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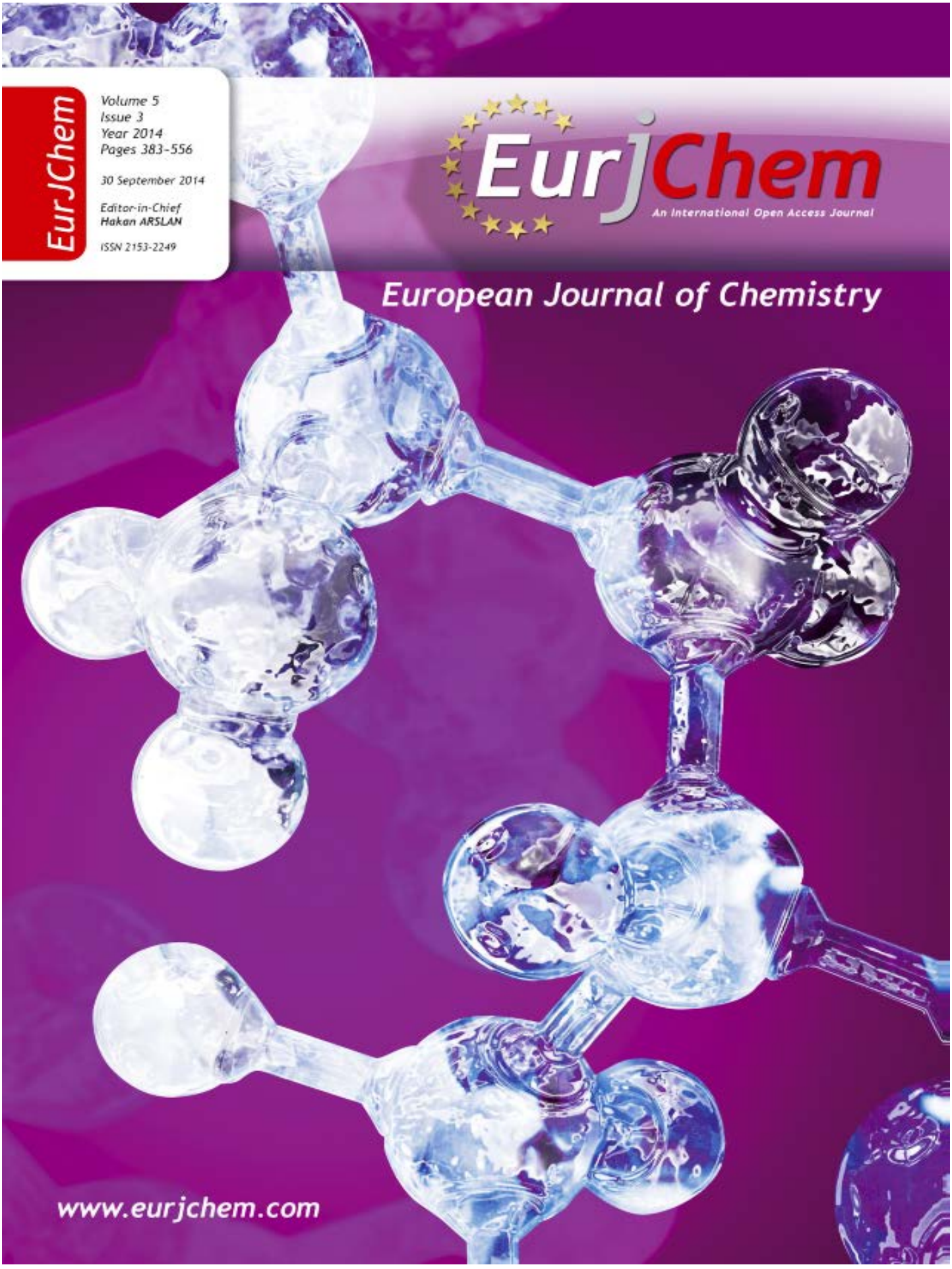

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Phytochemical constituents, hypoglycemic and haematological effects of methanolic *Acalypha wilkesiana* leaves extract on streptozotocin-induced diabetic rats

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ABSTRACT

Recently, there has been more interest with antidiabetic agents commencing natural products from plants as a better treatment than currently used synthetic drugs. The high performance liquid chromatography analysis of methanolic *Acalypha wilkesiana* leaves extract revealed occurrence of twenty one polyphenolic compounds. Catechin is the main constituent (29.91%), followed by apigenin (20.96%), then, ellagic acid, quercetin, kampferol, ferulic acid, luteolin, pyrogallol, rutin, caffeic acid, chlorogenic acid, protocatechouic acid, *p*-coumaric acid, caffiene, syrigic acid, catechol and *p*-OH-benzoic acid in percentages of 17.54, 10.45, 7.63, 4.67, 4.61, 4.30, 3.21, 3.15, 2.10, 1.48, 1.43, 1.13, 1.09, 1.08 and 1.05%, respectively. The antidiabetic and heamatological effects of methanolic *Acalypha wilkesiana* leaves extract (10, 20 and 40 mg/100 g body weight) in streptozotocin diabetic rats were investigated comparing with metformin HCl (50 mg/100 g body weight) for 30 days. Our results indicated that the most effective dose of methanolic *Acalypha wilkesiana* leaves extract was 40 mg/100 g body weight, which decreased blood glucose level to about 77.37% in diabetic rats, compared with a percentage of 76.50% achieved by administration with metformin HCl. Furthermore, liver functions activity, kidney functions, lipid profile, atherogenic indices and haematological parameters were scrutinized in diabetic rats treated with methanolic *Acalypha wilkesiana* leaves extract. These results indicate that the methanolic leaves extract of *Acalypha wilkesiana*, have favorable effects in bringing down the severity of diabetes and justify its use in traditional medicine for the treatment of diabetes and its complications.

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia and disturbances in fat and protein metabolism due to deficiency of insulin secretion and/or action [1]. In addition to hyperglycemia, diabetes mellitus leads to long term multi-organ complications and causes damage to eye, heart, kidneys, nerves system and blood vessels [2]. Streptozotocin (STZ), an antibiotic produced by *Streptomyces achromogenes*, is the most widely used agent in experimental diabetes [3]. The main cause of streptozotocin induced β-cell death in the alkylation of DNA by nitrosourea moiety of this compound. However, reduction of nitric oxide and reactive oxygen species may also be involved in DNA fragmentation and other deleterious effect of streptozotocin such as increasing in serum level of lipid peroxide due to oxidation of cells [4].

Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increase demand by patients to use the natural products with anti-diabetic activity [5]. Herbal products can improve glucose metabolism and the overall condition of individuals with diabetes, not only by hypoglycemic effects, but also by improving lipid metabolism, antioxidant status and capillary function [6]. *Acalypha wilkesiana* is one of a number of medicinal herbs that have potential for use in the management of diabetes mellitus. It is a member of the spurge family *Euphorbiaceae* and belongs to the genus *Acalypha*, comprising about 570 species. The plant has wide uses in the traditional medicines for the treatment of bacterial and fungal skin infections, neonatal jaundice, and gastrointestinal disorders [7]. *Acalypha wilkesiana* has been reported to possess antimicrobial [8], antihypertension [9] and anticarcinogenic properties [10]. It has also been reported by Udobang *et al*., [11] that the crude leaf extracts and fractions of this plant exhibit antiparasitic and analgesic properties. Though this plant is not edible, it is found to contain alkaloids, tannins and resins [12]. The chemical constituents of *Acalypha wilkesiana* leaves included acalyphamide, aurantiamide, succinimide calypho-lactate, 2-methyl anthraquinone, tri-*o*-methyl ellagic acid, β-sitosterol and β-D-glucoside [13]. Four kaempferol glycosides namely: mauritianin, clitorin, nicotiflorin and biorobin have also been isolated from the flowers and leaves of *Acalypha wilkesiana* [14]. Not much pharmacological research has been carried out on this plant despite its importance in traditional medicine. Therefore, the aim of this study is to examine the efficiency of

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methanolic leaves extract of *Acalypha wilkesiana* as hypoglycemic agent and some blood constituents within STZ-induced diabetic rats.

2. Experimental

2.1. Materials

The present investigation was carried out using *Acalypha wilkesiana* leaves belong to family *Euphorbiaceae*. The samples collected from the experimental farm of the Faculty of Agriculture, Mansoura University, Mansoura, Egypt (April, 2013). Streptozotocin and methanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA), while metformin hydrochloride was obtained from Chemical Industries Development (CID) for pharmaceutical industries, Giza, Egypt. All other chemicals were of the highest available commercial grade.

2.2. Methods

2.2.1. Preparation of methanolic Acalypha wilkesiana leaves extract

Acalypha wilkesiana leaves samples were air dried in the shade and ground into a fine powder, containing 11.65% moisture. Powdered air dried leaves (1 Kg) were extracted by soaking at room temperature for six times with methanol (20 L). The methanolic extract was filtrated using Whatman filter paper (No. 1) and then concentrated to nearly dryness under reduced pressure by using the rotary evaporator at 45 °C to achieve the crude methanolic extract which kept at 4 °C for further investigation and the percentage yield of the extract was 4.54% (*w*:*v*).

2.2.2. Analysis of polyphenols by HPLC technique

Phenolic compounds of *A. wilkesiana* leaves extract were identified and quantified at the Central laboratory, National Research Center, Giza, Egypt, according to the method described by Goupy *et al.*, [15] using reversed phase high performance liquid chromatography (RP-HPLC) with direct injection. Detection and quantification were performed with the help of the isocratic Varian system, equipped with Alltima column 5mm (C18, Hypersil MOS, 5 µm, 200 × 2.1 mm, Hewlett Packard) 4.6 × 250 mm and a detector namely Photo Diode Array (PDA) type. A gradient elution was employed using two solvent systems. Solvent A consisted of: acetic:water (2:98, *v*:*v*), solvent B: acetonitrile:water (70:30, *v*:*v*) with 1% of formic acid (*v*:*v*). The solvent flow rate was 1 mL/min, volume of the injected sample was 10 µL and separation was performed at 35 °C. The amounts of phenolic compounds in the extract were assayed by external calibration curves, comparing their retention times with known standards (caffeic acid, *p*-coumaric acid, catechin and quercetin). For example, 100 mg standard quercetin was taken into 100 mL volumetric flask, dissolved in the mobile phase and make up to the mark. The flask was shaken for 10 min and the volume was made up to the mark to obtain a standard stock solution of quercetin (1000 µg/mL). A stock solution was filtered through a 0.1 µm membrane filter, and then the 10 ppm working standard solution of quercetin was prepared. Suitable aliquots of stock solution were pipette out and volumes were made up to the mark with the mobile phase. To prepare a stock solution of sample, 1 g of accurately weighed methanolic *Acalypha wilkesiana* leaves extract were taken in a 100 mL volumetric flask, dissolved in the mobile phase and made up to the mark. After setting the instrument 10 µL of standard solution was injected and chromatogram recorded. From this, the area of chromatogram and the percentage of quercetin content were calculated at 280 nm and expressed as mg/100 g dry matter.

2.2.3. Determination of antidiabetic activity of methanolic A. wilkesiana leaves extracts

2.2.3.1. Experimental animals

Male Sprague-Dawley albino rats, weighing 180-200 g were obtained from the animal house of Faculty of Pharmacy, Mansoura University, Egypt. The rats were kept for adaptation under normal laboratory conditions for 7 days before the beginning of the experiment. All rats were fed on a normal diet and allowed free access to water. After acclimatization, the rats were housed in metabolic cages and divided randomly into six groups having six animals in each group. Animal experimentation described in the study was strictly conducted in accordance with the guidelines prescribed by the University Ethical Committee.

Group 1, represents normal rats by means non diabetic, which were fed with normal diet and received saline solution for 30 days. The remaining rats were fasted for 24 hours, then intraperitoneally injected with streptozotocin freshly prepared in 0.10 M citrate buffer, pH = 4.5 at a dose of 4.5 mg/100 g body weight (b. wt.) to induce diabetes mellitus, according to Ghasemi *et al.*, [16]. In order to stave off the hypoglycaemia during the first day after the streptozotocin injection, diabetic rats were given 5% glucose solution orally as reported by Orhan *et al.*, [17]. Blood glucose level of rats was determined 72 hours post-injection; rats were fasted for 18 hours before determination. Rats with blood glucose levels over 300 mg/dL were considered sufficiently as streptozotocin-diabetic rats and ready for treating with the extract. Then, diabetic rats were randomly divided into 5 groups (6 rats for each). Group 2, represents diabetic control rats, received a normal diet for 30 days without any treatment. Group 3, represents diabetic rats treated with metformin hydrochloride powder as a reference drug in dose 50 mg/100g b. wt. Groups 4, 5 and 6 were diabetic rats administered with crude methanolic *Acalypha wilkesiana* leaves extract in doses of 10, 20 and 40 mg/100g b. wt., respectively. Reference drug and crude methanolic extract were dissolved in saline solution (Sodium chloride, 0.9%) and given orally by a stomach tube after fasting for 2 hours, daily for 30 days.

Blood samples were collected from the eye canthus by heparinized tubes every 10 days after the beginning of the experiment. Then, each blood sample was divided into two portions. Centrifugation was carried out to a first portion to obtain clear serum and the fasting serum glucose levels were determined immediately. Serum blood samples were kept at refrigerator under freezing conditions for the determination of the other parameters included liver functions (ALT and AST), kidney functions (creatinine and urea) and lipid profile (Triglycerides, total cholesterol, HDL-C, LDL-C and VLDL-C). The second portion was treated with 10% of ethylene diamine tetracetic acid (EDTA) with a good shaking to determine the complete blood count (CBC) as a haematological analysis.

