Toxicology Assessment of *Ocimum tenuiflorum* L. Leaves Extracts on Streptozotocin-Induced Diabetic Rats

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Abstract

The present study was aimed out to investigate the effects of different solvent extracts from *Ocimum tenuiflorum* L. leaves on lipid profile, liver function, and kidney functions of streptozotocin-induced diabetic rats and toxicology assessment. In this study *Ocimum tenuiflorum* leaves solvent crude extracts were used for an acute test for 14th days. Diabetes was induced in Spargue-Dawley rats by intraperitoneal (i.p.) injection of streptozotocin. All the diabetic rats were under treated by oral admonition of extracts (1000 mg/kg) for fourteen days. After 14th days of treatment blood was withdrawn for biochemical and haematological tests. Kidney, pancreas and liver discarded for histopathology assessment of toxicity effect on organs. Blood glucose level and other biochemical measurements in streptozotocin-induced rats compared with the control group showed fluctuating levels in a different parameter. Histological analysis of tissues indicated there were no adverse changes in the liver, kidneys, and pancreas compared to the control group. According to our result, *Ocimum tenuiflorum* methanol leaf extract as a medium polarity solvent extract had possibly exerts of an antihyperglycaemic effects through major bioactive compounds that be able to control diabetes. A clinical study is needed to confirm the antidiabetic potential of this plant.

Keywords: Biochemical, Histopathology, Haematology parameters, *Ocimum tenuiflorum* L., Sprague Dawley rats, streptozotocin (STZ)

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Introduction

Medicinal plants in medication for various diseases results in positive outcomes, the toxic consequences to it should also be considered. Therefore, the proper choice of the dose is required. Although there is also an extensive use of these sources for food, economic, and medicinal purposes, unfortunately, the availability of information regarding the toxicology of these plants is scarce. In addition, the potential of these substances to inflict damage on organs mostly results from the interaction of a series of complex cellular processes which takes place in their metabolism activity. However, based on a scientific research conducted in the last 40 years, it was shown that numerous vital side effects come with plants which are utilized for food or traditional medicine. Besides, it was also found that some components of

these plants can lead to potentially toxic such as mutagenic, and carcinogenic [1,2]. It is important to conduct toxicity investigations on medicinal plants in order to assess their effects and mode on human and to prevent chronic medical problems which are possibly the results from the accumulation of toxic compounds within the cells and tissues of human body. Moreover, the use of traditional medicines amongst the world population amounts to 60% both in developing and developed countries where modern medicines are primarily used [3]. In most cases, the recipes are prepared by practitioners from a combination of two or more plant products, which could be used in order to treat more than one disease [4]. Based on long-term investigation conducted on the most disease, it was found that without the practice of proper dosage of medicinal plant product and consideration regarding the toxic effects, prolonged usage will occur. Due to the risks associated with the potential toxicity from long-term usage of such therapy and other herbal therapies, it is important for practitioners to be constantly informed of the report regarding the occurrence of renal and hepatic toxicity as a result from the consumption of medicinal herbs [5]. Severe toxicity has been associated with the potential of a substance to inflict biological harm or death soon after a single dose, or any poisonous effects which are due to a single short-term exposure to a toxic substance [6]. The purpose of defining severe toxicity is to determine the symptomatology implications of the administration conducted on the ranges of lethal dose intake for the best substance. It is also in order to give out a prompt warning upon the involvement of a highly toxic compound [7].

Usually, studies on toxicities of traditional medicine plant in animals are conducted for any pharmacological purposes. There are several useful advantages to the acquirement of the information from these studies, in terms of the choice of doses for repeat-dose studies. These advantages also allow the preliminary identification of the organs where this toxicity occurs, and information regarding the biologic activity of a chemical can be acquired. Besides, insights into the chemical's mechanism of action are obtained [6]. Toxic effects are associated with "harmful response of a biological system to a toxic compound". This can risk in the death of cells or the whole organism [8]. On the other hands, there are several major groups of compounds which have a role in the phytomedicine activity from plants. This includes alkaloids, terpenoids, coumarins, tannins, flavonoids, saponins, quinones, phenolic acids, and phenolics, that we can extract by the different polarity of the solvent. Generally, compounds with a low polarity such as some alkaloids, waxes, and fatty acids are extracted by n-hexane and chloroform [9]. Meanwhile, butanol, methanol, and ethyl acetate are known for extracting both medium polarity and some polar compounds such as some terpenoids, tannins, and flavonoids [10]. On the other hand, water is known for its extraction activity on highly polar compounds such as amino acids, carbohydrates, glycosides, and their derivatives [10].

Notwithstanding, there is a paucity of literature exploring the toxic effects of *Ocimum tenuiflorum* leaves and their effects on the biochemical parameters and activities of enzymes associated with diabetes mellitus (DM). The present study was carried out to determine the biochemical, haematological and histopathological toxicity of non-polar and polar solvent extracts of *Ocimum tenuiflorum* leaves. This study therefore aimed to examine the effects of various oral crude *Ocimum tenuiflorum* plant leaf extracts that were administered sub chronically in rats to determine their biochemical and haematology effects as well as to systematically evaluate the effects of these crude extracts on streptozotocin (STZ)-induced diabetic rats to determine their roles in reducing the toxic effects of DM on vital organs.

Material and Methods

Sample preparation

Fresh plants or plant parts were collected from Perak (Malaysia). Voucher specimens have been deposited at the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia (USM Herbarium number 11400). Flowers and leaves were removed from the stems and tap water used for washing them, leaves and stems were frozen for three days, (millwork Technology, LD53, Kingston, USA) as explained by [11]. After that, a dried sample was used for blending (Panasonic, MX 335, Malaysia). Following that, powder samples, were kept in vacuum package and kept at 4 ºC (Toshiba, GR-M48MP, Minato-Ku, Japan) before the analysis process.

Preparation of Crude Extracts

To prepare the crude extract, dried powder samples (500 g) were soaked for seven days in individual glass jars containing non-polar to polar solvents, including chloroform, n-hexane, ethyl acetate, methanol and water, at room temperature. After this soaking period, the solvent was filtered, and the residue of each extract was kept in a fume cupboard until it was dried and ready for solvent extraction. The extracts obtained from each solvent were filtered using filter paper and concentrated using a rotary evaporator (Buchi Lsbortechnik AG, Switzerland) [11].

Animals

Forty-two male Sprague Dawley rats (weight 200–300 g) were obtained from the animal house of the Animal Research and Service Centre, Universiti Sains Malaysia. The rats were housed in standard cages (22±3 ºC) with food and water provided *ad libitum*. After a period of acclimation, the animals were divided into seven groups with each group comprised of six rats in each group. Groups one to seven were injected with a single dose of Streptozotocin (STZ) 55 mg/kg (Sigma Aldrich Chemical Co., USA) and were monitored for a period of one week to allow for the stabilization of their diabetes. The first and second groups were kept as diabetic controls and were given an oral anti-diabetic drug standard (metformin 500 mg/kg.) (Glucophageas ® Lipha Pharma Ltd., United Kingdom) as a positive control and vehicle groups, respectively. The experimental protocol was approved by the Institutional Animal Ethical Committee (USM/ Animal Ethics Approval / 2013/ (89) (479)).

Induction of Experimental DM and Treatment Protocol

The animals were divided into seven groups of six animals as follows:

- Normal Group 1: Vehicle control treated with 10 mg/kg normal saline.
- Diabetic Group 2: Diabetic standard treated with 500 mg/kg metformin.
- Diabetic Group 3: Treated with 1000 mg/kg hexane extract.
- Diabetic Group 4: Treated with 1000 mg/kg chloroform extract.
- Diabetic Group 5: Treated with 1000 mg/kg ethyl acetate extract.
- Diabetic Group 6: Treated with 1000 mg/kg methanol extract.
- Diabetic Group 7: Treated with 1000 mg/kg water extract.

