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Toxicological evaluation of leaf essential oil of *Citrus sinensis* on alloxan-induced diabetic rats

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Abstract

This study investigated the toxicity effect of leaf essential oil of Citrus sinensis (Rutaceae) on alloxan –induced diabetic rats, having confirmed its antidiabetic potential in previous study.
Forty (40) male albino rats were randomly selected into 4 groups (A, B, C and D) of 10 rats each, representing (respectively) the Normal Control, Diabetic Control, Diabetic treated with 14.2 mg/kg b. wt. Metformin (reference drug), and Diabetic treated with 110 mg/kg b.wt. leaf essential oil of Citrus sinensis. Experimental animals apart from the normal, diabetic, and referenced drug treated groups were treated with the 110 mg/kg b.wt of the oil for 15 days. Effect on hematological parameters, liver and kidney function tests were evaluated. Results showed that there was a significant increase ($p<0.05$) in the concentration of red blood cell, packed cell volume and no significant difference ($p<0.05$) in White blood cell, lymphocyte and neutrophil when compared to the normal control. Citrus sinensis essential oil-treated diabetic rats also showed a significant increase ($p<0.05$) in total protein concentration, significant decrease ($p<0.05$) in albumin, total bilirubin and direct bilirubin concentrations compared to the normal control. Concentrations of serum creatinine and urea in the essential oil-treated diabetic rats significantly increased and decreased respectively when compared with the normal control group. A significant increase ($p<0.05$) was observed in the activity of heart alanine transaminase. Results of this study suggest that the leaf essential oil of Citrus sinensis at a dose of 110 mg/kg b.wt administered for 15 days might be toxic to the tissues study.

Keywords: Leaf essential oil, *Citrus sinensis*, Toxicological, Diabetic, Alloxan.

Introduction

Diabetes mellitus is a metabolic disorder characterized by persistant hyperglycemia and alteration. in the lipids, carbohydrates and proteins metabolism. Diabetes mellitus is a major global health problem. According to International Diabetic Federation, its incidence and prevalence is on the increase globally [IDF, 2006]. World Health Organization estimated that in year 2000, 171 million people had diabetes, this represented 2.8% of the world's population, and she predicted that this number will increase to 366 million (4.4%) by 2030 [IDF, 2006]. The complications arising from diabetes mellitus include vascular diseases, eye disorders, renal disorders and a host of secondary infections [ADA, 2009]. Currently, diabetes mellitus is managed with synthetic drugs, which is generally not preferred because of its high cost and undesired side effects. Hence, investigation into the development of alternative medicine has been ongoing. Herbal drugs constitute an important part of traditional medicine and literature showed that there are more than 400 plant species showing antidiabetic activity (Anwar *et al*., 2008).*Citrus* belong to the *Rutaceae* family. It comprised of about 140 genera and 1,300 species. *Citrus paradisi* (Grapefruit), *Citrus limon* (Lemon),*Citrus reticulata* (tangerine) and *Citrus sinensis* (Sweet Orange) are some important fruits of the genus (Anwar *et al*., 2008).*Citrus* fruits and their by-products are valued for their high economic and

medicinal and multiple uses, such as in the food industry, cosmetics and folk medicine (Silalahi, 2002; Saidani*et al.*, 2004). In a previous study by Muhammad *et al.* (2013), authors reported that Leaf essential oil of *Citrus sinensis* possessed significant antihyperglycemic effect on alloxan – induced diabetic rats. *Citrus* seed oil had also been reported to reduce blood glucose level significantly ($p<0.01$) in alloxan-induced diabetic rats (Chilaka*et al*., 2015). However, there is no scientific information on the toxicity effect of the leaf essential oil of *Citrus sinensis* on diabetic rats yet, which is what this study sought to investigate.

Materials and methods

Male wistar albino rats of Norvegicus strain were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin , Nigeria. The rats were housed in well ventilated cages and allowed to acclimatize to Animal House conditions for 7 days, during which they were fed with normal rat chow and allowed access to free water *adlibitum.* The chemical used were dimethylsulfoxide (product of Sigma Chemical Company, St. Louis, Mo,
USA), Alloxan monohydrate (Product of Sigma Chemical Company, St. Louis, Mo,
USA), OHAUS analytical balance (Ohaus Corporation, NJ, USA), Accu-chek active glucometer and strips (Product of Roche Diagnostic, Mannheim, Germany). All other chemical were of analytical grades and prepared in all glass distilled water.*Citrussinensis* leaves (Fresh) were obtained from the Junior Staff quarters of the University of Ilorin, Nigeria. It was Identified and authenticated at the department of Plant Biology, University of Ilorin.

