70^{b}	1.14 ± 0.16^{bc}	0.52 ± 0.15^{bc}	0.90 ± 0.10^{c}	1.22 ± 0.18^{a}
T2	Glucophage only	44.70 ± 1.23^{bc}	39.38 ± 3.79^{ab}	1.07 ± 0.74^{b}	0.48 ± 0.03^{bc}	0.93 ± 0.29^{d}	1.14 ± 0.35^{a}
T3	Extract + Lisinopril	48.70 ± 0.96^{c}	56.98 ± 1.72^{c}	1.29 ± 0.53^{c}	0.71 ± 0.26^{c}	0.78 ± 0.13^{b}	1.62 ± 0.59^{a}
T4	Glucophage + Lisinopril	47.42 ± 2.03^{b}	49.12 ± 2.15^{bc}	1.26 ± 0.65^{c}	0.68 ± 0.31^{c}	0.81 ± 0.03^{b}	1.05 ± 0.44^{a}
T5	Lisinopril only	60.25 ± 4.63^{d}	55.88 ± 2.07^{c}	1.39 ± 0.05^{d}	0.97 ± 0.13^{d}	1.07 ± 0.20^{b}	1.83 ± 0.34^{b}

Results are mean ± SD of seven determinations
Values in the same column with similar superscripts (a, b, c or d) are not significantly ($p > 0.05$) different from each other

lisinopril was higher than the control value and that observed in the rats treated with the extract alone.

Histopathology

An overall histopathology shown in Fig. 1a-g indicates that there were no alterations in the histological presentation of the tissue of the normal control group after the experimental period (1a). The photomicrograph of the model group however showed a fatty liver (1b). The photomicrograph of the extract treated (1c), the glucophage treated (1d), glucophage +lisinopril treated (1f) and lisinopril treated rats showed a manifestation that were similar to that of the control group. When the diabetic rats were however co-administered with the extract and lisinopril, the liver histology (1e) showed a fatty liver with cholestasis. The histology of the control rats showed normal manifestation of the histoarchitecture of the pancreas (2a) whereas large interlobular duct and diminished expression of islet cells were observed in the pancreatic tissue of the model group (2b). The histoarchitecture of other treatment groups (2c-g) were similar to that of the diabetic model group.

Discussion

Streptozotocin (STZ)-fructose type 2 model shares a number of features with human type 2 diabetes mellitus (T2DM) both histologically and metabolically and is characterized by moderate stable hyperglycaemia, hence, STZ-fructose induced diabetes model was used in this study. Streptozotocin injection caused β cells degeneration in rats, resulting in decrease in the release of insulin by the pancreas. Furthermore, high fructose ingestion causes insulin resistance (IR) thereby resulting in hyperglycaemia. Result of fasting blood glucose of ≥200 mg/dl obtained in this study confirms induction of type 2 diabetes in STZ-fructose administered rats. This is similar to the findings of earlier researchers [16, 19].

The ethanolic bark extract of *E. chlorantha* was found to cause no toxicity during the acute toxicity study even at 5000 mg/kg dose hence, a dose of 200 mg Kg^{-1} was used in this study for the sub-acute toxicity evaluation. In the present study, untreated diabetic rats showed increased activities of liver enzymes. Numerous studies have reported that diabetes is associated with raised serum activities of liver enzymes [10, 23]. A large clinical study reported that patients who were overweight (BMI 25–30 kg/m^2) and obese (BMI > 30 kg/m^2) are more likely to have elevated serum levels of liver enzymes [9]. Results from this study show that concurrent use of both lisinopril and *E. chlorantha* did not produce a better effect in restoring the serum liver biomarkers when compared with the single treatment of the extract. ACE-Is such as lisinopril are known to induce liver injury, and two cases of ACE-I induced cirrhosis has been

Table 3 Effect of combined administration of lisinopril and ethanolic bark extract of *E. chlorantha* on serum urea, creatinine, Na^+ and K^+ level in type 2 diabetes rats

