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Ameliorative Potentials of Aqueous and Hydro-Ethanol Extracts of *Citrullus colocynthis* L Fruit on Hyperglycemia and Some Serum Biochemical Parameters on Alloxan-induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was carry out to determine the dose effect of aqueous and hydro-ethanol *C. colocynthis* fruit extracts on some properties and serum biochemical parameters in alloxan-induced diabetic rats.
Study Design: Random trial.
Place and Duration of Study: University of Douala-Cameroon. Faculty of Science. Laboratory of Biochemistry, between April 2017 and June 2017.
Methodology: Forty two albino male rats (*Rattus norvegicus* var. albinus), aged 2 to 3 months and weighing 150- 200 g were used in this study. Animals were randomly distributed into 7 experimental groups of six rats each. One group was used as healthy control and diabetes was

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induced in the 6 others groups by intraperitoneal injection with alloxan monohydrate into tail veins. Five diabetic groups received oral glibenclamide (3mg/kg), 50 and 100 mg/kg bw of aqueous or hydro-ethanol fruit extract of *Citrullus colocynthis* respectively The remaining group was assigned as diabetic control rats. Body weight and serum biochemical parameters (glucose, lipids, transaminases and creatinine) were recorded weekly *during 3 weeks.*

Results: Diabetes induction of with alloxan significantly (p = .05) increases blood glucose, triglyceride, cholesterol, transaminases (AST, ALT) and creatinine blood level. Treatment of rats with hydro-ethanol extract (100 mg/kg.bw) steadily reduces (80.44%) glycemia but cause a significant increase of the liver relative mass, AST (138.38 IU/l) and ALT (152.35 IU/l) blood levels (p = .05). Administration of 50 mg/kg bw hydro-ethanol and aqueous extracts significantly reduce the glucose (22-46.44%), triglycerides, total cholesterol, transaminases and creatinine blood levels (72 to 85 %) (p = .05).

Conclusion: The administration of glibenclamide (3mg/kg), 50 and 100 mg/kg bw aqueous extracts and 50 mg/kg bw hydro ethanol extracts significantly reversed the damage associated with Alloxan induced diabetes close to the normal. The dose of 100 mg/kg bw hydro ethanol extracts should be avoided because of it toxic effects.

Keywords: Diabetes mellitus; Citrillus colocynthis; solvent extraction; toxic effect.

1. INTRODUCTION

Many decades ago, diabetes was not considered as a public priority in sub-Saharan Africa. But when the results of epidemiological studies revealed that diabetes was a huge and growing threat to health in Africa, actions have been taken to stem the epidemic for instance, diabetes education, reduction of the cost of insulin in Type 2 diabetes (85%–95% of cases), promotion of prescriptions of diabetes drugs in their generic names [1,2]. Despite all, diabetes treatment remains expensive resulting in avoidable complications. Healthcare expenditure often presents a challenge to the economic sustainability of household. Therefore, there is an increase tendency for traditional medicine in a context that out of pocket (OOP) remains the main method of healthcare payment in sub-Saharan Africa [2,3]. There are several reports regarding the antidiabetic activity of crude extracts prepared from plants [4,5]. *Citrullus colocynthis* (CCT) fruit also known as bitter cucumber is native to dry areas of North Africa, the Mediterranean Basin and Asia. It is distributed among the west coast of northern Africa, eastward through the sahara, until India [6]. The fruit is a folk remedy for diabetes, cancer, inflammatory and infectious diseases in tropical and subtropical countries. This plant can induce insulinotropic and mild immunostimulating effect due mainly to the presence of bioactive compounds [4,7]. Extraction procedures are an important step in processing bioactive constituents from plant materials using selective solvents. The solvent extraction has been widely used to extract compounds from *C. colocynthis* fruits. The type of solvent has been the most studied factor to ensure the efficiency of extraction [8]. Water, ethanol or the mixture of both are the most common hydrophilic solvent used. According to epidemiological studies, there is a positive association between the type of solvent, amount of total extractible compounds, therapeutic property and toxic effect of the plant. Many studies reported a dose dependent hypoglycemic and toxic effect of aqueous, ethanol or hydro-ethanol extracts of *C. colocynthis* fruit seed or pulp [9]. Considerable efforts have been made to extract and identify bioactive compounds responsible for the antidiabetic properties of *C. colocynthis*. The present study is therefore conducted with the objective to investigate the influence of aqueous and hydro-ethanol fruit solvent extracts and the drug dose on hypoglycemic properties and serum biochemical parameters in normal and alloxan-induced diabetic rats using glibenclamide as reference drug.

2. MATERIALS AND METHODS

2.1 Animals

Forty two albino rats (*Rattus norvegicus* var. albinus), aged 2 to 3 months and weighing 150-200 g were used in this study. They were purchased from animal house, Department of Animal Biology, Faculty of Science of the University of Dschang-Cameroon. The rats were placed in aerated polyethylene baskets and transported by road to the Animal Lab Centre of the Laboratory of Biochemistry, University of

Douala-Cameroon. Animals were randomly distributed into 7 experimental groups of six rats each and housed in separate polyethylene cages (36cm × 25cm), with free access to food and water for a total of five weeks including two weeks of adaptation period and three weeks of testing period. A thick layer of wood chips was deposited at the bottom of the cages. This chip was renewed after two days interval. They were kept at 25°C in an aerated environment on a 12: 12 hour light: dark cycle. During this period, rats received standard balanced chow for rodents and tap water ad libitum.

2.2 Source of Samples and Preparation of Extracts

Citrullus colocynthis fruits were collected in April 2017 at Ouaddaï region in the Center Est of Chad; Precisely at 50 km in the North-Est of Abeché town (E 20°30' - E 20°45' / N 13°45' - N 13°30). Fruits were washed under running tap water to remove the adhering dirt, cut in small pieces and dried at 50°C in an oven dryer (Binder). The dried slices were crushed to course powder by using mechanical grinder (robot mixer) and the powder was then sieved to 500µm particule size. Fifty grams of powder was mixed into 500ml of distillated water for aqueous extraction and 500ml of distillated water: ethanol solution (50:50v/v) for hydro-ethanol extraction and then submitted to dynamic maceration for 3 days at room temperature. The extracts were then filtered with a qualitative cellulose filter paper (particle retention: 20-25 µm). After filtration, the hydro-ethanol filtrate was concentrated by evaporating the solvent under reduced pressure 300-500 mmHg at 50-60°C using Rotary evaporator (Buchi). The aqueous filtrate and the concentrated hydro-ethanol extract were lyophilised and the dried extracts stored in an air tight container, keep at - 80°C for future analysis.

2.3 Preliminary Phytochemical Screening of Extracts

The preliminary phytochemical screening was performed by the methods described by Evans [10] for triterpenes/steroids (Libermann burchard Test), alkaloids (Dragendroff's Test), flavonoids (Shinoda test), saponins (foam test), phenol (Ferric chloride Test) and glycosides (Keller-killiani test). The color intensity or the precipitate formation was used as analytical responses to these tests [10]. The qualitative results were expressed as (+) for the presence and (−) for the absence of phytochemicals.