The crude extracts and metformin were administered to the respective animal groups every morning for 14 days through gastric intubation with a force-feeding needle (1 ml/kg). On the fourteenth day, after overnight fasting, blood samples were withdrawn directly from the heart for analysis after the animals were euthanized. The body weight of each experimental animal was recorded daily prior to treatment using a digital scale, and the animals were weighed again prior to being euthanized. Blood glucose levels were recorded on the first, seventh- and fourteenth-days by glucometer (Accu-check Advantage II clinical glucometer) (Roche Diagnostics Co., USA) during treatment in the overnight-fasted animals from the tail. Whole blood was collected into EDTA tubes and then centrifuged. The resulting supernatant was plasma and immediately transfer into a clean polypropylene tube using a Pasteur pipette. The samples were maintained at 2–8°C while handling.

Acute toxicity study

In accordance to the guideline provided by the Organization of Economic Cooperation and Development (OECD) for testing of chemicals 425 (2001) (up and down dosing procedure), five healthy female SD rats were picked for this experiment to administration of each crude extract. Prior to the administration of crude extracts (hexane, chloroform, ethyl acetate, methanol, water) of *Ocimum tenuiflorum* to all rats in each group, only one rat from each group, after fasting for 16 hours, feed by oral administration of crude extract (2000 mg/kg) using a ball-tipped stainless-steel feeding needle. The observation was made on one rat during 1, 4, 12, and 24 hours after treatment. The same dose was fed to another four additional rats after finding out that the first rat survived, which totaled the fed rats to five altogether. Toxic symptoms, was observed.

Haematology and biochemical assays

Blood samples were analyzed for red blood cells (RBC), haemoglobin (Hb) content, packed cell volume (PCV), white blood cells (WBC), and their differentials (i.e., neutrophils, eosinophils, basophils, lymphocytes, and monocytes). Biochemical parameters [aspartate aminotransferase (AST), alkaline phosphatase (ALK), alanine aminotransferase (ALT) activities, creatinine, urea, uric acid, albumin, globulins and total proteins] were examined. Furthermore, the electrolytes Na^+, K^+, Cl^+, Ca^{2+}, and P^{3+} were evaluated in serum samples. All the results were compared with results from the control group blood test results.

Haematological and biochemical parameters were measured at Gribbles Pathology (M) Sdn. Bhd. (Pulau Pinang, Malaysia), using an automated haematology analyzer (Sysmex-XT-1800, Kobe, Japan) and an automated chemistry analyzer (Olympus 640 Biochemistry Analyser, Tokyo), respectively.

Histopathological examination of liver, kidney and pancreas tissues

The liver, kidneys, and pancreas from each rat were posthumously excised, blotted and weighed for microscopic evaluation. Tissues were fixed in a fixative (i.e., 60% absolute ethanol, 30% formaldehyde and 10% glacial acetic acid) and embedded in paraffin. Samples were subsequently sectioned at 4 µm and subsequently stained with haematoxylin/eosin [13]. The sections were studied under a light microscope (DIALUX 20 EB) at 10x and 40x magnifications.

Anti-hyperglycaemic test in STZ-Induced Diabetic Rats (SDRs)

Diabetic rats were equally divided into seven groups (n = 6). The first group was treated with metformin (500 mg/kg). The second group was treated with normal 0.9% saline (10 ml/kg) as a negative control, and the third, fourth, fifth and sixth groups were treated with chloroform, ethyl acetate, methanol and water extract 1000 mg/kg), respectively, for fourteen days. BGLs were measured before and 14 days after treatment.

Statistical Analysis

Values were represented as a standard error of the mean (±SEM). A one-way ANOVA, was employed for the statistical analysis [14]**.**

Results

Effects of different extracts of Ocimum tenuiflorum L. on organ weights in STZ-induced treated rats

Table 1 indicated some differences in the organs of the treatment groups, these differences did not display significant changes compared to the control group. Although, the weight of the left testis showed a slightly increase in almost the same range when the animals were treated with all crude extract's administrations except water extract treatment group. The pancreas was the next organ that indicated a slightly increase when treated with water extract.

Parameters (g)	Normal Control	Diabetic Control	Various extracts of *Ocimum tenuiflorum* (1000 mg/kg)				
			Metformin	Chloroform	Ethyl acetate	Methanol	Water
Liver	4.46±0.09	5.03±0.00	4.03±0.07	4.09±0.4	4.43±0.09	4.45±0.5	4.33±0.3
Spleen	0.23±0.01	0.33±0.01**	0.23±0.02	0.22±0.03	0.20±0.02	0.21±0.04	0.24±0.02
Thymus	0.07±0.01	0.13±0.01	0.13±0.02	0.18±0.03	0.15±0.08	0.10±0.02	0.10±0.01
Right kidney	0.46±0.02	0.56±0.02	0.56±0.11	0.45±0.03	0.44±0.02	0.48±0.04	0.50±0.04
Left kidney	0.44±0.02	0.40±0.00	0.40±0.01	0.47±0.04	0.43±0.01	0.47±0.04	0.50±0.04
Stomach	3.33±0.05	3.69±0.05	3.69±0.34	2.80±0.24	2.95±0.10	3.30±0.60	3.29±0.40
Heart	0.38±0.01	0.37±0.02	0.37±0.04	0.38±0.01	0.30±0.00	0.38±0.03	0.44±0.03
Lung	0.71±0.02	2.08±0.10*	0.78±0.41	0.71±0.03	0.73±0.02	0.71±0.08	0.71±0.03
Right testis	0.39±0.01	0.51±0.00***	0.31±0.04	0.30±0.10	0.37±0.01	0.38±0.07	0.30±0.04
Left testis	0.45±0.03	0.50±0.00	0.50±0.01	0.58±0.04	0.58±0.01	0.57±0.01	0.35±0.04
Adipose tissue	0.18±0.00	1.02±0.07***	0.15±0.01	0.10±0.01	0.14±0.05	0.17±0.06	0.23±0.03
Pancreas	0.20±0.02	0.22±0.00	0.22±0.07	0.23±0.02	0.21±0.01	0.24±0.08	0.25±0.03

Table 1 Effects of different extracts of *Ocimum tenuiflorum* L. on organ weights in STZ-induced treated rats.

Values are expressed as Mean ± SEM. (n = 6).* $p < 0.001$, ** $p < 0.01$ and *** $p < 0.05$ between diabetic treated groups and control (Analysed by One-way ANOVA)

Body weight and behavior observations of acute toxicity study

Table 2 Shows that the limited dose (2000 mg/kg) given to the group of rats did not cause any changes or strange behavior throughout the period of fourteen days except hexane crude extract administration which shows that weight loose and they were too weak but after seven days of oral administration few rats died during these fourteen days observation some of the rats show diarrhea. Besides the gradual growth of the weight of the rats, there were no deaths and among the recording daily behaviors the rats did not show any symptoms of sickness, cramps and they behaved as the normal control rats. This means that the dose of the extract had no effect in changing the patterns of behave or leading to the appearance of any cases of illness in rats of both sexes (male 1000 mg/kg and female 2000 mg/kg).

Table 2 Acute toxicity assessment of hexane, chloroform, ethyl acetate, methanol and water extracts of *Ocimum tenuiflorum*

leaves (dose 2000 mg/kg) on body weight of control and treated rats

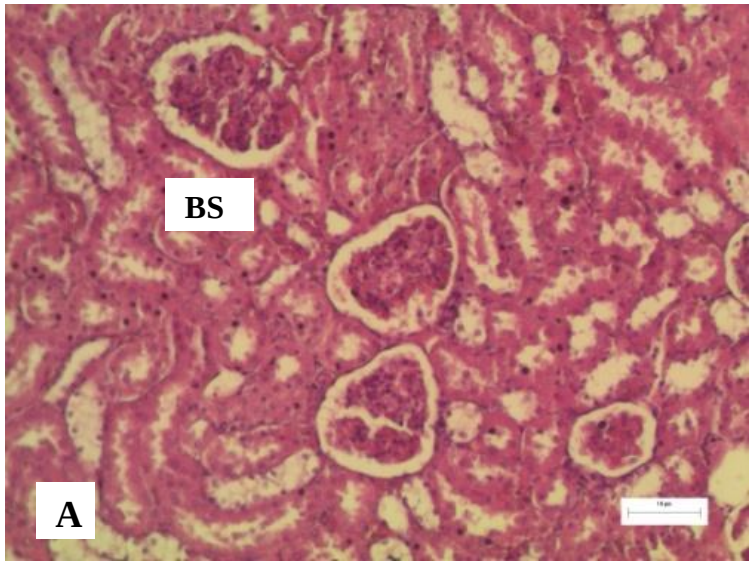
Female / 2000 mg/kg	crude extracts administration					
	Control	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Day 0	210± 3.08	221±1.09	232±1.85	215±4.84	235±4.08	243±3.33
Day 7	221±3.01	200±2.03	222±2.30	220±5.9	230±3.08	240±3.04
Day 14	233±4.06	210±3.40	241±1.70	232±8.8	245±4.15	251±3.7
Gain weight (g)	23±2.30	-11±1.80	9±1.30	17±7.2	10±3.14	8±4.04

Values were expressed as Mean ± SEM, compared the normal control.