Methods
Essential oil extraction

Citrus sinensis leaves were Pulverised and hydrodistilled for 3hr in a Clevenger- type apparatus according to the British Pharmacopeia Specification (1980). The resulting oil was collected, preserved in a sealed sample tube and stored under refrigeration until required for analysis.

Five (5 %) v/vstock concentration of the oil was prepared by dissolving 5 ml of the stock oil in 100 ml of 12.5 %v/v dimethylsulfoxide in normal saline (Lahlou, 2003).

Experimental animals

Forty (40) male albino rats of norvegicus strain (150–200 g) were housed in standard cagesand allowed to acclimatize to animal house for 14 days. Rats were maintained understandard laboratory conditions (12-h light/dark cycle, 25±2ºC). They were fed with rat chow and tap water *ad libitum*. Animals were then randomly selected into 4 groups (i.e.A, B, C and D) of 10 rats each representing (respectively) the Normal Control, DiabeticControl, Diabetic treated with 14.2 mg/kg b. wt. Metformin (reference drug), a product of Merck Santé S.A.S., an associate of Merck KGaA of Darmstadt, Germany, Licensed to Bristol-Myers Squibb Company and Diabetic treated with 110 mg/kg b.wt. leaf essential oil of *Citrus sinensis*. Essential oil was then administered to the animals every other day for a period of 15 days.

Ethical clearance

Ethical clearance for this study was obtained from the University of Ilorin ethical committee with protocol identification code UERC/LSC 067.

Induction of experimental diabetes

Animals were fasted for 8 hours and diabetes was induced by a single intraperitoneal injection of freshly prepared 150 mg/kg body weight alloxan monohydrate in sterile distilled water. Forty-eight (48hrs) after alloxan injection, fasting blood glucose was determined using AccuChek active glucometer and compatible strips. Rats showing glucose concentration above 110 mg/dl were considered diabetic.

Administration of oil

All treatments were intraperitoneally (IP) administered as follows:

Standardization of oil extracts

Group A (Normal control): Non-diabetic rats administered with distilled water.

Group B (Diabetic control): Diabetic rats administered with distilled water.

Group C (Diabetic + reference drug): Diabetic rats treated with 14.2 mg/kg body weight of metformin.

Group D (Diabetic + essential oil): Diabetic rats was treated with 110 mg/kg body weight of leaf essential oil of *Citrus sinensis.*

Collection and treatment of blood samples

Animals were sacrificed 24 hours after the last day treatment. They were anaesthetized with ethyl ether and sacrificed by simply incising the jugular vein. Blood samples were collected into EDTA and plain sample bottles for haematological and serum analysis respectively.

Haematologicalparameters

The following haematological parameters were analysed using automated hematologic analyzer: hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), neutrophils and lymphocytes employing the methods of Dacie and Lewis (1991).The analyzer uses whole blood samples to produce values for the parameters. It uses two fields, the cell packs (which functions as a detergent and self-rinses the system to avoid introduction of errors) and the stromatolyzer (which works on the cells).

Liver function test

Serum albumin concentration was quantified by the method described by Doumas*et al*. (1971). The method of Evelyn and Malloy (1938) was used tomeasure the concentration of Total and direct bilirubin concentration in the serum. Protein concentration in tissues was determined using the biuret method (Plummer, 1978).

Kidney function parameters

The method as described by Tietz*et al*. (1994) was used to determine the levels of urea and creatinine in serum.

Marker enzymes assay

The method of Wright *et al.* (1972) was used to determine the activity of ALP. AST activity was assayed using the method of Schmidt and Schmidt (1967) while Alanine Aminotransferasewas assayed for using the method of Schmdit and Schmdit (1963).

Statistical analysis

All data were expressed as the mean of five replicates $\pm$ standard error of mean (S.E.M). Statistical evaluation of data was performed by SPSS version 16.0 using one way analysis of variance (ANOVA), followed by Duncan's multiple range test for multiple comparison. Values were considered statistically significant at $p < 0.05$ (confidence level = 95%).