2.4 Proximate Composition of Extract

Moisture, crude protein, total ashes were determined in lyophilised extracts flour according to the AOAC official methods [11]. Moisture was determined by drying at 103 °C until constant weight is achieved. Ash was determined by heating dried sample at 550°C for 24 hours. Crude protein content of flour has been analysed according to Kjeldahl method. Total proteins were calculated by multiplying the evaluated nitrogen by 6.25. Crude fat content were quantified according to Bourely et al. method [12].

2.5 Experimental Induction of Diabetes

Diabetes was induced in 6 of the seven groups by injection with alloxan monohydrate (Sigma Chemicals Co., USA) dissolved in sterile normal saline at a dose of 150 mg/kg bw intraperitoneally into tail veins [13]. The remaining one was used as healthy control group. After alloxan injection, animal had free access to food. Glucose solution (5% (w/v)) was given to rat overnight to avoid hypoglycemic shock [14]. Only animals with clinical signs of severe diabetes and fasting glucose ≥ 200mg/dL in two successive determinations (18 and 24 hours after diabetes induction) were used.

2.6 Intervention

Five of the six diabetic groups were assigned as intervention group and the other one as diabetic control rats. Intervention groups received oral glibenclamide (daonil 3mg/kg) (Sigma Chemicals Co., USA), 50 and 100 mg/kg bw of aqueous or hydro-ethanol fruit extract of *Citrullus colocynthis* respectively with intragastric tube. The animals were divided into 7 groups (n=6) as follows:

Group1: HCR: Healthy Control rats;
Group 2: UD: Diabetic control rats without hypoglycemia treatment;
Group 3: DTD: Diabetic rats given aqueous solution of glibenclamide;
Group 4: DTAE 50: Diabetic rats given 50mg/kg bw aqueous extract;
Group 5: DTAE 100: Diabetic rats given 100mg/kg bw aqueous extract;
Group 6: DTHE 50: Diabetic rats given 50mg/kg bw hydro-ethanol extract;

Group 7: DTHE 100: Diabetic rats given 100mgkg /bw hydro-ethanol extract;

2.7 Clinical Study

Body weight was recorded weekly for 3 weeks. Blood samples were drawn from the tail vein and the blood glucose level was determined at 1 and 2 h after administration of test extracts and glibenclamide for short-term fasting blood glucose levels determination (mg/dl) and at 7, 14, 21 days interval for long-term fasting blood glucose determination. Careful considerations were used to avoid hemolysis during sampling and transfer. In order to separate the plasma serum, blood specimens were centrifuged at 3000 rpm for 10 minutes. The serum was then transferred to test tubes by eppendorf sampler and blood glucose level was measured by using glucose oxidase-peroxidase reactive strips and glucometer (One Touch Ultra). Biochemical parameters (total cholesterol, triglycerides, AST, ALT, creatinine) were analysed at the end of experimentation after sacrifice. Sacrifice was performed by jugular vein rupture under general anesthesia using intramuscular ketamine (100 mg/kg bw, Ketalar, Sankyo, Tokyo, Japan) after 14 hours of fasting. Fresh liver, kidney, lungs and heart were removed after exsanguinations, washed with NaCl 0.9 % and weighed using an analytical balance and recorded. Blood biochemical tests were performed using commercial kits Triglycerides were determined after enzymatic hydrolysis by lipases and cholesterol measured by enzymatic method (Hospitex Diagnostics). AST, ALT and creatinine were analysed using commercial kits (Biolabo SA).

2.8 Statistical Analysis

The results were expressed by the means ± standard deviation. Analyses of clinical and biochemical variables and fresh organs weights in experimental groups, were conducted using analysis of variance in a completely randomized design, followed by LSD multiple range test for comparisons. Multivariate analysis (Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA)) was done to analyse variability and classify extracts according to their blood glucose, lipids, creatinine and transaminase lowering effect and their impact on lung, heart, kidney and liver weight. STATGRAPHICS centurion version XV.II and XLSTAT 8.04 were used as software.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Extraction yield

Results of extraction yield (Table 1) showed that the amount of crude extract of *C. colocynthis* fruit depends upon the solvent nature. It varied from 9.9 to 16.36 % with a descending order of water: alcohol > water. Extraction with a mixture of water: alcohol resulted in the highest amount of total extractable compounds whereas the extraction yield with water was small. Higher extraction yield in water: alcohol mixture might be due to the fact that it easily penetrates the cellular membrane and extracts the intracellular ingredients from the plant material. These results showed that *C. colocynthis* fruit contains more of non polar compounds.

3.2.2 Phytochemical screening and proximate composition of extracts

3.2.2.1 Phytochemical screening

The preliminary phytochemical screening of aqueous and hydro-ethanol extracts of *C. colocynthis* fruit (Table 1) revealed the presence of various compounds such as phenols, sterols, terpenoids and glycosides. Hydro-ethanol extract are abundant in glycosides and terpenoids. Sterols were detected only in hydro-ethanol extract. Flavonoids, alkaloids and saponins were not detected in the extracts.

Table 1. Phytochemical screening of extracts

	Extracts	
	Aqueous extract	**hydro-ethanol extract**
Extraction yield (%)		
	9.9	13.6
Compounds		
Flavonoids	-	-
Phenols	+++	+++
Alkaloids	-	-
Sterols	-	+
Glycosides	+	+++
Saponins	-	-
Terpenoids	+	+++

+ + +: High presence; +: low Presence; - : Absence

3.2.2 2 Some proximate composition of extract

The amount of macronutrients (Table 2) presents in the extract depends upon the solvent nature. The aqueous extract contains more water than hydro-ethanol extracts. In contrary, hydro-ethanol extract has a lot of proteins (22%), lipids (21%) and ash (3%). The level of lipids is 2 times higher in hydro-ethanol extract showing that *C colocynthis* fruits contain more non polar substances easily extracted by the mixture of water and ethanol solvent.

3.1.2 Clinical study

3.1.2.1 Mortality during experimentation

All the experiment animals became diabetic after a single injection of alloxan. Mortality was highest (33%) in rat after administration of 100 mg/kg bw of hydro-ethanol extract (Table 3) and followed respectively by those receiving glibenclamide, 50 mg/kg bw hydro-ethanol extract and aqueous extracts (50 and 100 mg/kg bw). No death was recorded for diabetic and healthy control rats after three weeks of experiment. The clinical results include data only from the survivors after study period as shown in Table 3.

3.1.2.2 Effect of C. colocynthis extract on the blood glucose in short-term

Variation of blood glucose level two hours after administration of glibenclamide or *C. Colocynthis* fruit extracts in alloxan-induced diabetic rats are shown in Table 4. From this table, it appears that blood glucose levels of rats treated with glibenclamide "daonil (3mg/kg bw)" and hydro-ethanol extracts decrease significantly after the first hour of drug intake. It decreases from 255.50 to 193.40 mg/dl (24.3%) and 270.40 to 194.20 (36.83%) respectively and continues after the second hour but not significantly (36.2 and 28.18%). Glycemia of rats treated with the aqueous extracts decreases significantly from the first hour of feeding (35.49%) and then tends to increase after the second hour, but not significantly (36.83%). This result shows the effectiveness of daonil and fruit extracts in reducing blood glucose level in short term (0-2 hours).