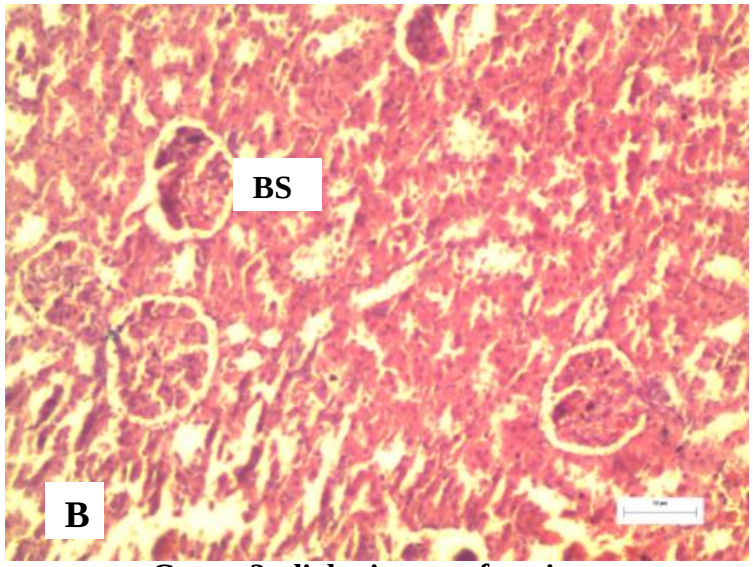
Histology assessment

Histopathology of the kidneys

The effects of *Ocimum tenuiflorum*: chloroform (OTCH), ethyl acetate (OTEE), methanol (OTME) and water (OTAQ) extracts on the histomorphology of Streptozotocin-induced rat kidneys can be seen in Figure 1. A mild thickening in the basement membrane of the arterioles of the glomeruli, along with mild changes in the density of the mesangial mesangium was observed in the diabetic rats. No other significant changes were seen (Figure 1). After treatment with various crude extracts, these changes in the diabetic rats improved towards normal conditions.



Group 1: normal control



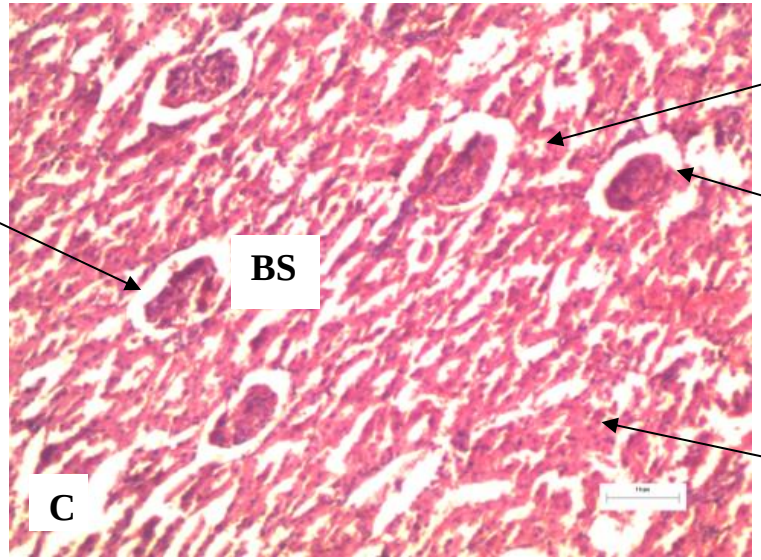
Group 2: diabetic + metformin

Per vascular space enlargement

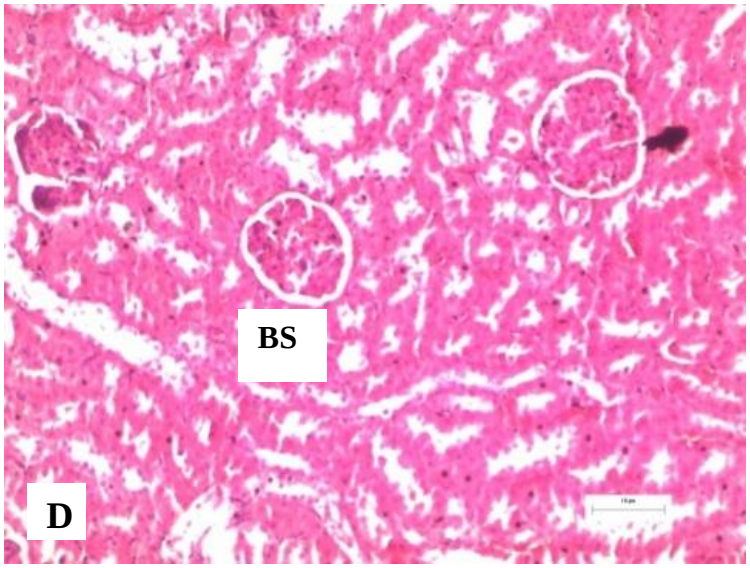
Mild changes in the density of Mesangium-Intra glomerula cell

Bowman Capsule

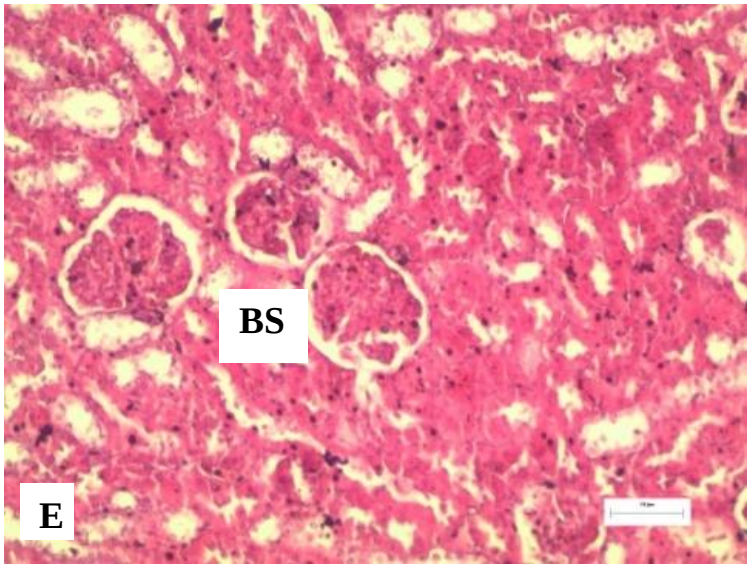
Mild changes in the density of Mesangium-Extra glomerula cell



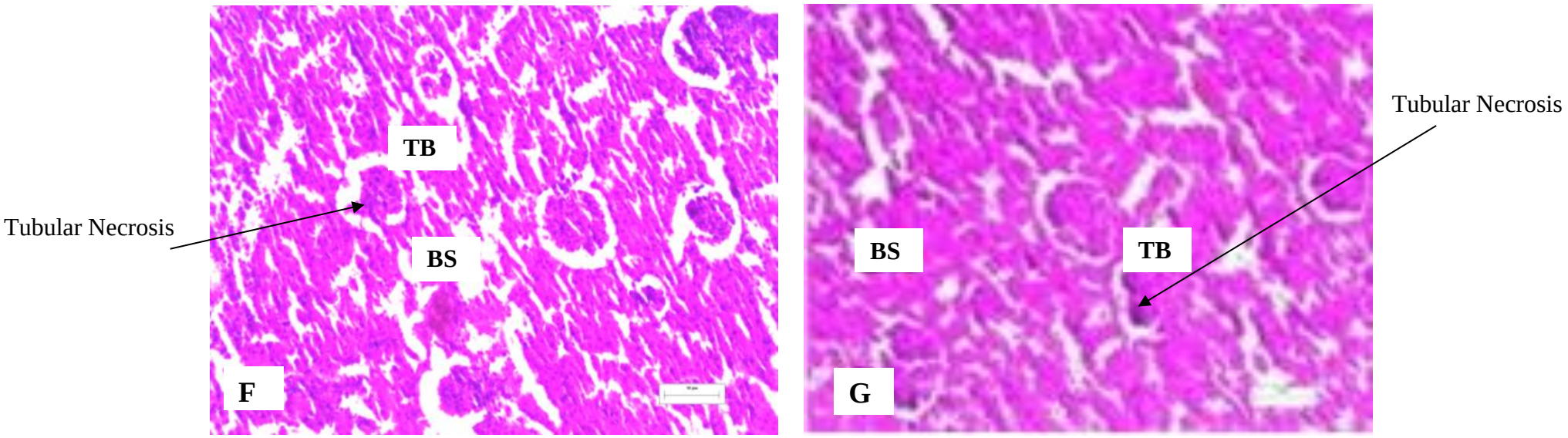
Group 3: diabetic control



Group 4: diabetic + OTCH (100mg/kg)