Results

Effect of leaf essential oil of *Citrus sinensis*on hematological parameters

Table 1 shows the effect of administration of leaf essential oil of *Citrus sinensis* on experimental animals. Diabetic control rats showed a reduction in RBC, PCV and Hb, which was significant ($p<0.05$) for RBC and PCV. There is no significant difference ($p<0.05$) in the concentrations of RBC, PCV and Hb of the rats treated with the essential oil and metformin when compared with the non-diabetic control rats.

Table 2 represents the effect of administration of leaf essential oil of *Citrus sinensis* on leukocyte indices of alloxan-induced diabetic rats. There is a significant decrease ($p<0.05$) in the concentration of WBC of all the diabetic rats when compared with the non-diabetic group. There is a significant reduction ($p<0.05$) in the concentration of neutrophils and lymphocytes of the diabetic control and the essential oil-treated diabetic rats when compared with the non-diabetic control rats. Only the diabetic rats treated with the standard drug showed no significant difference ($p<0.05$) in the concentration of neutrophils and lymphocytes when compared with the non-diabetic control rats.

Table 1: Erythrocyte indices of alloxan-induced diabetic rats administered with leaf essential oil of *Citrus sinensis*

Treatment	RBC (x1012µ/L)	PCV %	Hb (g/dL)
Non-diabetic + Water	5.26±0.09[a]	47.33±1.76[a]	14.80±1.06[a]
Diabetic + Water	4.59±0.40[b]	43.33±1.33[b]	13.67±1.22[a]
Diabetic + Standard drug	4.97±0.61[ab]	45.00±1.15[ab]	14.03±0.78[a]
Diabetic + EO	5.08±0.60[ab]	44.67±1.45[ab]	14.27±0.46[a]

Values are expressed as mean of five replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$)

Standard drug used was metformin

EO – Essential oil

Table 2. Leukocyte indices of alloxan-induced diabetic rats administered with leaf essential oil of *Citrus sinensis*

Treatment	WBC (x1012µ/L)	Neutrophils%	Lymphocytes%
Non-diabetic + Water	12.27±0.15[a]	42.00±0.58[a]	68.33±1.71[a]
Diabetic + Water	7.13±1.42[b]	31.33±1.96[b]	57.33±1.48[b]
Diabetic + Standard drug	7.53±0.45[b]	42.67±1.48[a]	65.33±1.33[a]
Diabetc + EO	7.47±0.64[b]	31.67±1.71[b]	58.00±1.15[b]

Values are expressed as mean of five replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$)

Standard drug used was metformin

EO – Essential oil

Effect of leaf essential oil of *Citrus sinensis*on liver function indices

Total and Direct Bilirubin levels of diabetic untreated rats increased significantly ($p < 0.05$) when compared to the non-diabetic rats. Rats treated with leaf essential oil showed a significantly decrease ($p < 0.05$) in total and direct bilirubin levels when compared to the non-diabetic rats and the diabetic untreated rats (Table 3).

Only the diabetic group treated with the standard drug showed no significant difference ($p < 0.05$) in total protein concentration when compared with the non-diabetic group. All the diabetic rats showed a significant reduction ($p < 0.05$) in albumin concentration when compared with the non-diabetic group (Table 4)

Table 3: Total and direct bilirubin concentrations of alloxan-induced diabetic rats administered with leaf essential oil of *Citrus sinensis*

Treatment	Total bilirubin(mg/dl)	Direct bilirubin(mg/dl)
Non diabetic + Water	13.80±1.03[a]	1.92±0.17[a]
Diabetic + Water	19.30±0.25[b]	2.41±0.45[b]
Diabetic + standard drug	12.79±0.22[a]	1.64±0.29[ac]
Diabetic + EO	11.28±0.84[c]	1.30±0.23[cd]

Values are expressed as mean of five replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$)
Standard drug used was metformin
EO – Essential oil

Table 4: Total protein and albumin concentrationsalloxan-induced diabetic rats administered with leaf essential oil of *Citrus sinensis*