2.2.3.2. Chemical analysis of blood

Serum glucose was determined by a colorimetric enzymatic method glucose oxidase (GOD) described in commercial kits by Spinreact (Spain) according to Trinder [18]. Liver functions (ALT and AST) were determined as described in commercial kits by Randox (United Kingdom) according to the method of Reitman and Frankel [19]. Kidney functions (Serum creatinine and urea) were determined by a colorimetric method according to Larsen [20]; Fawcett and Scott [21], respectively, as described in commercial kits by Human (Germany). The lipid profile, triglycerides (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) were determined by enzymatic colorimetric method of [22-24] described in a commercial kits by Human (Germany).

Table 1. HPLC analysis of polyphenols of methanolic *Acalypha wilkesiana* leaves extract.

Compound	Concentration (mg/100 g)	Compound	Concentration (mg/100 g)
Apigenin	624.15	Caffeic acid	94.03
Caffeine	33.78	Catechin	890.66
Catechol	32.21	Chlorogenic acid	62.61
Chrysin	-	Cinnamic acid	-
Coumarin	29.16	Ellagic acid	522.36
Ferulic acid	139.27	Gallic acid	11.70
Hisperidin	-	Kampferol	227.16
Luteolin	137.52	Naringenin	10.55
p-Coumaric acid	42.76	*p*-OH-Benzoic acid	31.24
Protocatechouic	44.18	Pyrogallol	128.16
Quercetin	311.25	Rosmarinic acid	9.15
Rutin	95.74	Salicylic acid	-
Syringic acid	32.62	Vanillic acid	-

Serum low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) concentrations were calculated using the Friedewald [25] equations as follows:

$$VLDL–C = TG/5 \quad (1)$$

$$LDL–C = TC – (HDL–C + VLDL–C) \quad (2)$$

The atherogenic indices were calculated as reported by Ikewuchi and Ikewuchi [26] using the following equations:

$$\text{Cardiac risk ratio} = \frac{TC}{HDLc} \quad (3)$$

$$\text{Atherogenic coefficient} = \frac{\left[TC-\left(HDLc\right)\right]}{\left[HDLc\right]} \quad (4)$$

$$\text{Atherogenic index of plasma} = \log \frac{TG}{HDLc} \quad (5)$$

2.2.3.3. Haematological analysis

All haematological tests were made using apparatus namely ABX Micros 60 which a fully automated hematology analyzer from Sysmex Corporation international company [27]. Micros 60 is an ideal choice for the determination of up to 18 fully automated parameters including hemoglobin (Hb), red blood cells count (RBC), hematocrit (Hct), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets count (Plt), platelets hematocrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW), white blood cells count (WBC) and WBC differential: Lymphocytes (LYM), Monocytes (MON) and Granulocytes (GRA). It can perform 60 samples per hour in open or closed tube forms for sample volume of 10 µL of whole blood per cycle.

2.2.4. Statistical analysis

Statistical analyses for obtained data were done using the SPSS statistical software package. All comparisons were first subjected to one way analysis of variance (ANOVA) and significant differences between treatment means were determined using Duncan's multiple rang test at $p < 0.05$ as the level of the significance.

3. Results and discussion

3.1. Analysis of polyphenols by HPLC

High performance liquid chromatography procedure was used for qualitative and quantitative analysis of polyphenolic compounds in methanolic *Acalypha wilkesiana* leaves extract. Twenty six polyphenolic compounds were available as authentic samples namely: apigenin, caffeine, catechol, chrysin, coumarin, ferulic acid, hisperidin, luteolin, *p*-coumaric acid, protocatechouic, quercetin, rutin, syringic acid, caffeic acid, catechin, chlorogenic acid, cinnamic acid, ellagic acid, gallic acid, kampferol, naringenin, *p*-OH-benzoic acid, pyrogallol, rosmarinic acid, salicylic acid and vanillic acid were used to identify the corresponding components in *Acalypha wilkesiana* leaves extract polyphenols.

Data in Table 1 reveal that twenty one compounds with different retention times were recognized in HPLC chromatogram. From the same table, it could be noticed that catechin, apigenin, ellagic acid, quercetin and kampferol were the predominant indentified component in *Acalypha wilkesiana* leaves extract as percentages of 29.91, 20.96, 17.54, 10.45 and 7.63%, respectively. Followed by ferulic acid (4.67%), luteolin (4.61%), pyrogallol (4.30%), rutin (3.21%), caffeic acid (3.15%), chlorogenic acid (2.10%), protocatechouic acid (1.48%), *p*-coumaric acid (1.43%), caffiene (1.13%), syrigic acid (1.09%), catechol (1.08%) and *p*-OH-benzoic acid (1.05%), in addition to traces of coumarin, gallic acid, naringenin and rosemarinic acid. While, five polyphenolic compounds, namely: chrysin, hisperidin, cinnamic acid, salicylic acid and vanillic acid were absence in the methanolic leaves extract of *Acalypha wilkesiana*.

Ours results were agreed with those obtained by Ikewuchi *et al.,* [28] who detected twenty nine known flavonoids in aqueous *Acalypha wilkesiana* leaves extract, consisting mainly of apigenin, quercetin, naringenin, kaempferol, epicatechin, catechin, ellagic acid, butein, myricetin, biochanin, baicalein, gallocatechin, robinetin, silymarin, and epigallocatechin 29.77, 14.97, 11.12, 10.62, 9.05, 4.37, 2.36, 2.34, 2.25, 2.08, 1.76, 1.72, 1.14, 1.13 and 1.11%, respectively. However, the preliminary phytochemical screening of the methanolic *A. wilkesiana* leaves extract revealed the presence of alkaloids, flavonoids (catechins and flavones), saponins and tannins (Table 1), all of which have potential health promoting effects, at least under some circumstances [29].

3.2. Effect of crude methanolic of Acalypha wilkesiana leaves extracts on blood glucose level

The obtained data in Table 2 indicate that the injection of streptozotocin at the dose 4.5 mg/100 g body weight caused a highly significant ($p < 0.05$) increase in blood glucose level from 102 to 387 mg/dL for non-diabetic and diabetic rats at zero time, respectively. In addition, gradual increasing was observed during the experimental periods (10, 20 and 30 days) until reaching the maximum level of 464 mg/dL for diabetic rats at the end of the experiment. This increase may be due to the destructive effect of streptozotocin on β-cells of islets of Langerhans which lead to insulin deficiency.

Table 2. Effect of crude methanolic leaves extract of *Acalypha wilkesiana* on levels of blood glucose (mg/dL) in diabetic rats.

Treatment period	Normal control	Diabetic Control	Diabetic + metformin	Diabetic + *A. wilkesiana* extract at a dose		
				10 mg/100 g b. wt.	20 mg/100 g b. wt.	40 mg/100 g b. wt.
Zero time	102±3.75	387±14.08 [a]	300±12.96 [a,b]	390±6.42 [a]	388±15.22 [a]	378±11.03 [a]
10 days	104±3.87	400±11.42 [a]	230±6.92 [a,b]	355±13.46 [a,b,c]	300±9.42 [a,b,c]	281±13.76 [a,b,c]
20 days	99±2.65	428±16.42 [a]	151±4.42 [a,b]	298±15.99 [a,b,c]	229±6.42 [a,b,c]	169±6.42 [a,b,c]
30 days	103±2.05	464±20.20 [a]	109±3.15 [b]	183±4.13 [a,b,c]	120±7.01 [a,b,c]	105±4.66 [b]

[a] The values are mean±SD of 6 rats in each group, significantly different from normal control at $p < 0.05$.
[b] The values are mean±SD of 6 rats in each group, significantly different from diabetic control at $p < 0.05$.
[c] The values are mean±SD of 6 rats in each group, significantly different from diabetic + metformin at $p < 0.05$.