Group 5: diabetic + OTEA (1000mg/kg)



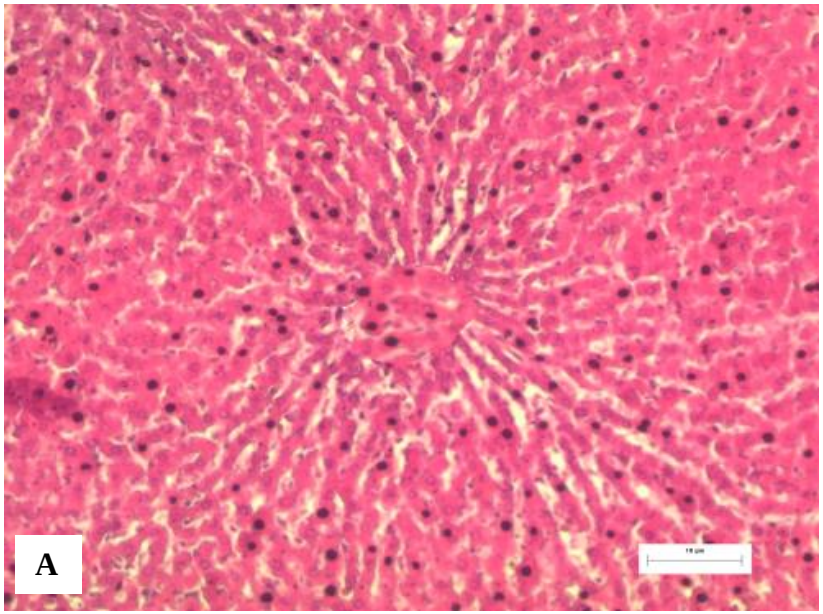
Group 6: diabetic + OTME (1000 mg/kg)

Group 7: diabetic +OTAQ (100mg/kg)

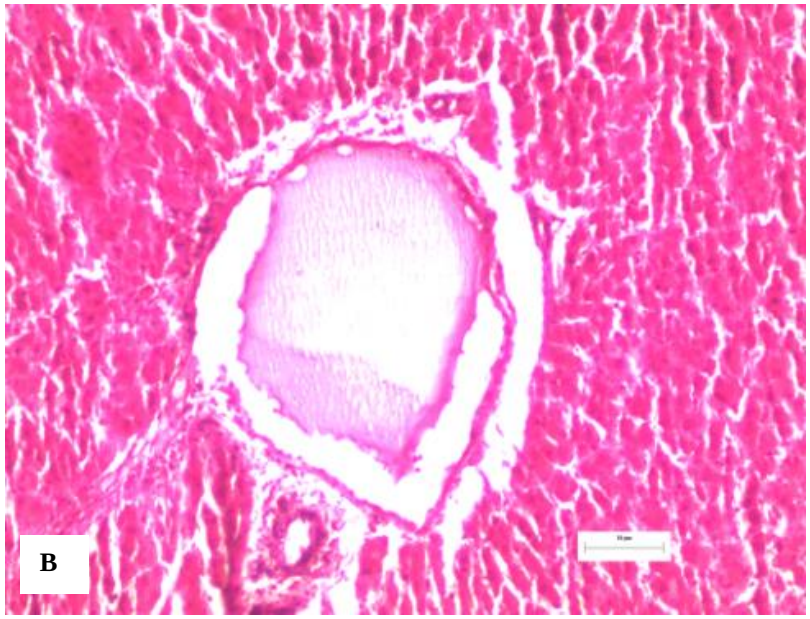
Figure1. Microphotographs of kidney histology of different groups after treatments with *Ocimum tenuiflorum* L. leave extracts made with chloroform (OTCH), ethyl acetate (OTEE), methanol (OTME) and water (OTAQ) in STZ-induced rats (H&E staining 10x). The glomerulus shows suffused capillary loops and expansion from red cell spillage in Bowman's space (BS)**,** Tubular Necrosis (TB)

Histopathology of the liver

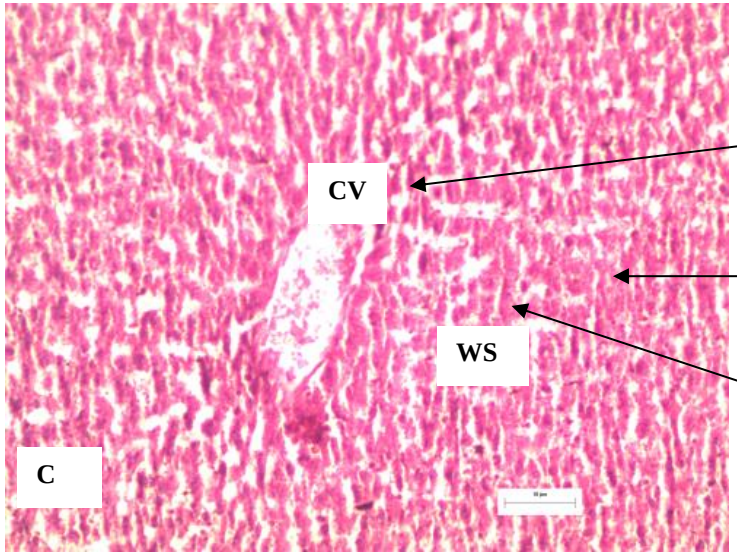
STZ-induced toxicology in the liver was observed at a morphological level by performing histology with H&E saline. The histomorphological effects of chloroform (OTCH), ethyl acetate (OTEE), methanol (OTME) and water (OTAQ) in STZ-induced rats can be seen in Figure 2. Congestion was noted in the hepatic central venule. Moreover, steatosis was observed around the central venue with a widening of the sinusoids and the infiltration of inflammatory cells. Congestion in the central venule and mild inflammatory cell infiltration was observed in the lower level in the methanol extract-treated group compared to other treatment groups. Nevertheless, the histology results demonstrated a range of pathological signs following treatment with various crude extracts, and these pathological signs included cell vacuolization, fatty deposits and lymphocyte invasion.