Treatment	Total protein(g/dl)	Albumin (g/dl)
Non diabetic + Water	4.68±0.31[a]	3.96±0.11[a]
Diabetic + Water	5.74±0.06[c]	2.67±0.10[b]
Diabetic + standard drug	4.48±0.04[a]	3.04±0.07[c]
Diabetic + EO	5.36±0.24[d]	2.48±0.60[bc]

Values are expressed as mean of five replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$)

Standard drug used was metformin

EO – Essential oil

Effect of leaf essential oil of *Citrus sinensis* on kidney function

The effect of administration of leaf essential oil of *Citrus sinensis* on serum creatinine and urea concentrations in alloxan-induced diabetic rats is presented in Table 5. There was a significant increase ($p<0.05$) in serum creatinine level for all the diabetic rats when compared with the non-diabetic control group. The diabetic untreated rats and the standard drug-treated diabetic rats showed a significant increase ($p<0.05$) in serum urea concentration while the essential oil-treated diabetic rats showed a significant reduction ($p<0.05$) in serum urea levels when compared with the non-diabetic control group.

Table 5: Serum creatinine and urea concentrations of alloxan-induced diabetic rats administered with leaf essential oil of *Citrus sinensis*

Treatment	Creatinine (mg/dl)	Urea (mg/dl)
Non diabetic + Water	1.83±1.06[a]	11.19±0.86[a]
Diabetic + Water	8.25±0.85[b]	35.05±2.15[b]
Diabetic + standard drug	4.42±0.30[c]	27.77±3.87[c]
Diabetic + EO	6.76±0.64[b]	4.52±0.74[d]

Values are expressed as mean of five replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$)

Standard drug used was metformin

EO – Essential oil

Effect of leaf essential oil of *Citrus sinensis* on marker enzymes of tissue damage

The effect of leaf essential oil of *Citrussinensis*on serum and tissue alkaline phosphatase is presented in Table 6. The diabetic untreated rats showed a significant increase ($p<0.05$) in serum alkaline phosphatase activity when compared to the non-diabetic control and the diabetic animals treated with standard drug and the essential oil. There was also observed concomitant significant decrease ($p<0.05$) in liver and kidney alkaline phosphatase activity in the diabetic untreated rats when compared with the non-diabetic control and the diabetic treated with standard drug and the essential oil.

Table 6: Effect of administration of leaf essential oil of *Citrus sinensis* on serum and tissue alkaline phosphatase (ALP) activity (nm/min/mg protein) in alloxan-induced diabetic rats

Treatment	Serum	Liver	Kidney
Non diabetic + Water	1.18±0.14[a]	5.42±0.40[a]	6.34±0.12[a]
Diabetic + Water	2.37±1.14[b]	1.42±0.63[b]	0.72±0.27[b]
Diabetic + standard drug	1.08±0.33[a]	4.46±0.62[a]	6.22±0.01[ac]
Diabetic + EO	1.10±0.40[a]	4.50±0.19[a]	6.57±0.71[ad]

Values are expressed as mean of five replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$)

Standard drug used was metformin

EO – Essential oil

Table 7 shows theeffect of administration of leaf essential oil of *Citrus sinensis* on serum and tissue aspartate transaminase (AST) activity (U/L) in alloxan-induced diabetic rats. The diabetic untreated group showed a significant increase ($p<0.05$) in serum AST activity and a concomitant significant decrease ($p<0.05$) in liver and heart AST activity when compared with the non-diabetic control and the diabetic groups treated with metformin and the essential oil.

The effect of the essential oil on serum and tissue ALT in alloxan-induced diabetic rats is presented in Table 8. Diabetic untreated rats showed a significant increase ($p<0.05$) in serum ALT activity with a concomitant significant decrease ($p<0.05$) observed in the liver. Heart ALT activity increased significantly ($p<0.05$) in the diabetic rats treated with both metformin and the essential oil when compared with the non-diabetic control and the diabetic control groups

Table 7: Effect of administration of leaf essential oil of *Citrus sinensis* on serum and tissue aspartate transaminase (AST) activity (U/L) in alloxan-induced diabetic rats.