Table 3. Effect of crude methanolic *Acalypha wilkesiana* leaves extract on liver functions (IU/L) activities in diabetic rats *.

Liver functions (IU/L)	Treatment period	Normal control	Diabetic control	Diabetic + metformin	Diabetic + *A. wilkesiana* extract at a dose		
					10 mg/100 g b. wt	20 mg/100 g b. wt	40 mg/100 g b. wt
ALT	Zero time	26±2.96	49±4.44 [a]	51±3.26 [a,b]	47±4.71 [a,b,c]	49±3.22 [a,b,c]	48±3.84 [a,b,c]
	30 days	27±1.16	71±5.09 [a]	34±4.11 [a,b]	33±4.25 [a,b]	27±2.57 [b,c]	26±3.02 [b,c]
AST	Zero time	34±2.11	54±6.06 [a]	61±5.72 [a,b]	64±5.12 [a,b]	57±4.65 [a,b]	53±5.04 [a]
	30 days	40±4.13	84±8.14 [a]	47±3.55 [a,b]	53±4.41 [a,b,c]	41±4.89 [b,c]	31±1.37 [a,b,c]

* The values are mean±SD of 6 rats in each group.
[a] Significantly different from normal control at $p < 0.05$.
[b] Significantly different from diabetic control at $p < 0.05$.
[c] Significantly different from diabetic+metformin at $p < 0.05$.

Moreover, the absence of available insulin in blood circulation, these may be the main causes of hyperglycemia, which observed in the treated rats with streptozotocin as achieved by Vessal *et al*., [4]. Also, Lenzen [30] reported that the significant increase in the levels of blood glucose in STZ-induced diabetic rats could be due to a beta cytotoxic induces chemical diabetes through damaging insulin-secreting cells.

It could be reported that the most effective dose of reducing blood glucose level was demonstrated at 40 mg/100 g b. wt. which decreased this parameter to 105 mg/dL after 30 days, where the maximum reduction of about 77.50% was obtained compared with diabetic control rats. While, treated rats with metformin HCl achieved a 76.50%, reducing blood glucose level at the dose 50 mg/100 g b. wt. after 30 days. The initial antihyperglycemic activity was observed after ten days, then the blood glucose level gradually decreased for all groups during the experimental periods. The methanolic *A. wilkesiana* leaves extract at doses 10, 20 and 40 mg/100g b. wt. reduced blood glucose levels by 11, 25 and 30%, respectively, comparing with that observed in diabetic control rats after 10 days (Table 2). Our data were in accordance with those obtained by Ikewuchi *et al*., [28] who used 10, 20 and 30 mg/100g b. wt. of aqueous *A. wilkesiana* leaves extract, which decreased blood glucose levels by 8.20, 20.86 and 21.34%, respectively, compared with untreated diabetic rats after 10 days. Additionally, our results run parallel with those obtained by Al-Attar [31] who illustrated 11.6% as a reduced percentage of blood glucose level by using aqueous *A. wilkesiana* leaves extract in diabetic mice after 30 days. Furthermore, Odoh *et al*., [32] showed that the most significant reduction of fasting blood glucose level (48.36%) was observed for 200 mg/kg b. wt. of methanolic *A. wilkesiana* root extract in alloxan-induced diabetic rats. Moreover, these previous comparisons demonstrated that hypoglycemic activity of the methanolic *A. wilkesiana* leaves extract was more effective than the aqueous ones at the same experimental period. Also, the higher percentage reduction in blood glucose levels, produced by the extract in this study, supports the use of *A. wilkesiana* leaves in the management of diabetes mellitus.

Several studies reported that the *A. wilkesiana* leaves contain polyphenols as bioactive compounds such as querecetin which produces an increase in the number of pancreatic islet cells, probably increase insulin release in STZ-diabetic rats and induces the hepatic glucokinase enzyme. Thus, the lowering property of plasma glucose could also be attributed to the ability of querecetin to regenerate pancreatic β-cells and to increase insulin release [4]. Finally, it could be concluded that lowering blood glucose levels, which was observed in the diabetic animals may be due to the stimulation of β-cells of pancreatic islets and mediated through stimulation of insulin release resembling the oral hypoglycemic drugs or peripheral glucose utilization [33].

3.3. Effect of crude methanolic extracts of Acalypha wilkesiana leaves on liver functions

Alanine amino transferase (ALT) and aspartate amino transferase (AST) activities are known as cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after damaging of the cell membrane; therefore both enzymes are used as indicators for hepatic damage [34]. Liver functions during this investigation were examined through the determination of ALT and AST activities in serum of non-diabetic and STZ-diabetic rats. It is clear that ALT and AST increased significantly from 26 and 34 IU/L in non-diabetic rats to 49 and 63 IU/L, respectively in diabetic control rats (Table 3). Such a significant increase of ALT and AST activities as shown suggest the possible necrotic injury of the liver or cholestasis with hepatocellular necrosis [35].

Data in Table 3 clearly indicate no significance differences between normal and treated rats with the methanolic leaves extract of *A. wilkesiana* at both doses 20 and 40 mg/100 g b. wt. for ALT activity values at the end of the experimental period. In addition, AST activity value differences were not significant between normal control and treated rats with *A. wilkesiana* leaves extract at dose 20 mg/100g b. wt. after 30 days. From the same table, it could be observed that treatment with methanolic *A. wilkesiana* leaves extract in all doses was more effective for gradually reducing of ALT and AST levels than treated diabetic rats with metformin HCl.

Our results for ALT levels were more effectual than those obtained by Ikewuchi *et al*., [28] who found no significant differences between untreated diabetic and diabetic rats treated with aqueous *A. wilkesiana* leaves extract. Furthermore, methanolic *A. wilkesiana* leaves extract reduced AST values to about 63% compared with Ikewuchi *et al*., [28] who offered a lower reduction value of 33% using an aqueous extract at the end of the experimental period. The restoration of AST and ALT activities to their respective normal levels after supplementation of methanolic leaves extract of *A. wilkesiana* further strengthens the antidiabetogenic effect of this extract. Moreover, it has been concluded that the decrease in the serum transaminase enzymes in STZ-induced diabetic rats by *A. wilkesiana* leaves extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity.

Table 4. Effect of crude methanolic *Acalypha wilkesiana* leaves extract on kidney functions (mg/dL) levels in diabetic rats *.