3.1.2.3 Effect of C. colocynthis extract on the blood glucose in long-term

The effect of *C. colocynthis* extract on long-term blood glucose level in alloxan-induced diabetic rats is shown in Table 5. Alloxan injection caused a significant increase in mean fasting blood glucose level in all intervention groups in comparison with healthy controls. The influence of the treating drugs in reducing blood glucose level depends upon the type of drug intake. The chemical drug stabilises blood sugar. The values range from 222.5 ± 58.569 to 232.33 ± 33.561 mg/dl. The effect of extracts in reducing blood glucose level depends upon the dose, the nature of the solvent used for extraction and the duration of experimentation. The aqueous extract (50 mg/kg bw) significantly decreases blood glucose level (22.43%) only after 21 days of treatment while 7 days of an administration are sufficient for 100 mg/kg bw aqueous and 50 mg/kg bw hydro-ethanol extract to significantly reduce glycemia at 42.67 and 46.19% respectively. The hydro-ethanol extract (100 mg/kg.bw) steadily reduces glycemia all along the duration of experimentation. This blood glucose level decreased significantly after 7 days of administration (58.89%) and then after 14 days (80.44%) to be below the values recorded for healthy control rats. Normal rats did not exhibit any significant alterations in their blood glucose concentration during the experimentation.

3.1.2.4 Body weight

Body weight of rats group is shown in Table 6. The body weight of healthy controls rats increases during the study period showing good food intake. Change in body weight is not significant in diabetic rats and in intervention group.

Table 2. Proximate composition of *C. colocynthis* fruits

Extracts	Eau %	Protein %	Lipids %	Ash %
Aqueous	20^{a} ± 0.1	18.6^{b} ± 0.2	11^{b} ± 0.1	2^{b} ± 0.12
Hydro-ethanol	10^{b} ± 0.15	22.2^{a} ± 0.18	21^{a} ± 0.1	3^{a} ± 0.16
P value	*.0001*	*.00015*	*.0009*	*.0002*

Results of n= 4 number of replicate; Mean ± S.D values with the same alphabets in a category are not significantly different

Table 3. Survival and mortality of rats three weeks after alloxan injection

	Total number of rats at the beginning	Number of death			Rats alived	
Groups		**Week 1**	**Week 2**	**Week 3**	**end of experimentation**	**Percentage (%)**
Group1: HCR: Healthy Control rats	6	0	0	0	6	100
Group 2: UD: Diabetic control rats without hypoglycemia treatment	6	0	0	0	5	100
Group 3: DTD: Diabetic rats given aqueous solution of glibenclamide	6	0	1	2	3	50
Group 4: DTAE 50: Diabetic rats given 50mg/kg bw aqueous extract	6	0	0	3	3	50
Group 5: DTAE 100: Diabetic rats given 100mg/kg bw aqueous extract	6	0	0	3	2	50
Group 6: DTHE 50: Diabetic rats given 50mg/kg bw hydro-ethanol extract	6	1	2	0	3	50
Group 7: DTHE 100: Diabetic rats given 100mgkg /bw hydro-ethanol extract	6	2	2	0	2	33.33

AE: aqueous extract. HE: hydro-ethanol extract

Table 4. Variation of blood glucose (mg/dl) level in alloxan-induced diabetic rats after drugs administration

	Administration		
Time (h)	**Chemical molecule (3 mg/kg bw)**	**Aqueous extract (100 mg/kg bw)**	**Hydro ethanol extract (100 mg/kg bw)**
0	$255.50^{a} \pm 14.978$	$298.67^{a} \pm 18,175$	$270.40^{a} \pm 19,932$
1	$193.40^{b} \pm 24.131$	$188.67^{b} \pm 16,503$	$194.20^{b} \pm 14,464$
2	$163.0^{b} \pm 8.660$	$192.67^{b} \pm 28,868$	$186.00^{b} \pm 12,247$
P value	*.000*	*.000*	*.000*

Results of n= 6 number of replicate; Mean ± S.D values with the same alphabets in a category are not significantly different

Table 5. Variation of blood glucose in alloxan-induced diabetic rats after 21 days of different treatment exposures

Groups	Number of rats	Days				*P value*
		0	7	14	21	
		Blood glucose level (mg/dl)				
Group1: HCR: Healthy Control rats	6	$81.17^{b} \pm 5,27$	$79.33^{b} \pm 4,46$	$78^{b} \pm 5,18$	$80.83^{b} \pm 5.57$	*.001*
Group 2: UD: Diabetic control rats without hypoglycemia treatment	6	$322^{b} \pm 32.44$	$272.5^{b} \pm 38.63$	$435.2^{a} \pm 25.22$	-	*.001*
Group 3: DTD: Diabetic rats given aqueous solution of glibenclamide	6	222.5 ± 58.57	269.33 ± 44.06	232.33 ± 33.56	-	*.48*
Group 4: DTAE 50: Diabetic rats given 50mg/kg bw aqueous extract	6	$266.2^{a} \pm 23.91$	$268.33^{a} \pm 29.32$	$250^{a} \pm 5.83$	$206.5^{b} \pm 8.89$	*.002*
Group 5: DTAE 100: Diabetic rats given 100mg/kg bw aqueous extract	6	$266^{a} \pm 53.75$	$109.75^{b} \pm 29.95$	$152.5^{b} \pm 38.85$	-	*.000*
Group 6: DTHE 50: Diabetic rats given 50mg/kg bw hydro-ethanol extract	6	$302^{a} \pm 48.62$	$149.67^{b} \pm 32.95$	$231.25^{b} \pm 15.52$	$162.5^{b} \pm 8.89$	*.001*
Group 7: DTHE 100: Diabetic rats given 100mgkg /bw hydro-ethanol extract	6	$317^{a} \pm 35.09$	$186.67^{b} \pm 18.45$	$62^{c} \pm 17$	-	*.000*

Results of n= 6 number of replicate; Mean ± S.D values with the same alphabets in a category are not significantly different.

- No value recorded because of death

AE: aqueous extract. HE: hydro-ethanol extract

Table 6. Body weight of alloxan-induced diabetic rats after 21 days of drugs administration

Group of rats	Time (days) of experimentation				*P value*
	0	7	14	21	
	Mass (g)				
Group1: HCR: Healthy Control rats	172.00[a] ± 34.7	180.33 [a] ± 40.45	184.67[a] ± 39.55	185.67 [a] ± 37.75	*.97*
Group 2: UD: Diabetic control rats without hypoglycemia treatment	185.33 [a] ± 30.75	145.67 [a] ± 13.05	140.67 [a] ± 18.15	133.67 [a] ± 24.11	*.08*
Group 3: DTD: Diabetic rats given aqueous solution of glibenclamide	135.67 [a] ± 29.01	139.33 [a] ± 24.01	137.33 [a] ± 21.01	130.67 [a] ± 19.01	*.97*
Group 4: DTAE 50: Diabetic rats given 50mg/kg bw aqueous extract;	161.33 [a] ± 5.51	159.00 [a] ± 7.00	166.33 [a] ± 22.81	154.33 [a] ± 35.25	*.92*
Group 5: DTAE 100: Diabetic rats given 100mg/kg bw aqueous extract;	164.00 [a] ± 18.08	163.67 [a] ± 12.66	181.00 [a] ± 4.36	179.00 [a] ± 5.57	*.2*
Group 6: DTHE 50: Diabetic rats given 50mg/kg bw hydro-ethanol extract;	167.33 [a] ± 27.47	138.67 [a] ± 20.01	137.67 [a] ± 22.59	133.33 [a] ± 20.74	*.32*
Group 7: DTHE 100: Diabetic rats given 100mgkg /bw hydro-ethanol extract;	152.67 [a] ± 24.44	150.00 [a] ± 26.67	136.67 [a] ± 35.56	131.00 [a] ± 32.05	*.78*