Group1: normal control



Group2: diabetic + metformin

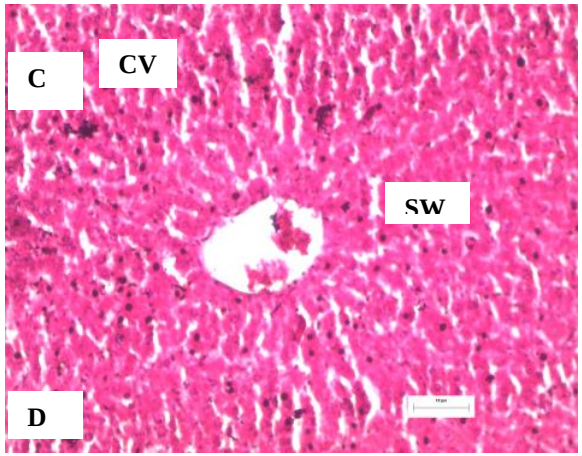


Central Venule

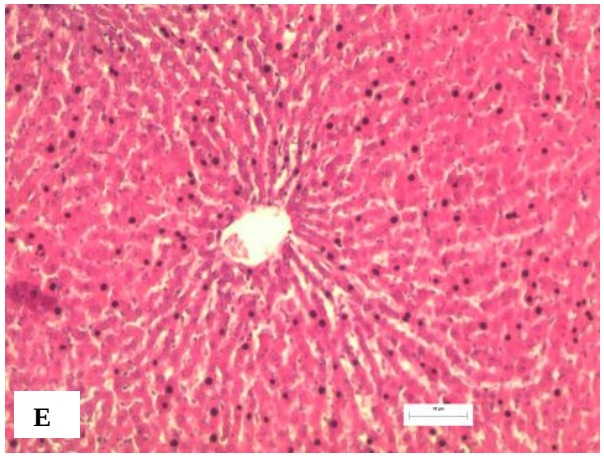
Congestion Steatosis observed with the

Widening sinusoids

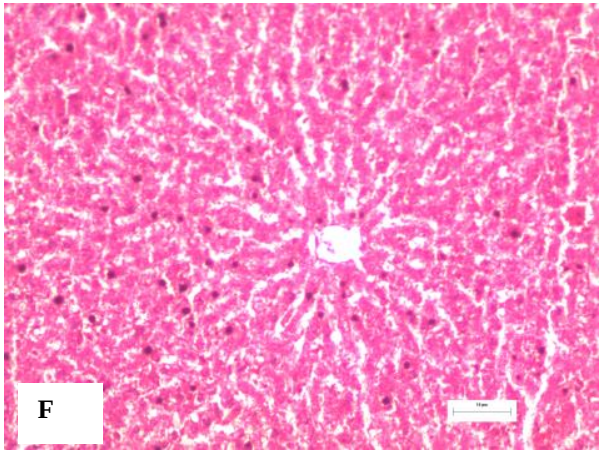
Group 3: diabetic + control



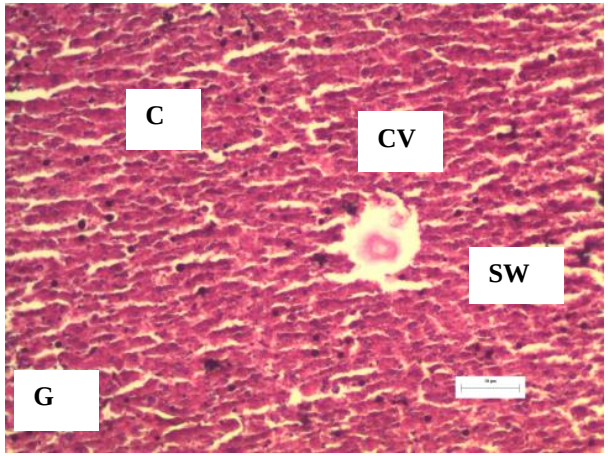
Group 4: diabetic + OTCH (1000 mg/kg)



Group 5: diabetic + OTEE (1000 mg/kg)



Group 6: diabetic + OTME (1000 mg/kg)

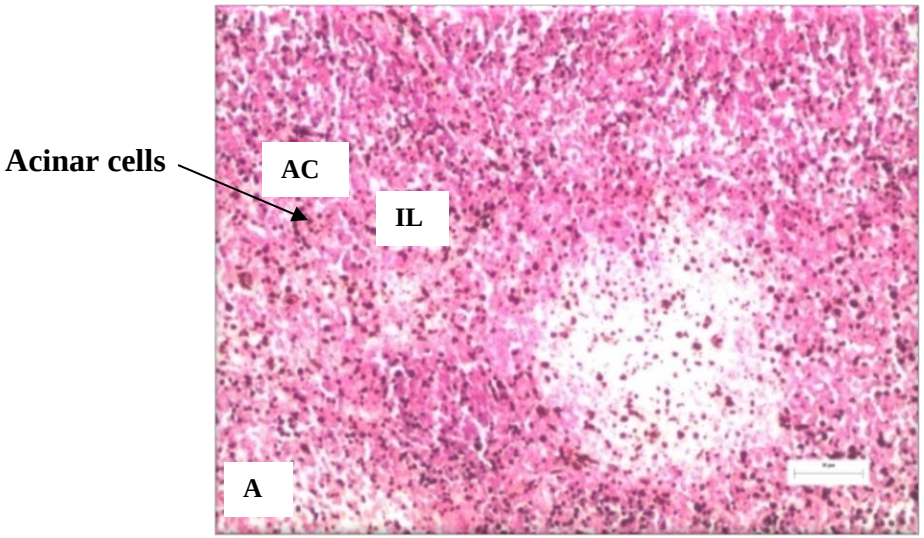


Group 7: diabetic + OTAQ (1000 mg/kg)

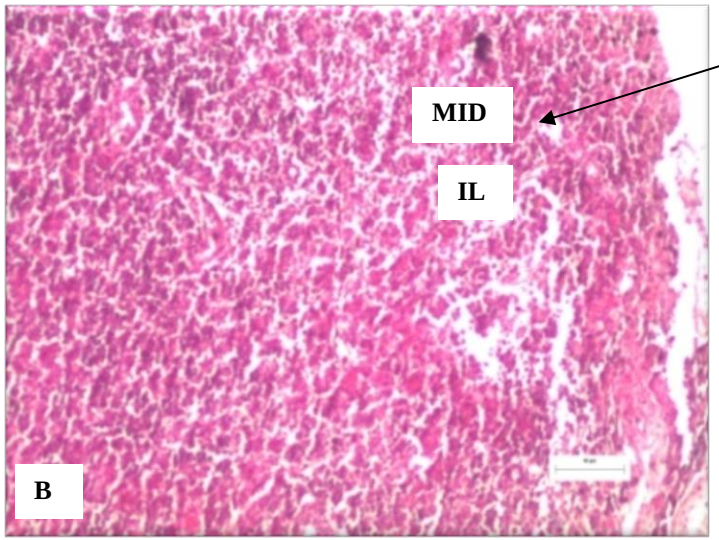
Figure 2. Microphotographs of liver histology of different groups after treatments of *Ocimum tenuiflorum* L. leaves extracts of chloroform (OTCH) , ethyl acetate (OTEE), methanol (OTME) and aqueous (OTAQ) in STZ-induced rats (H&E staining 10x). CV: centralvenule, S: sinosides, SW: sinusoids widening.

Histopathology of the Pancreas

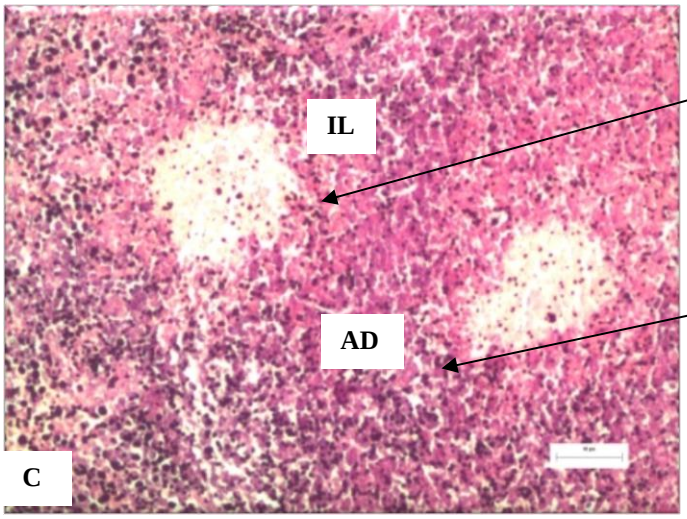
A histopathological examination of the pancreas (Figure 3) revealed a normal histological pattern among the rats in the negative control group. The diabetic control rats treated with STZ 55 mg/kg revealed atrophy in the order of 50–60% in the Islets of Langerhans accompanied by marked vascular degeneration of the islet cells and mild-to-moderate multifocal mononuclear cell infiltration in the pancreas. Regardless of which crude extract was treated, the diabetic rat pancreas islets demonstrated at least some form of treatment response. In addition, all crude extracts resulted in degenerative and atrophic changes characterized by β-cell vacuolation and a marked reduction in the size of vacuoles.