Kidney functions (mg/dL)	Treatment period	Normal control	Diabetic control	Diabetic + metformin	Diabetic + *A. wilkesiana* extract at a dose		
					10 mg/100 g b. wt	20 mg/100 g b. wt	40 mg/100 g b. wt
Creatinine	Zero time	1.3±0.05	1.6±0.03	1.9±0.02	1.5±0.07	1.4±0.05	1.6±0.02
	30 days	1.2±0.01	2.3±0.08	1.4±0.01	1.1±0.02	1.2±0.03	1.0±0.02
Urea	Zero time	51±2.03	77±2.31 [a]	72±1.56 [a,b]	70±1.15 [a,b]	70±1.77 [a,b]	71±2.72 [a,b]
	30 days	55±1.45	99±3.11 [a]	69±2.08 [a,b]	73±3.20 [a,b]	61±1.66 [a,b,c]	52±1.48 [b,c]

* The values are mean±SD of 6 rats in each group.
[a] Significantly different from normal control at $p < 0.05$.
[b] Significantly different from diabetic control at $p < 0.05$.
[c] Significantly different from diabetic+metformin at $p < 0.05$.

Table 5. Effect of crude methanolic leaves extract of *Acalypha wilkesiana* on lipid profile (mg/dL) levels in diabetic rats *.

Lipid profile (mg/dL)	Treatment period	Normal control	Diabetic control	Diabetic + metformin	Diabetic + *A. wilkesiana* extract at a dose		
					10 mg/100 g b. wt	20 mg/100 g b. wt	40 mg/100 g b. wt
Triglycerides	Zero time	171±3.67	289±11.45 [a]	280±8.20 [a,b]	276±7.33 [a,b]	280±6.38 [a,b]	28±8.33 [a,b]
	30 days	168±5.15	301±9.78 [a]	188±9.64 [a,b]	205±6.21 [a,b,c]	183±4.05 [a,b,c]	171±6.96 [b,c]
Total cholesterol	Zero time	195±4.04	380±9.85 [a]	377±9.72 [a]	383±7.13 [a,c]	375±5.74 [a,b,c]	386±8.88 [a,b,c]
	30 days	199±6.51	408±12.55 [a]	200±7.51 [a,b]	301±5.45 [a,b,c,f]	210±4.42 [a,b,c]	190±3.91 [a,b,c]
HDL–C	Zero time	47±1.76	26±2.16 [a]	28±3.11 [a,b]	29±2.60 [a,b]	30±2.88 [a,b]	29±4.01 [a,b]
	30 days	39±1.45	23±4.33 [a]	40±3.96 [b]	32±3.84 [a,b,c]	48±4.36 [a,b,c]	43±4.55 [a,c]
LDL–C	Zero time	114±2.60	296±3.10 [a]	293±2.67 [a]	299±3.04 [a,c]	289±3.44 [a,b,c]	301±4.20 [a,b,c]
	30 days	126±1.36	325±5.26 [a]	122±2.96 [b]	228±5.11 [a,b,c]	125±2.85 [b,c]	113±3.71 [a,b,c]
VLDL–C	Zero time	34±0.66	58±1.11 [a]	56±0.85 [a]	55±1.12 [a]	56±0.55 [a]	56±1.97 [a]
	30 days	34±0.35	60±0.99 [a]	38±0.77 [a,b]	41±1.08 [a,b]	37±0.64 [b]	34±1.04 [b,c]

* The values are mean±SD of 6 rats in each group.
[a] Significantly different from normal control at $p < 0.05$.
[b] Significantly different from diabetic control at $p < 0.05$.
[c] Significantly different from diabetic+metformin at $p < 0.05$.

3.4. Effect of crude methanolic Acalypha wilkesiana leaves extracts on kidney functions

Determination of serum creatinine and urea were used as indicators for kidney functions. The effect of methanolic *A. wilkesiana* leaves extract on serum creatinine and urea levels in STZ-diabetic rats is illustrated in Table 4. It could be noticed that injection with streptozocin induced a significant increase in serum creatinine and urea levels from 1.3 and 51.0 to 1.6 and 77.0 mg/dL respectively, compared with non-diabetic rats at the beginning of the experiment. These elevations may be attributed to the diverse hormonal and metabolic changes that accompany diabetes and the toxic effect of streptozocin on kidney [36]. It could be perceived from Table 4 that no significance differences between normal and treated rats with the methanolic extract at doses 10, 20 and 40 mg/100 g b. wt. for creatinine levels at the end of the experimental period. On the other hand, serum urea values were decreased with increasing the concentration of methanolic extract and the period of experiment to reach 52 mg/dL as a non significant value at the dose 40 mg/100 b. wt. compared with normal rats at the end of the experimental period.

Data in Table 4 show that methanolic *A. wilkesiana* leaves extract at doses 10, 20 and 40 mg/100 g b. wt. reduced serum urea levels to 26.3, 38.4 and 47.5%, respectively, after 30 days of the experiment comparing with diabetic control rats. Previous results run parallel with those of Ikewuchi *et al*., [28] who established that the reduction of blood urea represents 33.4, 47.8 and 56.5% at doses 10, 20 and 30 mg/100g b. wt. respectively of aqueous *A. wilkesiana* leaves extract at the end of the experimental period. The improvement of the kidney functions associated with treating the diabetic rats with the methanolic leaves extract of *A. wilkesiana* could be attributed to its antidiabetic action resulting in alleviation of altered metabolic status in animals and to its potent antioxidant potential which have scavenge free radicals and thereby may protect renal cells from oxidative stress.

3.5. Effect of crude methanolic Acalypha wilkesiana leaves extracts on lipid profile

Recorded data in Table 5 reveal that serum triglyceride, total cholesterol, LDL-C and VLDL-C values increased from 171, 195, 114 and 34 in non-diabetic rats to 289, 380, 296 and 58 mg/dL, respectively in diabetic rats by injection with streptozocin. On the other hand, Table 5 declares that there is a highly significant decrease in serum HDL-C level since it reached 26 mg/dL for STZ-diabetic rats, comparing with 47 mg/dL for normal rats at the beginning of the experiment. Many compositional abnormalities in the lipoproteins have been found in diabetic patients and the major cause of hypertriglyceridemia appeared to be the overproduction of VLDL, which is attributed to hyperglycemia and/or increased influx of free fatty acids in the liver [37]. Also, Fernandez *et al.,* [38] suggested that increasing in LDL cholesterol level (the risk factor for cardiovascular disease) may be attributed to some reasons such as an increase of intestinal absorption of lipid, cholesterol synthesis and liver lipid synthesis or liver dysfunction. In addition, the decrease in the serum HDL level may be due to the decrease of lecithin cholesterol acetyl transferase which responsible for estrification of cholesterol in HDL.