Results of n= 6 number of replicate; Mean ± S.D values with the same alphabets in a category are not significantly different.
AE: aqueous extract. HE: hydro-ethanol extract

Table 7. Body weight of alloxan-induced diabetic rat after 21 days of different treatment exposures (mg/g body weight)

Rats	Organ relative mass			
	Lung	Heart	Liver	Kidney
Group1: HCR: Healthy Control rats	0.010 [a] ± 0.001	0,003 [a] ± 0.000	0.029[c] ± 0.003	0.006 [a] ± 0.000
Group 2: UD: Diabetic control rats without hypoglycemia treatment	0.011 [a] ± 0.007	0,005 [a] ± 0.005	0.037[ab] ± 0.005	0.009 [a] ± 0.001
Group 3: DTD: Diabetic rats given aqueous solution of glibenclamide	0.011 [a] ± 0.003	0.004 [a] ± 0.001	0.039[ab] ± 0.004	0.010 [a] ± 0.000
Group 4: DTAE 50: Diabetic rats given 50mg/kg bw aqueous extract;t	0.013 [a] ± 0.000	0.003 [a] ± 0.000	0.032[bc] ± 0.002	0.010 [a] ± 0.003
Group 5: DTAE 100: Diabetic rats given 100mg/kg bw aqueous extract;	0.011 [a] ± 0.003	0.003 [a] ± 0.001	0.032[bc] ± 0.005	0.007 [a] ± 0.000
Group 6: DTHE 50: Diabetic rats given 50mg/kg bw hydro-ethanol extract;	0.011 [a] ± 0.001	0.004 [a] ± 0.001	0.040[a] ± 0.001	0.009 [a] ± 0.002
Group 7: DTHE 100: Diabetic rats given 100mgkg /bw hydro-ethanol extract;	0.014 [a] ± 0.006	0.004 [a] ± 0.000	0.043[a] ± 0.002	0.008 [a] ± 0.001
P value	*.92*	*.85*	*.001*	*.09*

Results of n= 6 number of replicate; Mean ± S.D values with the same alphabets in a category are not significantly different.
AE: aqueous extract. HE: hydro-ethanol extract

Table 8. Effect of different treatment exposures on total cholesterol and triglycerides blood level on alloxan-induced diabetic rats after 21 days

	Total cholesterol (g/l)	Triglycerides (g/l)
Group1: HCR: Healthy Control rats	1.78^{b} ± 0.035	0.65^{b} ± 0.054
Group 2: UD: Diabetic control rats without hypoglycemia treatment	2.28^{a} ± 0.117	1.52^{a} ± 0.142
Group 3: DTD: Diabetic rats given aqueous solution of glibenclamide	0.97^{d} ± 0.035	0.35^{d} ± 0.034
Group 4: DTAE 50: Diabetic rats given 50mg/kg bw aqueous extract	0.89^{d} ± 0.039	0.41^{cd} ± 0.016
Group 5: DTAE 100: Diabetic rats given 100mg/kg bw aqueous extract	$0,79^{e}$ ± 0.033	0.36^{d} ± 0.047
Group 6: DTHE 50: Diabetic rats given 50mg/kg bw hydro-ethanol extract	1.22^{c} ± 0.016	0.43^{cd} ± 0.021
Group 7: DTHE 100: Diabetic rats given 100mgkg /bw hydro-ethanol extract	1.27^{c} ± 0.061	0.47^{c} ± 0.046
P value	*.000*	*.000*

Results of n= 6 number of replicate; Mean ± S.D values with the same alphabets in a category are not significantly different.
AE: aqueous extract. HE: hydro-ethanol extract

Table 9. Effect of extracts on creatinine and transaminases AST and ALT blood level on alloxan-induced diabetic rats after 21 days

	Creatinine (mg/dl)	Aspartame aminotransferase AST(UI)	Alanine aminotransferase ALT(UI)	AST/ ALT
Group1: HCR: Healthy Control rats	6.7^{d} ± 0.629	$18,13^{d}$ ± 0.766	19.85^{d} ± 6.005	0.98^{b}±0.276
Group 2: UD: Diabetic control rats without hypoglycemia treatment	40.05^{a} ± 1.380	69.18^{b} ± 1.750	82.78^{b} ± 2.560	0.84^{bc}±0.012
Group 3: DTD: Diabetic rats given aqueous solution of glibenclamide	6.9^{d} ± 0.141	28.00^{c} ± 3.950	34.33^{c} ± 6.501	0.82^{bc}±0.048
Group 4: DTAE 50: Diabetic rats given 50mg/kg bw aqueous extract	7.5^{d} ± 0.535	15.70^{de} ± 0.535	23.13^{d} ± 0.695	0.68^{cd}±0,040
Group 5: DTAE 100: Diabetic rats given 100mg/kg bw aqueous extract	$6,6^{d}$ ± 0.469	14.38^{e} ± 0.946	34.25^{c} ± 1.500	0.42^{d}±0.041
Group 6: DTHE 50: Diabetic rats given 50mg/kg bw hydro-ethanol extract	10.6^{c} ± 0.410	28.83^{c} ± 1.602	25.50^{d} ± 8.240	1.22^{a}±0.357
Group 7: DTHE 100: Diabetic rats given 100mgkg /bw hydro-ethanol extract	23^{b} ± 1.055	138.38^{a} ± 5.023	152.35^{a} ± 4.331	0.91^{bc}±0.008
P value	*.000*	*.000*	*.000*	*.000*

Results of n= 6 number of replicate; Mean ± S.D values with the same alphabets in a category are not significantly different.
AE: aqueous extract. HE: hydro-ethanol extract

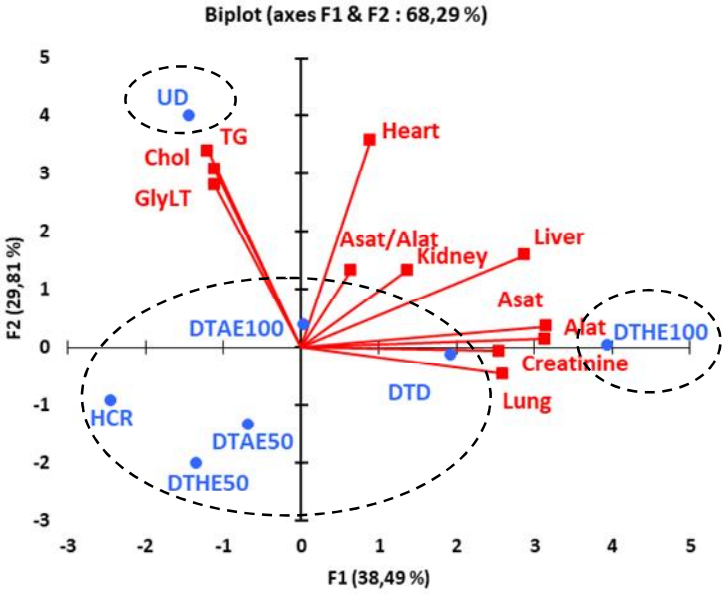

Fig. 1. Overall behavior of diabetic rats' organs and biochemical parameters as affected by treatments