Groups	Treatments	Urea (mg/ml)	Creatinine (mg/ml)	Sodium (Na^+) (mmol/L)	Potassium (K^+) (mmol/L)
NC	Distilled water	13.30 ± 2.67^{a}	1.030 ± 0.19^{a}	1.384 ± 0.94^{a}	3.54 ± 1.19^{a}
DM	STZ-fructose+ distilled water	45.69 ± 1.07^{b}	3.169 ± 1.06^{b}	0.965 ± 0.78^{b}	10.10 ± 0.90^{b}
T1	Extract only	19.89 ± 3.12^{c}	1.269 ± 0.46^{a}	1.375 ± 0.44^{a}	6.63 ± 1.65^{a}
T2	Glucophage only	27.44 ± 4.21^{c}	1.948 ± 0.44^{a}	1.255 ± 0.86^{a}	7.60 ± 0.74^{a}
T3	Extract + Lisinopril	15.56 ± 3.91^{a}	1.069 ± 0.93^{a}	1.177 ± 1.46^{a}	5.16 ± 1.16^{a}
T4	Glucophage + Lisinopril	18.82 ± 4.14^{a}	1.459 ± 0.37^{a}	1.153 ± 1.14^{a}	6.43 ± 1.89^{a}
T5	Lisinopril only	12.84 ± 0.83^{a}	1.392 ± 0.48^{a}	1.364 ± 0.61^{a}	5.51 ± 0.62^{a}

Results are mean ± SD of seven determinations
Values in the same column with similar superscripts (a, b or c) are not significantly ($p > 0.05$) different from each other

Table 4 Effect of combined administration of lisinopril and ethanolic bark extract of *E. chlorantha* on serum CK and LDH activities

Group	Treatment	Creatine kinase-MB	Lactate dehydrogenase activity ((U/L)
NC	Distilled water	13.57 ± 4.32[a]	0.837 ± 0.82[a]
DM	STZ-fructose+ distilled water	43.64 ± 5.36[b]	1.878 ± 0.13[b]
T1	Extract only	20.24 ± 1.89[c]	1.469 ± 0.20[b]
T2	Glucophage only	17.74 ± 4.74[a]	1.357 ± 0.50[a,b]
T3	Extract + Lisinopril	13.78 ± 3.74[a]	1.135 ± 0.17[a,b]
T4	Glucophage + Lisinopril	14.32 ± 0.87[a]	1.075 ± 0.87[a,b]
T5	Lisinopril only	16.07 ± 2.37[a]	1.121 ± 0.83[a,b]

Results are mean ± SD of seven determinations
Values in the same column with similar superscripts (a, b or c) are not significantly ($p > 0.05$) different from each other

previously reported [8]. The long term effect of co-administration as shown by the histology of the liver suggest that combined administration of lisinopril and *E. chlorantha* may potentiate hepatotoxicity. This may however not be the case when the extract is administered alone. Previous evidence suggests that diabetic condition is associated with changes in morphology and eventually functional alteration in kidneys [14]. The present results clearly support this report. Our study demonstrated increased levels of kidney function markers; creatinine and urea in serum of diabetic untreated group. In contrast, all extract treated rats showed significant reduction in these markers, thus suggesting the ability of *E. chlorantha* the treatment to protect against diabetes-induced kidney damage.

Data obtained from our study showed that co-administration of lisinopril and *E. chlorantha* attenuated the development of kidney impairment caused by the diabetic condition. The development of pathological changes including elevation of serum kidney biomarkers has been documented to occur in DM [30]. Our result indicates that the use of *E. chlorantha* in diabetes improved kidney function and that the efficacy may not be increase with simultaneous administration of lisinopril.

Our results also showed that cardiac function was restored in the treated groups compared with the untreated diabetic rats. Although administration of *E. chlorantha* in diabetic rats was shown in the present study to improve cardiac function, the result of our study suggest that the effect may be more pronounced when co-administer with lisinopril. This thus implies that a synergistic effect may occur from the interaction of both agents. Previous authors have reported that lisinopril administration may improve myocardiac injury in hypertensive patients [28]. Our result corroborates this report.