It could be noticed, from Table 5 that triglyceride decreased with increasing the extract concentration and the experimental period. Accordingly, the treatment of diabetic rats with 40 mg/100 g b. wt. of methanolic *A. wilkesiana* leaves extract was the most effective concentration, where the reduction percentage of triglyceride level achieves about 43% at the end of the experiment compared with diabetic control rats. These findings were in the same line of those reported by Ikewuchi *et al*., [28] who conducted that triglycerides value were reduced in percentages of 10, 14 and 24% by using the doses 10, 20 and 30 mg/100g b. wt. respectively, of aqueous *A. wilkesiana* leaves extract. Data presented in Table 5 show that the administration of diabetic rats with 40 mg/100g b. wt. of methanolic *A. wilkesiana* leaves extract for 30 days led to the highest reducing effect of total cholesterol level, which reached 190 mg/dL with a reduced percentage of 53% comparing with untreated diabetic rats. On the contrary, It has been reported that aqueous *A. wilkesiana* leaves extract have a low effect in reducing total cholesterol levels, which accomplished 4, 4 and 19% by using the doses 10, 20 and 30 mg/100 g b. wt., respectively [28].

Table 6. Effect of crude methanolic leaves extract of *Acalypha wilkesiana* on atherogenic indices in diabetic rats *.

Atherogenic indices	Treatment period	Normal control	Diabetic control	Diabetic + metformin	Diabetic + *A. wilkesiana* extract at a dose		
					10 mg/100 g b. wt	20 mg/100 g b. wt	40 mg/100 g b. wt
Cardiac risk ratio	Zero time	4.15±0.27	14.62±0.66 [a]	13.46±0.54 [a]	13.21±0.38 [a]	12.50±0.75 [a]	13.31±0.35 [a]
	30 days	5.10±0.17	17.74±0.43 [a]	5.00±0.14 [a,b]	9.41±0.27 [a,b,c]	4.38±0.11 [b]	4.42±0.13 [b]
Atherogenic coefficient	Zero time	3.15±0.11	13.62±0.46 [a]	12.46±0.32 [a]	12.21±0.41 [a]	11.50±0.29 [a]	12.31±0.36 [a]
	30 days	4.10±0.27	16.74±0.58 [a]	4.00±0.27 [a,b]	8.41±0.27 [a,b,c]	3.38±0.27 [b,c]	3.42±0.27 [b,c]
Atherogenic index of plasma	Zero time	0.56±0.04	1.05±0.11	1.00±0.07	0.98±0.06	0.97±0.08	0.99±0.07
	30 days	0.63±0.03	1.12±0.12	0.67±0.04	0.81±0.05	0.58±0.02	0.60±0.02

* The values are mean±SD of 6 rats in each group.
[a] Significantly different from normal control at $p < 0.05$.
[b] Significantly different from diabetic control at $p < 0.05$.
[c] Significantly different from diabetic+metformin at $p < 0.05$.

Table 7. Effect of treatment of crude methanol leaves extract of *Acalypha wilkesiana* on levels of Hb (g/dL) in diabetic rats *.

Treatment period	Normal control	Diabetic control	Diabetic + metformin	Diabetic + *A. wilkesiana* extract at a dose		
				10 mg/100 g b. wt	20 mg/100 g b. wt.	40 mg/100 g b. wt
Zero time	14.2±0.54	11.1±0.34	10.9±0.29	11.4±0.32	11.0±0.42	11.0±0.54
10 days	14.8±0.43	10.6±0.44	11.1±0.31	11.9±0.43	12.3±0.35	13.6±0.44
20 days	13.9±0.65	10.9±0.34	11.7±0.42	12.8±0.54	13.0±0.45	14.6±0.54
30 days	15.0±0.64	10.3±0.31	12.5±0.53	13.1±0.63	13.6±0.48	15.0±0.62

* The values are mean±SD of 6 rats in each group.

At the same time, a 87%, raising in the percentage of serum HDL-C has been achieved when diabetic rats administrated with the dose 40 mg/100 g b. wt., while treated diabetic rats with aqueous *A. wilkesiana* leaves extract have a moderate effect in increasing HDL-C value which reached 55, 46 and 66% by using the doses 10, 20 and 30 mg/100 g b. wt., respectively [28]. From the same table, it could be revealed that all doses of methanolic *A. wilkesiana* leaves extract caused a decrease in serum LDL-C and VLDL-C which became 65 and 43% after 30 days at a dose of 40 mg/100 g b. wt. respectively, compared with diabetic control rats, indicating the likely cardio-protective effect of the extract at this dose. These decreased values of both LDL-C and VLDL-C clarified that the methanolic extract of *A. wilkesiana* was more effective than aqueous one which realized a reduction of LDL-C and VLDL-C in 10 and 24%, respectively [28]. Moreover, the study of Odoh *et al.*, [32] indicated that a significant reduction ($p < 0.05$) in serum total cholesterol and triacylglycerol levels of 50% and 58%, respectively, was observed for the dose 200 mg/kg b. wt. of methanolic *A. wilkesiana* root extract in alloxan-induced diabetic rats. The beneficial effects of methanolic *A. wilkesiana* leaves extract on lipid profile of diabetic rats could be related to the insulinotropic effect or the insulin secretagogue activity of this extract by its components, especially flavonoids, which significantly increased LDL receptor mRNA levels, which, in turn, increase hepatic uptake and degradation of LDL causing a decrease in serum LDL levels, as well as its caroteinoides, which are thought to act mainly as antioxidants [39].

3.6. Effect of crude methanolic leaves extracts of Acalypha wilkesiana on atherogenic indices

Atherogenic indices are powerful indicators of the risk of heart disease. The higher values of atherogenic indices are a sign of the higher risk of developing cardiovascular diseases [40]. On the other hand, low atherogenic indices pointed to the protection against coronary heart diseases [41].

Clinical findings in Table 6 show the effect of crude methanolic *A. wilkesiana* leaves extract on atherogenic indices in diabetic rats. It could be concluded that no significance differences in both cardiac risk ratio and atherogenic coefficient between normal rats and diabetic rats administrated with the extract after 30 days. In addition, the results for atherogenic index of plasma appeared no significant differences in both normal and treated diabetic rats at all experimental periods. Our consequences for atherogenic indices were in the same line with those obtained by Ikewuchi *et al.*, [28] who accomplished that no significant differences in the atherogenic indices of the *A. wilkesiana* treated groups compared with control, except the atherogenic index of plasma which was significantly lower. In addition, these portends reductions of cardiovascular risk, according to clinical data as a caused by raising of HDL-C concentration in plasma [42]. Furthermore, treated diabetic rats showed decrease in atherogenic index and increase in percentage of protection against atherogenicity. Our data reported that a decrease in atherogenic index is due to an increase in HDL-C levels after the treatment with plant extract. HDL-C is known to play an important role in the transport of cholesterol from peripheral cells to the liver by a pathway termed reverse cholesterol transport, and is considered to be a cardio protective lipid. The existence of negative correlation between HDL-C and atherosclerosis resulted in improvement in the percentage of protection against atherogenicity in STZ-induced diabetic treated rats [43]. Thus, the antihyperlipidemic effect of methanolic *A. wilkesiana* leaves extract could play a protective role against the development of atherosclerosis and cardiovascular complications in diabetes mellitus.