Group 1: normal control



Group 2: diabetic + metformin



Group 3: diabetic control

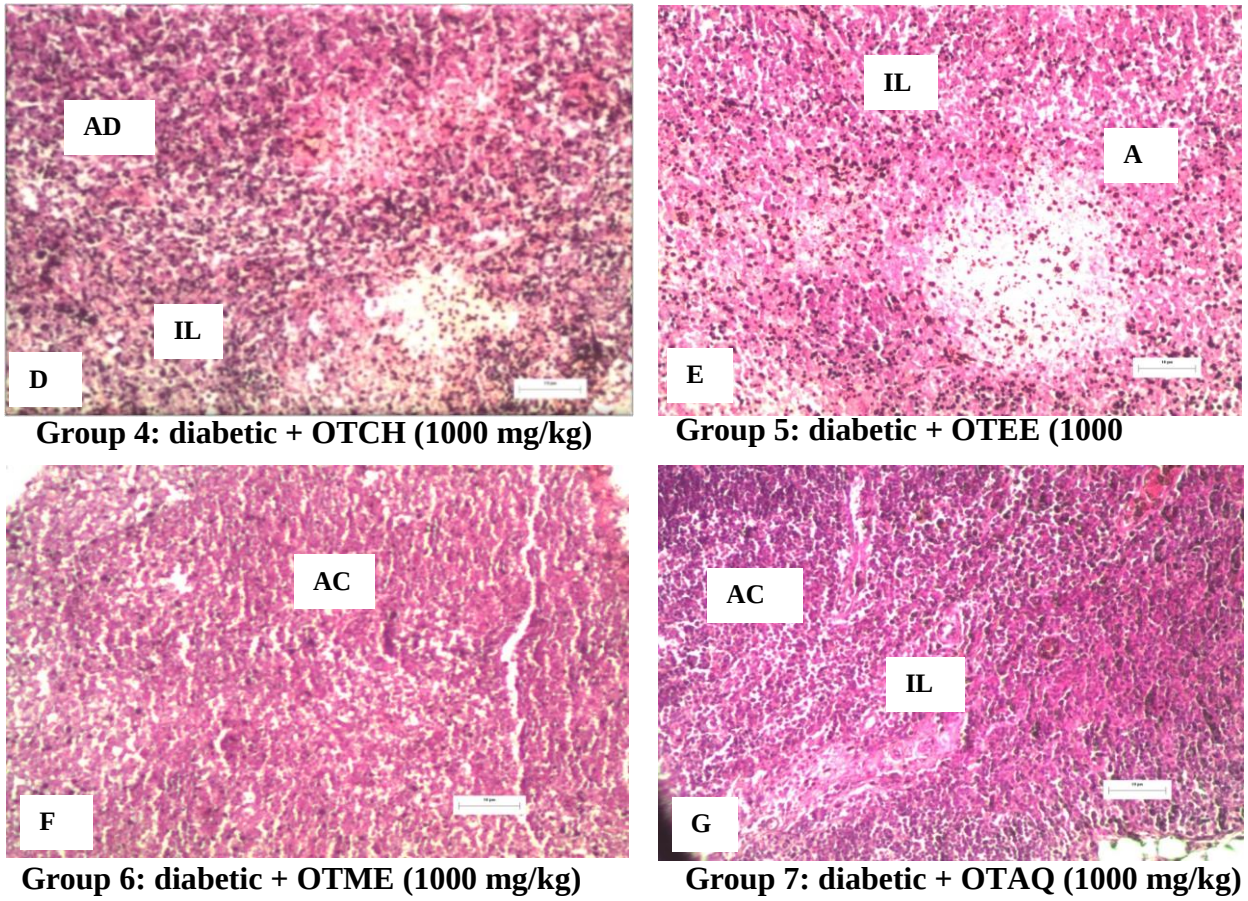


Figure3. Microphotographs of pancreas histology of different groups after treatments of *Ocimum tenuiflorum* L. leaves extracts of chloroform (OTCH) , ethyl acetate (OTEE), methanol (OTME) and aqueous (OTAQ) in STZ-induced rats (H&E staining 10x). IL: islet of langerhans, AC: acinar cells, MLD: mild langerhans disruption, AD: acinar disintegration, LD: langerhans disruption.

Biochemical parameters

Effects of different extracts of Ocimum tenuiflorum leaves on the plasma lipid profiles of STZ-induced diabetic rats

Table 3 shows the effects of chloroform, n-hexane, ethyl acetate, methanol and water extracts of OT leaves on serum triglycerides and total cholesterol in STZ-induced rats. The results show that the serum triglycerides and total cholesterol decreased but not significant ($p>0.05$) in diabetic-induced rats treated with crude extracts compared with the normal control group. According to Toma et al. (2015), a high plasma triglyceride level represents an independent and synergistic risk factor for cardiovascular disease and was associated with hypertension, abnormal lipoprotein metabolism, obesity, insulin resistance, and DM. The results of methanol-treated rats showed a significant reduction in the level of triglycerides. This effect may have been mediated by the flavonoid and vitamin C extracted by this solvent from the leaves [13]. Flavonoids and vitamin C have been shown to decrease plasma triglyceride levels [14]. In this study, rats treated with methanol and ethyl acetate extracts showed increased levels of high-density lipoprotein (HDL) compared to the negative control group.

Table 3 Effects of different extracts of *Ocimum tenuiflorum* L. leaves on the plasma lipid profiles of STZ-induced diabetic rats

Parameters (mmol/L)	Normal control	Diabetic control	Various extracts of *Ocimum tenuiflorum* (1000 mg/kg)				
			Metformin	Chloroform	Ethyl acetate	Methanol	Water
Total cholesterol	1.63±0.03	2.43±0.08	1.47±0.07	1.76±0.07	1.60±0.09	1.60±0.01	1.78±0.01
Triglycerides	1.25±0.02	3.09±0.04*	1.06±0.12	1.43±0.02	1.25±0.11	1.05±0.09	1.26±0.11
HDL	0.80±0.00	1.18±0.01	0.81±0.05	0.83±0.02	0.82±0.05	0.84±0.02	0.81±0.04
LDL	0.21±0.00	0.92±0.00	0.20±0.03	0.22±0.01	0.20±0.01	0.19±0.01	0.21±0.01

Values are expressed as Mean ± SEM. (n = 6)* $p < 0.001$ between diabetic treated groups and control (Analysed by One-way ANOVA).

Effects of oral administration of crude extracts of Ocimum tenuiflorum L. leaves on plasma hepato-specific markers and plasma electrolyte profiles of STZ-induced rats

Tables 4 indicate the effects of orally administered chloroform, ethyl acetate, methanol and water *Ocimum tenuiflorum* L. leaves extracts on plasma electrolyte profiles and the plasma hepato-specific markers of STZ-induced diabetic rats. The results showed that serum urea, uric acid, and creatinine increased compared with normal rats, but it is not significant except for the methanol group treatment. The administration of chloroform, n-hexane, ethyl acetate, methanol, water *Ocimum tenuiflorum* L. leaves extract and metformin did not indicate any significant changes at serum urea compared with the control rats. Methanol administered to STZ-induced diabetic rats indicated a decrease in the level of calcium compared to the control group but did not show significantly.

Table 4 Plasma urea, creatinine, bilirubin, Uric acid and Calcium levels and plasma electrolyte profiles in control and STZ-induced diabetic rats with different *O. tenuiflorum* L. leaves extracts

Parameters	Control	Diabetic group	Various extracts of *Ocimum tenuiflorum* (1000 mg/kg)				
			Metformin	Chloroform	Ethyl acetate	Methanol	Water
Urea (mg/dl)	9.57±0.05	10.23±0.38	9.42±0.31	9.95±0.32	9.15±0.09	8.35±0.30	8.78±0.40
Creatinine (µmol/l)	45.00±0.5	32.17±0.52	43.50±0.40	46.50±03	43.50±0.54	48.00±0.23	44.17±0.71
Bilirubin (mg/dl)	<2	<2	<2	<2	<2	<2	<2
Uric acid (µmol/l)	165.11±6.03	142.12±5.43	161.08±8.10	163.19±7.02	162.16±6.10	160.14±6.10	166.13±6.05
Calcium (mmol/l)	2.68±0.02	2.50±0.05	2.69±0.02	2.65±0.09	2.67±0.05	2.59±0.24	2.65±0.08
Phosphate (mg/dl)	2.56±0.32	3.66±0.04	2.11±0.17	2.63±0.02	2.30±0.04	2.71±0.16	2.79±0.11
Sodium	144.17±0.33	140.67±0.04	142.83±0.98	142.00±1.22	143.75±0.75	142.00±0.70	142.83±0.60
Potassium	5.17±0.00	6.93±0.05	5.10±0.13	5.00±0.05	5.20±0.14	5.45±0.15	5.83±0.32
Chloride	102.50±0.14	94.17±0.98	108.00±0.57	105.00±0.60	105.25±0.72	102.50±0.80	107.50±0.05

Values are expressed as Mean ± SEM. (n = 6, between diabetic treated groups and control (Analysed by One-way ANOVA).