Asat: Aspartame aminotransferase, Alat Alanine aminotransferase, HCR: Healthy control rats; UD: diabetic rats without treatment; DTD: diabetic rats treated with Daonil; DTAE 50mg/kg bw: diabetic rats treated with 50mg/kg bw aqueous extract; DTAE 100mg/kg bw: diabetic rats treated with 100mg/bw aqueous extract. DTHE 50mg/kg bw: diabetic rats treated with 50mg/kg bw hydro-ethanol extract; DTHE 100mg/kg bw: diabetic rats treated with 100mg/kg bw hydro-ethanol extract

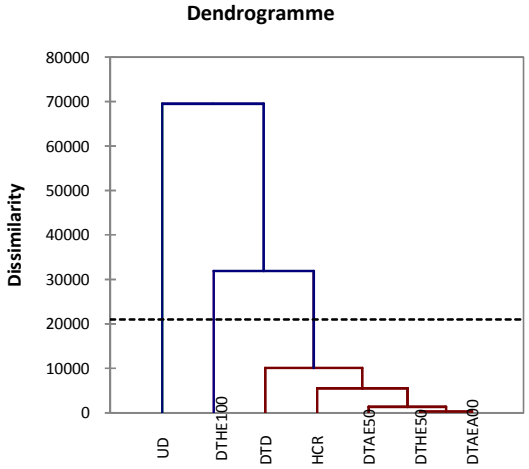

Fig. 2. Cluster dendogramme grouping drugs samples in classes

3.1.2.5 Organ's weight

Organ relative mass (weight/bodyweight ratio) of all animals used in the study after sacrifice are shown in Table 7. There were only significant changes in liver relative mass compared to the others organs. Indeed, the relative body weight of liver in healthy groups is significantly low after 21 days of intervention. In alloxan-induced diabetic rats, there was a significant increase in liver weight (0.039 mg/g bw). Treatment of diabetic rat with glibenclamide did not decrease

the relative mass (0.037 mg/g of bw) of liver while administration of aqueous extract reduces significantly the liver weight (0.032 mg/g bw) towards normal. The treatment of diabetic rats with the hydro-ethanol extract causes a significant increase of the liver relative mass thus suggesting a liver injury.

3.1.2.6 Effect of extracts on blood biochemical parameters

Effects of extracts on blood lipid parameters: Blood triglycerides and total cholesterol of rats group are shown in Table 8. Diabetic rats have significant increase (Table 8) in blood triglyceride and total cholesterol levels. Treatment of diabetic rats with daonil significantly decreased blood triglyceride (0.97g/l) and total cholesterol levels (0.35 g/l). The influence of extracts in reducing triglyceridemia or cholesterolemia depends upon the concentration of extracts. The dose 50 mg/kg bw hydro-ethanol extract with the lowest values, significantly reduce the blood triglycerides (0.79 g/l) and total cholesterol (0.36 g/l) levels. It is followed by 50 mg/kg bw aqueous extract (0.89 and 0.41 g/l respectively), 100 mg/kg bw aqueous extract (1.22 and 0.43 g/l respectively) and hydro-ethanol extract (1.27 and 0.47 respectively) at a dose of 100 mg/kg bw.

Effect of extracts on creatinine and transaminases AST and ALT

Renal dysfunction parameters: Creatinemia: Creatinine concentrations after sacrifice of rats are shown in Table 9. Healthy controls rats have blood creatinine levels (6.7 mg/dl) significantly low. Diabetes induction with alloxan significantly increases to 6 times the blood creatinine level (40.05 mg/dl) and the treatment of diabetic rats with daonil significantly decreases it close to the normal (6.7 mg/dl). Treatment of diabetic rats with 50 mg/kg bw aqueous or hydro-ethanol extract has similar decreasing effect as Daonil. The creatinine blood level decreases to 5.3 (7.5 mg/dl) and 6 times (6.6 mg/dl) respectively. At a dose of 100 mg /kg of bw, aqueous and hydro-ethanol extract decreases also the blood creatinine level, but in a lesser extend (4 and 2 times respectively) compared to the chemical molecule or 50mg /kg of bw extracts. In all cases, the blood creatinine level of rat receiving 100 mg/kg of bw hydro-ethanol extract is the highest among the intervention groups (23 mg/dl).

Hepatic dysfunction parameters: AST and ALT: AST and ALT transaminases levels after sacrifice of rats are shown in Table 4. Healthy controls rats have AST and ALT blood levels (18.13 and 19.75 IU/l, respectively) significantly low. Diabetes induction with alloxan significantly increases to four times the AST and ALT blood level (69.1 and 82.78 IU/l, respectively). Treatment of diabetic rats with daonil significantly reduced to 2.5 times the levels of AST and ALT (28.00 and 34.33 IU/l respectively). Extend of AST level reduction is more significant when diabetic rats are treated with 50 mg/kg bw aqueous (4.4 times) or hydro-ethanol (4.8 times) extract. Treatment of diabetic rats with 50 mg/kg bw hydro-ethanol extract has similar blood ALT decreasing effect as Daonil contrary to aqueous extract which reduced more (3.6 times) the blood ALT level of diabetic rats. Administration of 100 mg/kg bw aqueous extracts to diabetic rats decreases in similar manner as Daonil the blood AST level. This decreasing effect is even more significant for blood ALT level. Treatment of diabetic rats with 100 mg/kg bw hydro-ethanol extracts significantly increased the AST (138.38 IU/l) and ALT (152.35 IU/l) blood levels. These values are twice as high as the levels of diabetic rats. The AST/ALT ratios are less than 1 except for rat receiving 100 mg/kg bw aqueous extracts which is above 1 (1.22).The effect of extract on blood glucose and lipids depends upon the concentration and the nature of the extract. Analysis of variability and classification of extract according to their glucose and lipids lowering effect and their blood creatinine and transaminases level pattern and organ relative weight/mass ratio was done using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). Representation of variables and rat groups samples according to the first two principal components F_1 and F_2 which explain 68.29 % of variance are shown in Fig. 1. The first Principal Components (PCs) were able to explain 38.49 % of the total variance. Renal and hepatic dysfunction parameters (creatinine, AST, ALT), lung and liver relative weight/mass ratio are well correlated on this axis. The second axis F_2 which explain 29.81% of the total variance is highly correlated with heart relative weight/mass ratio, glycemia and lipids parameters (triglyceridemia and total cholesterolemia). Spatial distribution of samples well differentiates healthy control rats (on the left hand of F_1 axis) from diabetic control rats (on the positive hand of F_2 axis) and diabetic control rats treated with 100 mg/kg bw hydro-ethanol extract of *C. colocynthis* (on the right hand of F_1 axis). Healthy control rats are characterised by low blood creatinine, AST and ALT level as well as

lung and liver relative weight/mass ratio. Diabetic control rats have high blood glucose, triglycerides and cholesterol level. High blood creatinine, AST and ALT level as well as lung and liver relative weight/mass ratio characterised diabetic control rats treated with 100 mg/kg bw hydro-ethanol extract of *C. colocynthis*. Cluster dendogram which group samples into three classes according to the degree of similarity in blood biochemical parameters and relative weight/mass ratio are shown in Fig. 2. Diabetic rats treated with glibenclamide, aqueous extract (50 and 100mg/kg bw), hydro-ethanol extract (50mg/kg bw) with a similar profile as healthy control rats (HRC) form a single class characterise by low blood creatinine, AST and ALT level as well as lung and liver relative weight/mass ratio. Their biochemical parameters and organ relative weight/mass ratio profiles are different to that of diabetics rats treated with hydro-ethanol extract at a concentration of 100 mg/kg body weight which form another class. Diabetic's untreated rats with high blood glucose, triglycerides and cholesterol level form the last class. This result shows the effectiveness of daonil and fruit extracts of *C. colocynthis* in reducing blood glucose level close to the healthy control rats.