3.7. Haematological parameters (complete blood count)

The complete blood count (CBC) is used as a broad screening test to check for such disorders as anemia, infection and many other diseases. It is actually a panel of tests that examines different parts of the blood, which play an important role in metabolism and important indicators of health in both human or animals [44]. The complete blood count (CBC) includes the following tests.

3.7.1. Hemoglobin (Hb)

Hemoglobin (Hb) was measured to detect anemia and its severity. This is one of those tests that will vary in reference ranges, although it is fair to say that values around or less than 10 g/dL are usually seen in patients with some signs or symptoms of anemia such as shortness of breath or fatigue and pallor [45]. Our findings in Table 7 show a decrease in hemoglobin levels from 14.2 to 11.1 g/dL by the injection with STZ to induce diabetes mellitus. Then, the oral administration of methanloic *A. wilkesiana* leaves extract in doses 10, 20 and 40 mg/100 g b. wt. confirmed a non-significant dose-dependent increase in hemoglobin levels to reach 13.1, 13.6 and 15.0 g/dL, respectively at the end of the experimental period. Therefore, the bioactive components in methanloic extract healing effect on disorders of anemia, such as shortness of breath and pallor.

Table 8. Effect of treatment of crude methanol leaves extract of *Acalypha wilkesiana* on levels of RBC (10^6/µL) and RBC indices in diabetic rats *.

		Treatment period	Normal control	Diabetic control	Diabetic + metformin	Diabetic + *A. wilkesiana* extract ata dose: 10 mg/100 g b. wt.	20 mg/100 g b. wt.	40 mg/100 g b. wt.
RBC (10^6/ µL)		Zero time	8.33±0.24	6.15±0.17	5.93±0.14	6.69±0.21	6.43±0.23	6.83±0.30
		30 days	9.01±0.32	5.92±0.15 [a]	7.88±0.27	8.02±0.34	8.29±0.25	8.69±0.19
RBC indices	HCT (%)	Zero time	46.9±1.57	35.2±0.98 [a]	36.2±1.11 [a]	35.2±1.04 [a]	37.3±1.17 [a]	37.7±1.14 [a]
		30 days	44.2±1.32	30.0±0.89 [a]	39.6±1.13 [a,b]	43.7±1.36 [b,c]	44.7±1.21 [b,c]	45.1±1.54 [b,c]
	MCV (µm³)	Zero time	69±2.09	36±1.14 [a]	39±1.11 [a]	31±0.88 [a,c]	38±1.21 [a]	33±0.99 [a,c]
		30 days	70±2.31	33±1.11 [a]	51±1.45 [a,b]	67±2.01 [b,c]	68±2.14 [b,c]	69±1.99 [b,c]
	MCH (pg)	Zero time	20.7±0.77	14.4±0.33 [a]	13.6±0.32 [a]	14.3±0.44 [a]	14.8±0.23 [a]	15.2±0.42 [a]
		30 days	23.7±0.87	13.3±0.27 [a]	16.9±0.32	20.0±0.76 [a,b,c]	20.4±0.87 [a,b,c]	20.8±0.63 [a,b,c]
	MCHC (g/dL)	Zero time	36.4±1.21	22.7±0.76 [a]	23.1±0.87 [a]	26.2±0.45 [a]	25.3±0.54 [a]	25.7±0.74 [a]
		30 days	36.1±1.14	20.2±0.77 [a]	27.0±0.87 [a,b]	34.0±1.21 [b,c]	36.2±1.13 [b,c]	36.6±1.14 [b,c]
	RDW (%)	Zero time	20.7±0.64	12.7±0.32 [a]	12.6±0.24 [a]	13.0±0.24 [a]	12.9±0.35 [a]	13.3±0.32 [a]
		30 days	20.6±0.55	13.1±0.28 [a]	16.4±0.35 [a]	16.2±0.36 [a]	17.9±0.35 [a]	18.3±0.43 [b]

* The values are mean±SD of 6 rats in each group.
[a] Significantly different from normal control at $p < 0.05$.
[b] Significantly different from diabetic control at $p < 0.05$.
[c] Significantly different from diabetic+metformin at $p < 0.05$.

Table 9. Effect of treatment of crude methanolic leaves extract of *Acalypha wilkesiana* on levels of Plt (10^3/µL) and Plt indices in diabetic rats *.

		Treatment period	Normal control	Diabetic control	Diabetic + metformin	Diabetic + *A. wilkesiana* extract at a dose 10 mg/100 g b. wt.	10 mg/100 g b. wt.	10 mg/100 g b. wt.
Plt (10^3/ µL)		Zero time	1072±33.11	553±17.35 [a]	432±18.27 [a,b]	519±19.43 [a,b,c]	425±16.37 [a,b]	501±20.58 [a,b,c]
		30 days	1100±41.16	340±16.17 [a]	787±28.22 [a,b]	1000±38.55 [a,b,c]	1008±36.88 [a,b,c]	1050±42.56 [a,b,c]
PLT indices	PCT (%)	Zero time	0.9±0.09	0.33±0.03	0.42±0.04	0.34±0.02	0.36±0.02	0.34±0.02
		30 days	0.9±0.08	0.3±0.02	0.61±0.04	0.82±0.04	0.86±0.05	0.88±0.06
	MPV (µm³)	Zero time	9.7±0.33	5.6±0.31 [a]	5.3±0.19 [a]	5.5±0.21 [a]	5.8±0.28 [a]	5.5±0.21 [a]
		30 days	9.5±0.23	5.9±0.32 [a]	7.1±0.21 [a]	8.7±0.31 [b]	9.9±0.34 [b]	9.9±0.31 [b]
	PDW (%)	Zero time	14.6±0.55	4.7±0.17 [a]	5.2±0.21 [a]	4.3±0.15 [a]	4.4±0.21 [a]	4.5±1.17 [a]
		30 days	14.9±0.57	4.8±0.14 [a]	9.2±0.32 [a,b]	12.3±0.34 [a,b,c]	13.4±0.28 [b,c]	14.5±0.29 [b,c]

* The values are mean±SD of 6 rats in each group.
[a] Significantly different from normal control at $p < 0.05$.
[b] Significantly different from diabetic control at $p < 0.05$.
[c] Significantly different from diabetic+metformin at $p < 0.05$.

These results for hemoglobin levels were not agreed with those obtained by Ikewuchi *et al*., [28] who mentioned that no significant differences between normal and treated rats with aqueous *A. wilkesiana* leaves extract. Thus, extraction of bioactive compounds with methanol was more effective than water to cure symptoms accomplished with hemoglobin decline caused by diabetes mellitus.

3.7.2. Red blood cells (RBC)

The count of the actual number of red blood cells (RBC) per volume of blood. Both increase and decrease can point to abnormal conditions. Decreased count of RBC indices to anemia, while increasing count of RBC in the company of fluid loss indices to diarrhea, dehydration and burns [44].