Effects of oral administration of crude extracts of Ocimum tenuiflorum L. leaves on liver function profiles of STZ-induced rats

The results showed that except for the methanol extract, serum aspartate transaminase (AST) and alanine transaminase (ALT) levels increased with each extract compared with normal rats but no significant (Table 5). However, the administration of chloroform and methanol extracts resulted in a slight decrease in ALT and AST levels in diabetic rats compared to metformin-treated diabetic rats. On the other hand, the results also indicated that the ethyl acetate and water extracts led to a slight increase (no significant) in the levels of AST and ALT in diabetic rats compared to the control groups. The total proteins, albumin and globulin levels in the treated groups fluctuated somewhat but did not fall significantly below the levels of the control group.

Table 5 Effects of different extracts of *Ocimum tenuiflorum* L. leaves on the liver profile of STZ-induced treated rats.

Parameters	Negative Control	Diabetic Control	Various extracts of *Ocimum tenuiflorum* (1000 mg/kg)				
			Metformin	Chloroform	Ethyl acetate	Methanol	Water
TP (g/l)*	72.30±3.38	76.33±0.11	73.30±1.50	75.30±3.04	75.50±0.81	73.00±3.01	76.30±2.50
Albn (g/l)*	38.40±1.16	28.83±0.04	38.71±1.03	39.92±1.50	37.57±1.70	38.92±1.01	38.06±1.04
Glbn (g/l)*	23.25±2.50	54.17±3.33	24.66±1.5	24.22±1.5	26.15±3.43	23.12±3.75	27.76±1.10
ALP (U/l)*	112.26±5.11	102.17±4.14	116.38±6.01	113.47±7.16	112.62±5.61	110.47±11.02	111.80±7.20
GGT (U/l)*	<4	<3	<4	<4	<4	<4	<4
AST (U/l)*	72.50±2.11	268.67±2.81	78.83±2.71	77.5±0.54	78.5±5.01	72.15±0.61	73.43±2.17
ALT (U/l)*	25.05±0.08	77.50±1.09	24.40±0.33	28.50±1.04	27.25±1.14	25.32±0.02	28.98±0.80

Values are expressed as Mean ± SEM (n = 6, between diabetic treated groups and control (Analysed by One-way ANOVA).
*(TP): Total protein, (A): Albumin, (G):Globulin, (A/G): Albumin/Globulin, (ALP): Alkaline phosphatise,
*(GGT): Gamma Glutamyltransferase, (ALT): Alanine transaminase, (AST): Aspartate transaminase,

Haematology parameters

Effects of the chloroform, ethyl acetate, methanol and water extracts of the leaves of Ocimum tnuiflorum on the haematological indices of STZ-induced rats

Table 6 shows the effects of chloroform, ethyl acetate, methanol and water extracts of *Ocimum tnuiflorum* leaves on the haematological indices of STZ-induced rats. The results indicate that, all crude extracts administration to diabetic rats, had no significant effects on the RBC count compared to the diabetic and negative control groups. The WBC count of the crude extracts treatment groups did not indicate any significant difference compared to the normal control group, whereas the chloroform and water extracts led to a slightly increased WBC compared to the negative control groups. The administration of a methanol extract of *Ocimum tnuiflorum* leaves in the diabetic group resulted in a decrease in the monocyte count compared to the negative control groups but did not display any significant. The extracts did not produce any significant differences in the lymphocyte, neutrophil or basophile counts, or in the mean cell volume, compared to the diabetic and negative control groups.

Table 6 Effects of different extracts of *Ocimum tenuiflorum* L. leaves on the haematological results of STZ-induced treated rats.

Parameters	Negative Control	Diabetic Control	Metformin	Various extracts of *Ocimum tenuiflorum* (1000 mg/kg)			
				Chloroform	Ethyl acetate	Methanol	Water
Total WBC count ($\times 10^9$ cells/L)	9.38±1.12	11.38±0.94*	9.52±0.02	10.83±0.54	9.58±0.05	9.50±0.07	10.85±0.58
Neutrophils count (%)	22.50±1.08	29.50±2.57	21.33±0.75	24.50±0.71	23.75±0.52	23.75±0.43	25.50±2.08
Lymphocytes count (%)	63.67±3.82	61.67±1.70	64.83±4.81	66.25±3.30	65.00±2.01	64.75±1.08	67.00±4.02
Monocytes count (%)	2.83±0.31	13.83±1.50*	2.17±0.40	3.00±0.33	3.50±0.20	2.50±1.70	3.00±0.03
Eosinophils count (%)	1.19±0.22	1.50±0.22	1.17±0.22	1.1±0.54	1.25±0.50	1.00±0.33	1.33±0.20
Basophils count (%)	1.17±0.16	1.17±0.16	1.17±0.16	1.00±0.00	1.50±0.05	1.75±0.20	1.33±0.21
RBC ($\times 10^6$ mm^{-3})	8.09±0.12	8.09±0.09	8.12±0.07	8.66±0.07	8.33±0.03	8.78±0.09	9.03±0.25
HGB (gr/dL)	16.33±0.04	14.73±0.02	16.17±0.62	16.25±0.05	16.50±0.80	16.75±0.80	17.00±0.55
PVC (%)	0.48±0.02	0.48±0.01	0.50±0.00	0.52±0.31	0.42±0.00	0.52±0.00	0.45±0.00
MCV (fL)	89.67±0.14	59.67±1.87 *	87.33±0.06	88.75±0.21	88.75±0.21	85.75±0.20	89.17±0.65
MCH (ρg)	18.67±0.20	17.67±0.25	18.83±0.21	18.75±0.62	18.00± 0.62	18.25±0.20	18.33±0.23
MCHC (gr/dL)	21.67±0.85	32.16±4.25***	21.17±0.73	20.25±0.67	21.25±0.67	21.25±0.71	21.67±0.21

Values are expressed as Mean ± SEM (n = 6, between diabetic treated groups and control (Analysed by One-way ANOVA).

Discussion

Lack of scientific and clinical data for a better understanding of the efficacy and safety of the drugs and traditional herbal medicinal [17,18]. In fact, there are some inherently dangerous substances in plant extracts, such as naturally occurring toxins, which are probably cytotoxic or carcinogenic [19,20]. Therefore, the preparation of safety and side effect of traditional herbal medicine through toxicological assessments is important. As the liver is the primary organ for detoxification and distribution of drugs, the assessment could be performed on it to ensure its safety, as well as kidney, which is the major excretory organ [21,22]. Based on the current study, the assessment was performed on the function parameters of the liver, kidney, and pancreas of the animals which were treated with a sub-acute dose of *Ocimum tenuiflroum* crude extracts. There were a limited number of studies and data regarding the toxicity of the alcoholic *Ocimum tenuiflroum* extracts.

Besides, most of these studies focused on ethanolic as the solvent for extract [23]. This was the first study conducted on the toxicity of hexane, chloroform, ethyl acetae, methanol, and water extract. In accordance to World Health Organization (WHO) guideline and Organization for Economic Cooperation and Development (OECD) revised the up-and-down procedure for acute toxicity test [24,25], the results of the acute toxicity were obtained. Although the newest method applied for the measurement of acute toxicity was OECD 425, there were differences between this method and the previous ones, which were OECD 420 and 423. A smaller number of animals was required for estimation of the acute oral toxicity of chemicals. Both of Revision of Test Guideline 425 and revisions to the Test Guideline 420 AND 423 were conducted. This was the first application of OECD 425/2001 with the purpose of investigating the acute toxicity effects of *Ocimum tenuiflroum*. Only five animals with a dose limit of 2000 mg/kg or 5000 mg/kg were required for this study.