Treatment	Serum	Liver	Heart
Non diabetic + water	5.88±0.54[a]	12.69±1.15[a]	36.50±2.29[a]
Diabetic + Water	7.84±0.52[b]	4.43±0.93[b]	5.53±1.25[b]
Diabetic + standard drug	5.30±0.66[a]	12.34±1.17[a]	35.91±1.42[a]
Diabetic + EO	5.20±0.43[a]	12.05±0.03[a]	38.14±1.74[a]

Values are expressed as mean of five replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$)

Standard drug used was metformin

EO – Essential oil

Table 8: Effect of administration of leaf essential oil of *Citrus sinensis* on serum and tissue alanine transaminase (ALT) activity (U/L) in alloxan-induced diabetic rats

Treatment	Serum	Liver	Heart
Non diabetic + Water	1.10±0.48[a]	7.78±0.79[a]	4.04±0.46[a]
Diabetic + Water	2.68±0.28[b]	0.50±0.00[b]	4.93±0.42[a]
Diabetic + standard drug	0.95±0.11[a]	7.71±0.09[a]	7.50±2.05[b]
Diabetic + 7.00a.m EO	0.83±0.15[a]	7.32±0.09[a]	7.35±0.80[b]

Values are expressed as mean of five replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$)

Standard drug used was metformin

EO – Essential oil

Discussion

The result of this study showed a significant reduction ($p<0.05$) in the concentrations of erythrocyte parameters; RBC, PCV and Hb. This finding is similar to those of previous studies (Sheela and Augusti, 1992; Mohammed *et al*., 2009; Muhammad and Oloyede, 2009). Anaemia at the onset of diabetes mellitus has been documented and it was ascribed to increased non-enzymatic glycosylation of red blood cell membrane proteins (Oyedemi*et al*., 2011). Oxidation of these proteins and hyperglycaemia in diabetes mellitus results in increased production of free radicals through lipid peroxidation. This has a haemolytic effect on RBC (Arun and Ramesh, 2002 ; Maria *et al*., 2009). Upon administration of leaf essential oil of *Citrus sinensis,* therewassignificant increase in the concentration of RBC and PCV to normal levels with a performance similar to that observed with standard drug, metformin. This observation might be due to the presence of certain phytoconstituents of the oil, which was previously reported to be contain polyphenols, terpenes and hydrocarbons (Mohammad *et al.,* 2013). Some of these compounds may possess stimulatory ability on the stem cells in the bone marrow inducing erythropoietin formation and consequently resulting in RBC production. Another plausible explaination to this observation might be attributed to the antioxidant capacity of some of the phytoconstituents. Phenols has been reported to possess strong antioxidant capacity (Akah*et al*., 2007). Peroxidation of polyunsaturated fatty acids in the cell membrane could be inhibited and thus prevent further haemolysis of red blood cells in the diabetic animals by these constituents (Oyedemi*et al*., 2011; Torell*et al*., 1986). Reports from previous studies have also revealed the ability of the orange volatile oil to chelate Fe^{2+} which might serve to

prevent the metal from initiating lipid peroxidation (Mohammad *et al.*, 2013). The OH* scavenging ability of the volatile oil was attributed to the ability of the phenolic compounds in the oil as well, donating hydrogen since the radical can be neutralized by the hydrogen atom (Oyedemi*et al*., 2010; Faure *et al*., 1991).

Induction of diabetes in this study also led to a significant reduction in leucocyte parameters WBC, neutrophils and lymphocytes. This is in agreement with the work of Harsunen*et al*. (2013) where adult patients with newly diagnosed type 1 diabetes had significantly lower total WBC, neutrophil, basophil, monocyte and lymphocyte counts compared to control subjects. Our results therefore strengthens the suggestion of Harsunen*et al*. (2013) there is a general involvement of the innate immune system in the pathogenesis of type 1 diabetes.However, administration of metformin restored to normality the concentrations of neutrophils and lymphocytes while the essential oil could not bring about any ameliorative effect on the leucocyte parameters evaluated. Since white blood cells (WBC) or leukocytes are the mobile units of the body's protective system, a reduction of these parameters could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection (Oyedemi*et al*., 2010).