3.2 Discussion

Hydro-ethanol extract of *C. colocynthis* fruit contains higher quantity of terpenes and glycosides than aqueous extract. Alkaloids and saponin were not detected in any extract. This is in contradiction with the result obtained by Abdel-Hossam et al. [15] and Diwan et al. [16] who identified those compounds in aqueous extract of *C. colocynthis* fruit. Many authors attributed the anti diabetic property of *C. colocynthis* to the presence of compounds such as alkaloid, saponin and glycosidic components [15]. The hypoglycaemic effect of extracts could be related to the presence of glycosidic components since alkaloids and saponin were not detected. Sturm and Stuppner [17] identified glucosides of cucurbitacin E, I and L (a class of highly oxygenated, bitter tasting triterpenes) as the main constituents in the pulp of *C. colocynthis*. He reported that this bitter tasting triterpenes have shown to be responsible of wide range of their biological activities. Alloxan is a classic diabetogenic agent inducing a diabetic state in rats by intravenous administration. This state is characterised by an increase in blood glucose or hyperglycemia, due to its destructive action on pancreatic beta cells (severe necrotic changes) resulting in an absolute or relative deficiency of insulin production and an increase in blood glucose level [18]. Analysis of short-term fasting blood glucose data shows a hypoglycemic effect of *C. colocynthis* fruit extracts similar to daonil. The study of the hypoglycemic effect of fruit extracts during three weeks of treatment revealed that blood glucose level reduction depends upon the extraction solvent nature, dose of extracts and the duration of experimentation. At low concentrations (50 mg/kg bw) and regardless of the type of extract, blood glucose level is gradually decreased along the three weeks intervals of experimentation. High concentrations, however, cause significant drop in the blood glucose level and two weeks later, the blood glucose level is significantly low. Hypoglycemia is even recorded two weeks after administration of 100 mg/kg bw hydro-ethanol extract to alloxan diabetic rats. This sudden reduction in blood glucose level seems to be one of the reason of the early death, after 7 to 14 days of administration of rats receiving 100 mg/kg bw aqueous or hydro-ethanol extracts in comparison to those receiving low extracts concentration (50 mg/kg bw) which died later (after 14 to 21 days of study). The hypoglycemia property of *C. colocynthis* fruit extracts confirm the use of this plant in diabetes treatment as reported by several ethnobotanical and ethnopharmacological investigations [4,19,20]. The influence of the dose on anti-hyperglycaemic effect of *C. colocynthis* fruit extracts was also reported by Lamba et al. [21]. Many studies link the hypoglycemic effect of *C. colocynthis* to his action on the beta cells of the pancreatic islets (regeneration) leading to stimulation of insulin secretion [17]. It has been reported that alloxan induced diabetes by selective toxicity action on the beta cells due to its structural resemblance to glucose. Its mechanism of action is the production of free radicals which could, be deactivated by antioxidant molecules [7]. *C. colocynthis* fruit extracts contain antioxidant molecules such as phenols, terpenes and glycosides revealed by phytochemical tests and the antioxidant activity of these molecules could be responsible for their hypoglycemia properties. The high hypoglycemic activity of hydro-ethanol extracts compared to aqueous extracts could be explained by their high content in glycoside, terpenes and sterol. Indeed, Tannin-splitz et al., [22] demonstrated the antioxidant capacity of cucurbitacin glucosides present in *C. colycinthis* and their potential in the treatment of chronic degenerative diseases involving free radicals and oxidative damage. It is known that steroids are

molecules that regulate a variety of biological processes and possess diverse pharmacological properties including hypoglycemic effect [23]. Alloxan diabetic rats show weight loss compared to healthy rats. Similar results have been reported by Eman G.E. Helal et al. [24]. Treatment with the chemical molecule or extracts stabilises the body mass. Figueroa-Valverde et al. [25] reported that glibenclamide had no significant overall effect on weight gain or adiposity. Stability in body mass was recorded for Diabetic + HE (50 or 100mg/kg bw) and Diabetic + AE (100mg/kg bw) groups. Only rats receiving 50mg/kg bw of hydro-ethanol extract show a non significant increase in body mass. Stability in body mass is usually an advantage in diabetes management [26]. Alloxan diabetes - induced is associated with impaired lipid metabolism with an increase in blood lipid parameters including triglyceridemia and cholesterolemia since lipolysis in the adipose tissue is not inhibited in case of insulin deficiency and this causes hyperlipidemia. These abnormalities are one of major factor of risk of cardiovascular diseases [27]. Diabetic rats have high levels in triglycerides and total serum cholesterol compared to healthy controls rats. Similar results have been published by [28],[29],[30]. These authors stated that the high abnormal concentration of serum lipids observed in diabetic subjects is mainly due to an increase in fatty acids mobilisation from adipose tissue due to insulin deficiency or insulin resistance. Insulin deficiency results in higher cholesterolemia since insulin has a cholesterol-lowering effect by it inhibitory action on 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoA reductase), a key enzyme in the pathway of cholesterol synthesis [31] or the activation of LCAT (Lecithin-Cholesterol Acyltransferase), enzyme of its degradation. Glibenclamide significantly reduces triglyceridemia or cholesterolemia. Indeed, many studies reported that glibenclamide reduces the level of triglycerides and blood cholesterol by normalising the secretion of insulin [32]. The normalisation of the lipid profile of diabetic rats treated with extracts is due to their stimulating effect on the secretion of insulin from pancreatic beta cells [32]. It may also be due to the presence of hypolipidaemic constituents in *C. colocynthis* fruit as reported by Daradka et al. [9]. The influence of the extracts in reducing blood triglycerides or total cholesterol levels depends upon the concentration. Administration of 50 mg/kg bw extracts (aqueous or hydro-ethanol) significantly reduce triglyceridemia or cholesterolemia compare to glibenclamide or both extracts at high concentration (100 mg/kg bw). Cholesterol and triglycerides -lowering effect is an advantage in diabetes treatment. The possible mechanism of action of lipids alteration might be cholestatic effect of citrillus colocynthis in liver, enhanced removal or catabolism of lipoproteins and/or inhibition of lysosomal lipids hydrolytic enzymes secreted by the liver [9]. Alloxan induced diabetes causes an increase in relative liver weight/mass ratio (mg/g body weight). Similar results have been reported by Lucchesi et al. [33]. Oryan et al. [18] reported a degenerative changes in the hepatocytes in a histological studies of liver of alloxan induced diabetic rats. The reverse effect of extracts in lowering liver weight depends upon the dose. Administration of 50 mg/kg bw extract significantly decrease (*P=.01*) the liver weight to normal compared to higher concentration (100 mg/kg bw) which significantly increased the liver weight (*P=.01*). Serum transaminases AST and ALT are markers of liver and cell damage. Alloxan diabetes –induced rats have significantly (*P=.000*) high blood AST and ALT levels suggesting hepatocyte or cell damage due to insulin resistance which induces excessive synthesis of free fatty acids which are directly toxic to hepatocytes. Similar results have been reported by Lucchesi et al., [33]. Kim and Ha [34] reported that alloxan diabetes induced generally causes a significant increase in AST and ALT activity because diabetes alters the liver's normal ability to synthesise glycogen. The reverse effect of *C. colocynthis* fruit extracts in lowering AST and ALT blood levels is due to these enzymes activities inhibition. However, the extent depends upon the nature of the extract and concentration. Administration of 50 mg/kg bw extracts (aqueous or hydro-ethanol) and 100 mg/kg bw aqueous extract reduce significantly (*P=.000*) AST and ALT blood levels in contrary to 100 mg/kg bw hydro-ethanol extract which significantly increase (*P=.000*) the level of transaminases suggesting by this liver toxicity effect of this extract at high doses. This result confirms the suspected toxicity noticeable by the significant increase (*P=.01*) in liver weight/mass ratio of these rats. Several authors such as Oryan et al. [18] reported an influence of the dose on toxic effects of *C. colocynthis* at higher concentration. Azzi Rachid et al. [4] reported that toxicity was due to the presence of toxic components like saponine and alkaloids that induce toxicity according with the dose. Administration of *C. colocynthis* fruits extract at high concentration (100 mg/kg bw) significantly increases (*P=.01*) the relative liver