Haematological results in Table 8 illustrate decrease in RBC count from 8.33 to 6.15× 10^6/µL by the injection of rats with STZ to induce diabetes. Then, methanloic leaves extract of *A. wilkesiana* in doses 10, 20 and 40 mg/100g b. wt. caused an increase in RBC reckon to reach 8.02, 8.29 and 8.69×10^6/µL, respectively after 30 days. From the same table, it could be noticed that RBC indices (HTC, MCV, MCH, MCHC and RDW) were reduced by the injection with streptozocin. Afterward, RBC indices were gradually increased to almost the normal control rat's values by treatment with *A. wilkesiana* leaves extract. On the contrary, Ikewuchi *et al.,* [28] found no significant differences between normal and treated rats with aqueous *A. wilkesiana* leaves extract for both RBCs count and RBC indices. This gives an indication that the methanloic *A. wilkesiana* leaves extract may contain some phytochemicals that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells [46]. The stimulation of this hormone enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC [47]. Moreover, Table 1 indicates the presence of flavonoids, tannins, phenols and flavonols in methanloic *A. wilkesiana* leaves extract. These compounds have been reported to possess strong antioxidant capacity, therefore, could inhibit the peroxidation of polyunsaturated fatty acids in the cell membrane and haemolysis of red blood cells in the diabetic animals.

3.7.3. Platelets (Plt)

Platelets (Plt) are produced within the vascular channels (sinusoids) of the bone marrow by the fragmentation of the protruding cytoplasm of large bone marrow cells known as megakaryocytes. Platelets count and its indices (PCT, MPV and PDW) levels were reduced to reach about 50% of these values in normal rats when streptozocin was used to induce diabetes mellitus. Then, the oral administration of methanoic *A. wilkesiana* leaves extract established dose-dependent increase in platelet counts and its indices to achieve the highest percentage levels (about 90%) compared with diabetic rats at a dose of 40 mg/100 b. wt. at the end of the experimental period as shown in Table 9.

Our findings were in accordance with those obtained by Ikewuchi *et al*., [28] who calculated the increasing percentages of platelet counts to reach about 94, 57 and 31% of treated diabetic rats with aqueous *A. wilkesiana* leaves extract at doses 10, 20 and 30 mg/100 g b. wt., respectively. These increasing in platelets count and its indices imply the increase in clotting, which protect against bleeding and increase insulin resistance as the cause of predisposition to adverse cardiovascular events. Taniguchi *et al*., [48] reported that increased platelets count may independently predict insulin resistance among non obese type 2 diabetes mellitus patients. This effect indicated the ability of the methanoic *A. wilkesiana* leaves extract to stimulate the biosynthesis of clotting factors due to the presence of active compounds that might help to precipitate blood coagulation or clotting, especially during severe bleeding or haemorrhage [49].

Table 10. Effect of treatment of crude methanolic leaves extract of *Acalypha wilkesiana* on levels of WBC (10^3/µL) and WBC differential in diabetic rats *.

		Treatment period	Normal control	Diabetic control	Diabetic + metformin	Diabetic + *A. wilkesiana* extract at adose		
						10 mg/100 g b. wt	10 mg/100 g b. wt	10 mg/100 g b. wt
WBC (10^3/ µL)		Zero time	6.5±0.43 [d]	11.5±0.66 [a]	12.1±0.65 [a]	11.7±0.56 [a]	11.9±0. 54 [a]	11.5±0.55 [a]
		30 days	5.9±0.21 [d]	12.0±0.45 [a]	9.2±0.32 [a,b]	7.3±0.43 [b]	6.6±0.31 [b,c]	6.5±0.31 [b,c]
WBC differential	LYM (%)	Zero time	58.4±2.13 [c]	66.1±2.43 [a]	69.4±2.22 [a]	68.5±2.87 [a]	67.3±2.76 [a]	67.0±2.42 [a]
		30 days	51.7±2.09 [d]	75.3±2.54 [a]	60.1±1.89	51.5±2.03	53.1±2.11 [b,c]	52.7±1.99 [b,c]
	MON (%)	Zero time	1.3±0.02 [a]	1.9±0.09 [a]	1.8±0.08	1.1±0.07 [b]	1.0±0.08 [b]	1.2±0.08 [b]
		30 days	1.9±0.03 [a]	1.3±0.09 [a]	1.0±0.09 [a]	1.1±0.01 [a]	1.8±0.31 [c]	1.7±0.31 [c]
	GRA (%)	Zero time	40.3±0.25 [c]	32.0±0.31 [a]	28.8±0.17 [a,b]	30.4±0.21 [a,c]	31.7±0.19 [a,c]	31.8±0.21 [a,c]
		30 days	46.4±1.11 [a]	23.4±0.76 [a]	38.9±0.65 [a,b]	48.4±0.45 [b,c]	45.9±0.64 [b,c]	45.6±0.65 [b,c]

* The values are mean±SD of 6 rats in each group.
[a] Significantly different from normal control at $p < 0.05$.
[b] Significantly different from diabetic control at $p < 0.05$.
[c] Significantly different from diabetic + metformin at $p < 0.05$.

3.7.4. White blood cells (WBC)

White blood cells (WBC) were primarily formed in the bone marrow and may also be produced in organs of the lymphatic system such as spleen, thymus and lymph nodes. They serve to fight off infections and protect the body from diseases. This test serves to monitor diseases progression and/or response to chemotherapy. Moreover, the differential divided the various types of white blood cells based on size distribution. The differential is done to evaluate the body's capacity to resist and overcome infection [45]**.** Quantifiable findings in Table 10 explain the effect of streptozocin injection of white blood cells and its indices (LYM, MON and GRA which include neutrophils, eosinophils, and basophils). Drug poisoning is one of the main causes of raising white blood cell count, which plays important roles in the destabilization of coronary artery plaques at the onset of acute coronary syndrome [50]. However, an elevated white blood cell count in peripheral blood is a known risk factor of coronary artery disease [51]. Thus, the observed higher white blood cell count for diabetic rats suggestion the protection against onset of acute coronary syndrome and the increased risk of coronary artery disease. On the other hand, the white blood cell counts and its related indices were significantly restored to near normal after methanolic *A. wilkesiana* leaves extract administration at doses 20 and 40 mg/100 g b. wt. The presence of some phytochemicals in the *A. wilkesiana* leaves extract with the ability to stimulate the production of white blood count and its differentials could be responsible for the observed results in the treated diabetic rats. This observation of increasing levels of these parameters by *A. wilkesiana* extract suggests that the principal function of phagocytes, which is to defend against invading microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory processes of the animals, will be enhanced [52].

4. Conclusion

In conclusion, this investigation revealed the presence of pharmacologically active compounds in the methanoilc extract of *Acalypha wilkesiana* leaves. This extract had hypoglycemic activities and positively affected on the hematopoietic system, the integrity of liver and kidney functions and improved the lipid profile as a cardioprotective agent for STZ treated diabetic rats. Furthermore, extract had no deleterious effect on hemoglobin or red blood cell morphology. All of these highlights of methanolic *Acalypha wilkesiana* leaves extract support its uses as a potential source of discovery for new therapeutic agents for health care and management of diabetes mellitus and its complications.

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