On the other hand, investigation on the toxicity of *Ocimum tenuiflroum* was in accordance with the test guideline for OECD 420 and 423 [26]. Based on the literature survey and analyses conducted on female rats, it was shown that usually similar response was given by both sexes in acute oral toxicity tests. However, different responses were found due to the higher sensitivity of the females compared to males, unless if it was suggested that males had higher sensitivity towards given substances [24,27]. Therefore, female rats were chosen. The increase of the body weights of the treated and controlled rats was performed after the oral administration of a single dose of different crude extracts of *Ocimum tenuiflroum* (1000 mg/kg). As a result, it was found that there was a statistically minor change in their body weights, as shown in table 3. Based on the body weight gain in test animals; it was shown that there was no effect to the administration of the extracts on the growth of the animals. Furthermore, there was no death, cramps, nervousness, or other strange behavior displayed by the animals within the fourteen days of the investigation. This was probably since, the compounds which can cause damage to the nervous system or brain, were absent from the components of this extract at this dose level.

The breathing and vision of treated rats did not put through any changes which could influence their movement and behavior. In general, the purpose of the acute toxicity was to specify the therapeutic index ratio between the lethal dose and the pharmacologically effective dose within the same strain and species. Based on our study conducted on the acute toxicity among rats, besides them being alive, there was no unusual behavior or sign of illness displayed by them within the fourteen days of exposure to 1000 mg/kg of extracts dose. In terms of giving any cumulative effect on the toxic part of vital organs such as the

liver, kidney, and bone marrow, this was not effective. Based on the conclusion of this study, non-polar to polar crude extracts of *Ocimum tenuiflroum* was quite safe even when used at a higher dose.

Moreover, these extracts did not have acute toxicity and the female rats had a higher oral lethal dose (LD50) compared to 2000 mg/kg body weight. In addition, the acute toxicity of 1000 mg/kg of *Ocimum tenuiflroum* was conducted in diabetic rats to investigate the antihyperglycaemic effects, biochemical markers, histology of vital body organs and bioactive compounds in the active crude extract of *Ocimum tenuiflorum* leave extracts. Preliminary studies were conducted in normal rats by [28]. The antihyperglycaemic test showed the metformin reduced blood glucose level (BGLs) from the first day up to fourteen days after daily oral treatment. Metformin, a biguanide, acts as an insulin sensitizer *i.e.*, its blood-glucose lowering action does not depend on the presence of functioning pancreatic β-cells. Therefore, biguanides are appropriately called "euglycemic" agents [29,30]. Several studies have been found that flavonoids originate from foods and the herbal plant could lead to a recovery in glucose metabolism, the lipid profile, regulating hormones and enzymes in the human body, which could protect human beings from diseases such as obesity, diabetes, and their complications.

The current study of crude extracts given to rats resulted in fluctuating levels of glucose at different times after oral treatments given to the rats, but the results did not indicate any significant changes with the exception of the methanol extract, which had a significant effect on the level of blood glucose compared to the control group during the test conducted after the fourteenth day of treatment. In the current study, the body weight and normal state of vital organs in terms of weight/histology were accepted as non-toxic effects [31,32]. Usually, aside from one or two organs being involved in the major toxic effect, they represent the targeted organs of toxicity of the particular substance. From this, it was indicated that rats would be inflicted with damages on the targeted organs in a certain degree if they were exposed to toxic substances. In comparison to the control group, there was no significant change found in the behaviour of the diabetes groups and the body-weight in treatment groups. Therefore, it was suggested that there was no effect posed by the oral dose administration of *Ocimum tenuiflorum* different extracts on the growth of the rats.

In the evaluation of toxicity effects in medicinal plants experiments, the biochemical analysis was highly important as an index due to its high sensitivity and ability in reflecting the bio metabolic processes, such as anabolism or catabolism of the substances on certain internal vitals, especially liver and kidney. The first fire line was where metabolization and conversion to end products through enzymatic reactions conducted by substances. This was represented by these tow vital organs. This study was the first report where details on the effects of the acute toxicity of *Ocimum tenuiflorum* extracts on rats with 1000 mg/kg of dose during fourteen days were put in focus [33,34]. The results for plasma urea, creatinine and total bilirubin and plasma electrolytes were consistent with the findings of Campos et al. (2003) in rats. The increase in the activities of plasma AST, ALT, LDH indicated that diabetes may induce hepatic dysfunction. Supporting our findings, [12,36] showed that liver had steatosis around the central venule with a widening of the sinusoids and the infiltration of inflammatory cells. The kidney histopathology data of STZ-induced diabetic rats indicated mild tubular damage as well as haemorrhage in the Bowman's space due to glomerular damage. A similar result was reported by [37]. Despite, the fourteen-consecutive-day treatment of STZ-induced diabetic groups with different crude extracts was effective for restoring the activities of these enzymes to normal levels. A possible explanation for the

differential effects of these crude extracts on the activities of AST, ALP, and LDH in plasma and the liver is that these treatments might potentially inhibit STZ-induced liver damage [38,39]. The amelioration of liver damage via the oral administration of various crude extracts could be confirmed by studying their effects on bilirubin levels. This result showed the level of plasma bilirubin in response to each treatment. Regarding to the previous study, Narwal et al., (2011) reported that the increased plasma bilirubin (i.e., hyperbilirubinemia) may be the result of decreased liver uptake, conjugation or increased bilirubin production due to haemolysis, and these changes were reflected in a reduced RBC count. The excellent recovery of methanol *Ocimum tenuiflorum* extract can be explained by the regenerative capability of the renal tubules. Similar results were observed in *Trigonella foenumgraecum* seed powder administration in the treatment of STZ-induced diabetic rats [41]. The toxicology effects of the streptozotocin action in the β cells of diabetic rats was characterized by a histology study of β cells and changes in blood glucose levels after fourteen days of treatment. Histopathological investigation of diabetic rats indicated mild destruction of the islets of Langerhans. STZ is known to destroy the β-cells of the pancreas, which causes selective pancreatic islet β-cell cytotoxicity mediated through the release of nitric oxide (NO), methyl actions, methyl radicals, and reactive oxygen species (ROS).

Overall, with the exception of the chloroform extract, each of the crude OT leaf extract treatments was well-tolerated by the animals, and there were few toxic effects observed directly. These results were supported by the histological findings of Khan et al. (2009) and Sunil et al. (2012). The finding of biochemical parameters was further supported by [44], due to the vitamin C content of the leaves that led to increases in plasma HDL cholesterol [38,45]. Increased plasma HDL has been linked to reduced cardiovascular risks in a diabetic group [45,47]. These results of plasma hepato-specific markers indicated that the different extracts had different effects on the integrity and function of the liver and kidneys of STZ-induced diabetic rats. The different extracts improved the low plasma calcium levels in the diabetic rats. The methanol extract may have achieved this effect by affecting the secretion of parathyroid hormones, thereby promoting the tubular renal re-absorption and intestinal absorption of calcium by stimulating the renal production of 1,25-dihydroxyvitamin D or calcitriol as well as the re-absorption of bone [44,47]. Calcium fluxes are also important mediators of hormonal effects on target organs through several intracellular signaling pathways, such as the phosphoinositide and cyclic adenosine monophosphate systems [48]. Previous studies have suggested that WBC plays an important role in the disruption of coronary artery plaques, thus delaying the inception of the acute coronary syndrome [44,49]. Even though, a high peripheral WBC count is a known risk factor for coronary artery disease [44,50]. Consequently, there are two possible implications for the high blood cell counts seen in the experimental rats:(a) preservation versus the onset of the acute coronary syndrome, (b) an elevated risk of coronary artery disease. The heightened WBC count may have been produced by the immune stimulatory activity of anti-nutritional compounds, such as saponins and tannic acid [51]. On the other hand, an increase in the platelet count also has two implications. It can indicate increased clotting, which provides protection against bleeding. It can also be an indicator of increased insulin resistance and a predisposition towards adverse cardiovascular events.

Conclusions

In conclusions, *Ocimum tenuiflorum* leaf methanol extracts were more effective in the biochemical and haematology index in diabetic rats compared to another crude extract. A

toxicity study of crude extracts in vital organs showed there were low side effects for methanol extract in the histology studies.

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All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

Compliance with ethical standards
The work is compliant with ethical standards.

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