weights of the rats. The relative weight /mass ratio of the rats treated with aqueous or hydro-ethanol extracts at the concentration of 100 mg/kg bw were significantly increased (*P=.01*) but AST and ALT levels were significantly low (*P=.000*) in rats treated with 100 mg/kg aqueous extract. Neither saponin nor alkaloids were detected in extracts. If the hydro-ethanol extract exhibits hepatic toxicity unlike the aqueous extract at the same concentration, one could think about the high lipid and sterol content of hydro-ethanol extract. Some authors reported toxicity effect of sterol at high dose [35]. Indeed, feeding rats during two weeks with the hydro-ethanol extract (100mg/kg bw), put them under hyperlipidaemic and hypersterolemia diet. This excess of lipid and sterol intake along with hyperlipidaemic induced by the diabetic state could have an impact on the hepatocytes functioning despite of the normalisation of insulin secretion. The ratio of AST/ALT is of little benefit in sorting out the cause of liver injury except in acute alcoholic hepatitis, in which the ratio is usually greater than 1 [36]. The rats with elevated AST/ALT ration received 100 mg/kg bw aqueous but not alcoholic extract and further investigation may be carry out to understand this elevated ratio. The nephrotoxic effect of alloxan is well recognised. Alloxan-induced diabetic rats have significantly elevated (*P=.000*) creatinine levels compared to normal. Creatinine is a biochemical marker of the state of renal function [37]. The high creatinine level in diabetic rats is a result of renal dysfunction. Treatment of rats with glibenclamide or extracts significantly (*P=.000*) improves serum creatinine clearance by the kidneys. However, the extent depends upon the concentration of extracts. Diabetic rats treated with 50 mg/kg bw extracts have significantly lower (*P=.000*) creatinine levels compared to rats treated with 100 mg/kg bw extracts which have significantly elevated (*P=.000*) creatinine levels. This level is even higher when the rats are treated with 100 mg/kg bw hydro-ethanol extract. Although the relative kidneys weight of healthy control rats, diabetic or intervention group does not differ significantly (*P=.09*), this result reports a nephrotoxic effect of hydro-ethanol extracts at the concentration of 100 mg /kg bw. The administration of glibenclamide (3mg/kg), 50 and 100 mg/kg bw aqueous extracts and 50 mg/kg bw hydro ethanol extracts significantly (P<.05) reversed the damage associated with Alloxan induced diabetes close to the normal; revealing the hypoglycemic and hypolipidemic effects of *C. colocynthis* fruit at these doses. From the results obtained, it can be concluded that the dose 50 and 100 mg/kg bw aqueous extracts and 50 mg/kg bw hydro ethanol extracts are effective in diabetes healing and 100 mg/kg bw hydro ethanol extracts should be avoided because of it toxic effects.

4. CONCLUSION

Glibenclamide and *C. colocynthis* fruit extracts has short and long term blood glucose lowering effect depending of extraction solvent nature, dose of extracts and duration of treatment. These extracts significantly reduce lipidemia and creatinine levels. The administration of glibenclamide (3mg/kg), 50 and 100 mg/kg bw aqueous extracts and 50 mg/kg bw hydro ethanol extracts significantly reversed the damage associated with Alloxan diabetes induced close to the normal. The dose 100 mg/kg bw hydro ethanol extracts should be avoided because of it toxic effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors. The experiments were performed in accordance with the EEC directive of 1986; 86/609/EEC adopted by the Institutional Ethics Committee of the Cameroon Ministry of Scientific Research and Technology Innovation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pastakia SD, Pekny CR, Manyara SM, Fischer L. Diabetes in sub-Saharan Africa – from policy to practice to progress: targeting the existing gaps for future care for diabetes. Diabetes, Metabolic Syndrome and Obesity. 2017;10:247–263. DOI:10.2147/DMSO.S126314
2. Mutyambizi C, Pavlova M, Chola L, Hongoro C, Groot W. Cost of diabetes mellitus in Africa: A systematic review of existing literature. Globalization and Health. 2018;14(3):1-13.