The result of the haematological study shows that although diabetic condition may be associated with reduction in haemoglobin and erythrocytes level, combined administration of lisinopril and *E.chlorantha* restores these parameters similar to what may be achieve when the extract is administered singly. Combined administration of the two agents however proved to be better in restoring PCV than the administration of the extract alone. A study indicated that lisinopril has the potential to stimulate erythropoietin release from the kidney which is the humoral regulator of RBC production [27]. Our study thus demonstrated a synergistic interaction in restoring PCV value when lisinopril was co-administered

Table 5 Effect of combined administration of lisinopril and ethanolic bark extract of *E. chlorantha* on Erythrocyte count, Leucocyte count, Heamoglobin and PCV level in type 2 diabetic rats

Group	Treatments	Erythrocyte count million/μL	Leucocyte count × 10^9/L	Heamoglobin (g/dl)	PCV (%)
Control	Distilled water	5.41 ± 0.99[a]	8.14 ± 2.09[a]	13.00 ± 1.47[a]	45.14 ± 1.77[a]
Model	STZ-fructose+ distilled water	3.48 ± 0.49[b]	14.66 ± 0.98[b]	7.32 ± 2.50[a]	33.26 ± 2.27[b]
T1	Extract only	5.00 ± 0.27[a]	7.15 ± 1.52[a]	12.73 ± 0.96[a]	48.23 ± 3.44[a]
T2	Glucophage only	5.35 ± 0.49[a]	5.55 ± 0.66 [a]	11.79 ± 1.80[a]	46.83 ± 3.63[a]
T3	Extract + Lisinopril	5.75 ± 0.29[a]	5.47 ± 0.96[a]	14.07 ± 1.29[a]	52.25 ± 1.71[c]
T4	Glucophage + Lisinopril	5.84 ± 0.48[a]	4.88 ± 0.71[a]	14.91 ± 1.44[a]	50.14 ± 2.20[c]
T5	Lisinopril only	4.840 ± 0.11[a]	7.62 ± 1.16[a]	12.69 ± 0.76[a]	48.64 ± 2.49[a]

Results are mean ± SD of seven determinations
Values in the same column with similar superscripts (a, b or c) are not significantly ($p > 0.05$) different from each other

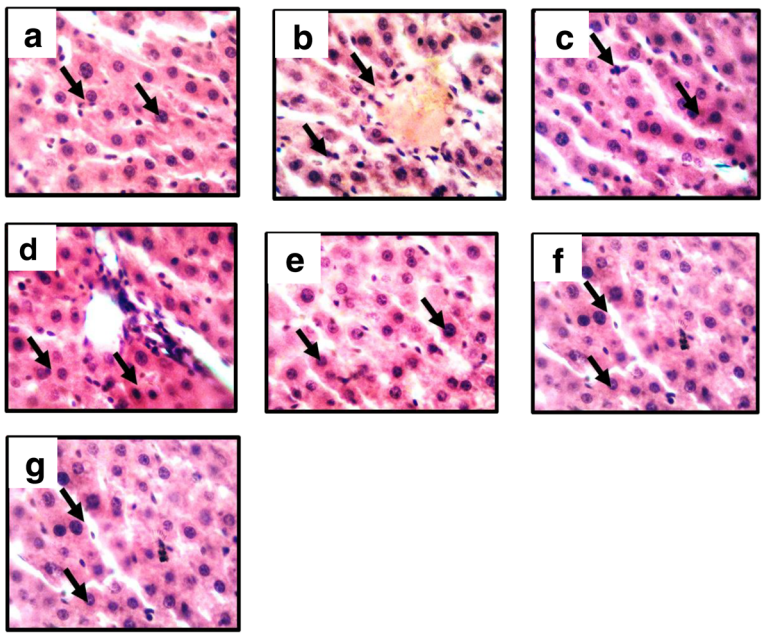

Fig. 1 Representative photomicrograph (× 400, Haematoxylin and eosin stained) of the liver of (**a**) control, (**b**) model group; (**c**) Extract treated; (**d**) Glucophage treated; (**e**) Extract + lisinopril treated (**f**) Glucophage + lisinopril treated; (**g**) Lisinopril treated rats