Result of this study showed that diabetic control animals in this study had increased levels of total and direct bilirubin concentration. Administration of the essential oil to diabetic rats led to a drastic reduction below normal levels of both total and direct bilirubin. Bilirubin (total and direct) are parameters used to assess the excretory function of the liver (Yakubu and Omoniwa, 2012). Severe hemolysis causes the release of bilirubin into the blood which manifests as elevated levels of unconjugated and total bilirubin. Unconjugated and total bilirubin can also increase in the event of low bilirubin conjugation. The essential oil may have been able to encourage glucose uptake thus preventing the glycosylation of hemoglobin which ultimately leads to hemolysis and also either inhibit normal hemoglobin breakdown or induce a rapid removal of bilirubin from the blood faster than normal through increased rate of conjugation and excretion into the bile.

In this study, total protein concentration was raised while albumin concentration was reduced. This results is similar with those documented by Pierpaolo*et al.* (1991). Metformin administration restored only total protein to control level while *Citrus sinensis*essential oil could not restore the liver protein synthetic functions.

In this study, induction of diabetes led to an increase in serum creatinine and urea levels. Administration of metformin and the essential oil did not restore the kidney function parameters to normal levels. Instead the essential oil led to a drastic reduction of urea below normal levels.

Urea is formed in the liver from the amino nitrogen of deaminated amino acids that are excess of requirements and transported to the kidney for excretion. High blood urea levels in renal disease are a consequence of impaired renal function (Murray *etal*., 2003). Creatinine is excreted by kidney through the blood. It is filtered in the glomerulus and then reabsorbed by the tubules. Blood creatinine level is raised in cases of renal failure (Murray *etal.*, 2003). Serum Urea and Creatinine are indicators of kidney functions. They are usually required to assess the normal functioning of different parts of the nephrons (Abolaji*et al*., 2007). Research has extensively documented that hyperglycemia leads to increase in serum urea and creatinine which is indicative of progressive renal damage (Bauza and Mosquera, 2003; Anjaneyulu*et al*., 2004; Emre*et al.*, 2006). Little attention have been given to Clinical conditions associated with low serum urea nitrogen (UN) concentrations in patients, therefore there is paucity of information available. Some causes of low serum urea are acute liver failure (decreased urea synthesis), overhydration (hemodilution), starvation, and repeated peritoneal dialysis (Sonnenwirth and Jarett, 1980). Since rats had free access to food and did not undergo peritoneal dialysis, the observed decrease in serum urea may be attributed to either acute liver failure which is a significant loss of the liver's synthetic functions or overhydration as a result of poor excretion of excess water by the kidneys.

Diabetes induction with alloxan led to a decrease in both liver and kidney ALP activity and a concomitant rise of same in the serum. Previous reports have also confirmed altered total ALP activity in diabetes mellitus (Hough *et al*., 1981; Weil and Russell, 1940). However, administration of the essential oil of *Citrus sinensis* leaf caused a reversal of ALP in the tissues and serum to normal levels (Table 6). This suggests that the essential oil might be able to restore the integrity of the plasma membrane and thus stop the leakage of ALP from the membranes of the liver and kidney cells into the serum.

Elevated activities of serum aminotransferases are common sign of liver disease and are observed more frequently among people with diabetes than the general population (Arkkila*et al*., 2001). Many studies have shown an association between specific diabetic complications and disturbances in various tissues, such as nephropathy and cardiovascular

diseases (Arkkila*et al*., 2001). In this study, the onset of diabetes was accompanied by a rise in serum AST activity with a simultaneous drop of activity in the liver and heart (Table 7). The administration of the essential oil and metformin caused a significant increase in the activity of ALT in the heart of experimental rats. This increase was however not reflected in the serum of the animals. This means there was no leakage arising from a derangement of cardiac cell membranes but though the essential oil and metformin might have induced excessive synthesis of ALT in the heart above the normal levels.

Conclusion

Essential oil of *Citrus sinensis* in this study was able to improved erythrocyte parameters in diabetic rats but has not effect on the leucocyte indices. The oil also could not normalize liver protein synthetic functions and kidney function indices but caused a reduction in serum urea concentration beyond normal levels. This is an indication that the essential oil may be able to cause a significant loss of liver synthetic functions and/or an impairment of kidney excretion of excess water. Finally, the essential oil was able to restore the integrity of plasma membranes of diabetic rat liver, kidney and heart but also induced excessive synthesis of heart ALT in diabetic rats. Therefore in the light of our findings, more phytochemical studies should be carried out on the essential oil of *Citrus sinensis* so as to maximize its antidiabetic potential with the consciousness of its toxicity properties.

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