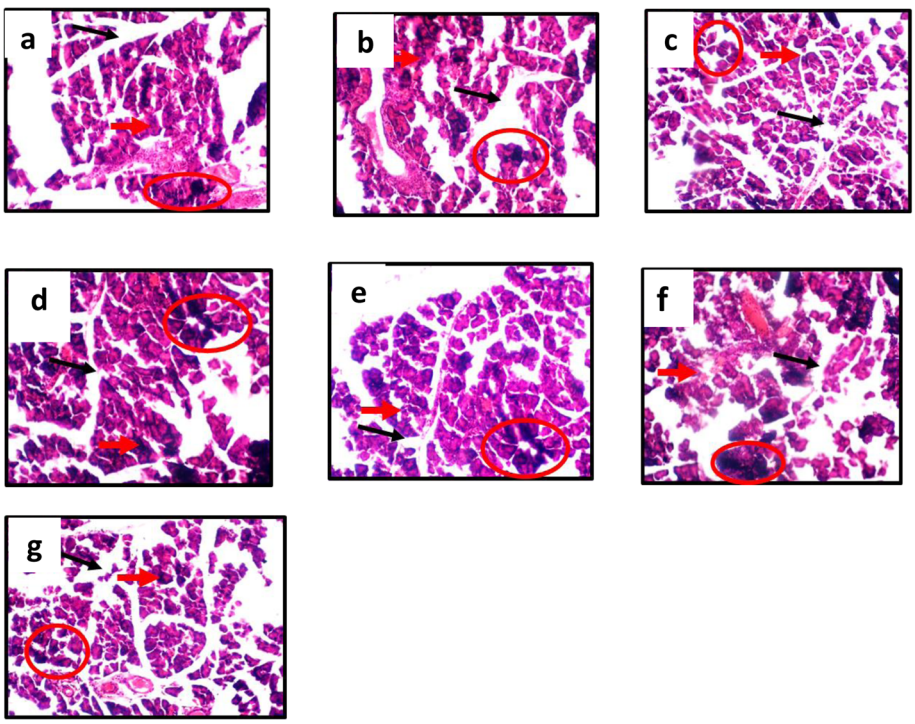

Fig. 2 Representative photomicrograph (× 400, Haematoxylin and eosin stained) of the pancreas of (**a**) control, (**b**) model group; (**c**) Extract treated; (**d**) Glucophage treated; (**e**) Extract + lisinopril treated (**f**) Glucophage + lisinopril treated; (**g**) Lisinopril treated rats–

with the *E. chlorantha* compared to single administration of the extract.

Histopathological examination of tissues is useful in identifying the type of lesions caused by xenobiotics and is acknowledged as the most sensitive end point for detecting organ toxicity [15]. It is also useful in providing information about acute or chronic effects of toxic substances that may not be detected by other biomarkers [4, 12]. In this study the histology of the pancreas showed diminished expression of islet cells in the diabetic rats in contrast to the control with normal histoarchitecture cells indicating that diabetes may be associated with cells damage. The liver histology of the model group and the extract and lisinopril co-administered group showed fatty liver while the other groups did not. This also buttress the fact that single administration of the extract had better therapeutic and less toxicity effect on liver function compared to co-administration of the extract with synthetic drug.

Conclusion

This study concluded that *E chlorantha* is not toxic at acute dose and that combine administration of *E chlorantha* extract and lisinopril at sub-acute dose in diabetes may aggravate liver dysfunction associated with diabetes.

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Authors' contributions

LBI carried out experimental work, plant collection, literature search and manuscript preparation. EOA supervised the study and revised the manuscript for publication. All authors have read and approved the manuscript.

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Availability of data and materials

Not applicable in this section.

Ethics approval and consent to participate

The study was designed and conducted following the Department Animal Ethics Committee guidelines (No. KSUMB/005/01/018).

Consent for publication

Not applicable in this section.

Competing interests

The author declare that they have no competing interest.

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