DOI:10.1186/s12992-017-0318-5
3. Farzaei MH, Rahimi R, Farzaei F, Abdollahi M. Traditional medicinal herbs for the management of diabetes and its complications: An evidence-based review. International Journal of Pharmacology. 2015;11:874-887.
4. Azzi Rachid, Lahfa Farid Boucif, Mezouar Dounia, Benmehdi Houcine, Djaziri Rabah. Acute toxicity, hypoglycemic and antihyperglycemic effect of ethanolic extract of *Citrullus colocynthis* L. seeds in normal and streptozotocin-induced diabetic rats. 2nd Int'l Conference on Advances in Environment, Agriculture & Medical Sciences (ICAEAM'15), June 11-12, 2015, Antalya -Turkey
5. Kim MJ, Ha BJ. Antihyperglycemic and antihyperlipidemic effects of fermented *Rhynchosia nulubilis* in Alloxan-induced diabetic rats. Toxicol. Res. 2013;29(1):15-19
6. Gurudeeban S, Satyavani K, Ramanathan T. Bitter apple (*Citrullus colocynthis*): An overview of chemical composition and biomedical potentials. Asian Journal of Plant Sciences. 2010;9:394-401.
7. Dashti N, Zamani M, Mahdavi R, Ostad Rahimi A. The effect of citrullus colocynthis on blood glucose profile level in diabetic rabbits. Journal of Jahrom University of Medical Sciences. 2012;9(4):24-28.
8. Singh M, Jha A, Kumar A, Hettiarachchy N, Rai AK, Sharma D. Influence of the solvents on the extraction of major phenolic compounds (punicalagin, ellagic acid and gallic acid) and their antioxidant activities in pomegranate aril. J Food Sci Technol. 2014;51(9):2070–2077.
9. Daradka H, Almasad MM, Qazan VSh, El-Banna NM, Samara OH. Hypolipidaemic effect of *Citrullis colocynthis* L. in Rabbits. Pakistan Journal of Biological Sciences. 2007;10(16): 2768-2771.
10. Evans WC. Trease and evans' pharmacognosy. 16th ed. London: Sauders Elsevier; 2009.
11. AOAC. Official methods of analysis of AOAC International, 17th Edition. Association of Official Analytical Chemists. 2000.
12. Bourely J. Observation sur le dosage de l'huile des graines de cotonniers. Coton et Fibres Tropicales. 1982;37(2):183-196. French.
13. Ananthan R, Latha M, Ramkumar K, Pari L, Baskar C, Bai V. Effect of Gymnemamontanum leaves on serum and tissue lipids in alloxan diabetic rats. Experimental Diabetes Research. 2003; 4:183–189.
14. Nipankar SU, Chitalange SS, Nagore D. Evaluation of anti-diabetic activity of AHPL/AYTAB/0513 tablet in streptozotocin induced diabetes in rats. Int J pharm Investig. 2017;7(1):25-32.
15. Abdel-Hassan I, Abdel-Barry J, Mohammeda S. The hypoglycaemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. J. Ethnopharmacol. 2000;71:325-330.
16. Diwan FH, Abdel-Hassan IA, Mohammed ST. Effect of saponin on mortality and histopathological changes in mice. East Mediterr Health J. 2000;6:345-351.
17. Sturm S, Stuppner H. Analysis of cucurbitacins in medicinal plants by high-pressure liquid chromatography– Mass spectrometry. Phytochem. Anal. 2000;11: 121–127.
18. Oryan A, Hashemnia M, Hamidi AR, Mohammadalipour A. Effects of hydro-ethanol extract of *Citrullus colocynthis* on blood glucose levels and pathology of organs in alloxan-induced diabetic rats. Asian Pac J Trop Dis. 2014;4(2):125-130.
19. Allali H, Benmehdi H, Dib MA, Tabti B, Ghalem S, Benabadji N. Phytotherapy of diabetes in west Algeria. Asian Journal of Chemistry. 2008;20(4):2701-2710.
20. Adam Sakine MN, Mahmout Y, Gbenou J, Agbodjogbe W, Moudachirou M. Effet antihyperglycémiant des extraits de *Boscia senegalensis* (Pers.) Lam. ex Poiret et de *Colocynthis vulgaris* (L.) Schrad, Phytothérapie. 2011;9:268-273 French.
21. Lamba S, Buch K, Lewis H, Lamba H. Phytochemicals as potential hypoglycemic agents. Studies in Natural Products Chemistry. 2000;21:457- 496.
22. Tannin-splitz T, Bergman M, Grossman S. *Curcubitacins glucosides*: Antioxidant and free-radical scavenging activity. Biochemical and Biophysical Research Communication. 2007;364:181-186.
23. Juárez-Rojopa IE, Tovilla-Zárateb CA, Aguilar-Domíngueza DE, Roa-de la Fuentec LF, Lobato-Garcíac CE, Blé-Castilloa JL, et al. Phytochemical screening and hypoglycemic activity of Carica papaya leaf in streptozotocin-induced diabetic rats. Rev Bras Farmacogn. 2014;24:341-347.

24. Eman G.E. Helal, Noran Abou- Aouf, ALsayeda Mohammed Khattab, Mohammad Abulhasan Zoair. Anti-diabetic effect of Artemisia Annua (Kaysom) in alloxan-induced diabetic rats. The Egyptian Journal of Hospital Medicine. 2014;57:422-430.
25. Figueroa-Valverdea L, Diaz-Cedillob F, Lopez-Ramosa M, Garcia-Cerveraa E, Pool-Gomeza E, Cardena-Arredondoc C, et al. Glibenclamide-pregnenolone derivative has greater hypoglycemic effects and biodistribution than glibenclamide-OH in alloxan-rats. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2012;156(2):122–127.
26. Weitgasser R, Lechleitner M, Luger A, Klingler A. Effects of glimepiride on HbA1c and body weight in Type 2 diabetes: Results of a 1.5-year follow-up study. Diabetes research and Clinical Practice. 2003;61.
27. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. Endocrinol Metab Clin North Am. 2010;39(3):481–497. DOI:10.1016/j.ecl.2010.05.011.
28. Eddouks M, Lemhadri A, Michel JB. Hypolipidemic activity of aqueous extract of *Capparis spinosa* L. in normal and diabetic rats. Journal of Ethnopharmacology. 2005;98 (3):345-350. DOI:https://doi.org/10.1016/j.jep.2005.01.053
29. Ravi K, Rajasekaran S, Subramanian S. Antihyperlipidemia effect of Eugenia Jambolana seed kernel on streptozotocin induced diabetes in rats. Food Chem. Toxicol. 2005;43:1433-1439.
30. Sharma M, Fernandes J, Ahirwar D, Jain R. Hypoglycemic and hypolipidimic activity of alcoholic extract of *Citrus aurantium* in normal and alloxan-induced diabetics rats. Pharmacologyonline. 2008;3:161-171.
31. Harris IR., Hoppner H, Sieften W, Farrell AM, Witter KP. Regulation of HMG-COA synthetase and HMG-COA reductase by insulin and Epidermal Growth Factor in HaCaT keratinocytes. Journal of Investigative Dermatology. 2000;114(1): 83-87.
32. Hossein A, Reza H, Vahid N, Minoo I. Effects of aqueous extract of berberis integerrima root on some physiological parameters in streptozotocin-induced diabetic rats. Iranian Journal of Pharmaceutical Research. 2013;12(2): 425-434
33. Lucchesi AN, Cassettari LL, Spadella CT. Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans. Journal of Diabetes Research. 2015;11. DOI:http://dx.doi.org/10.1155/2015/494578
34. Kim MJ, Ha BJ. Antihyperglycemic and Antihyperlipidemic Effects of Fermented Rhynchosia nulubilis in Alloxan-induced Diabetic Rats. Toxicol. Res. 2013;29(1):15-19
35. Radhakrishnan A, Rajaratnam MD, Helena Gylling, MD, Tatu A, Miettinen MD. Independent association of serum squalene and noncholesterol sterols with coronary artery disease in postmenopausal women. Journal of the American College of Cardiology. 2000;35(5):1185-1191.
36. Murali AR, Carey WD. Liver test interpretation - Approach to the patient with liver disease: A Guide to Commonly Used Liver Tests. Disease management. Center for clinic education. Cleveland clinic; 2017. Accessed 23 October 2018 Available:http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/hepatology/guide-to-common-liver-tests/
37. Amartey NA, Nsiah K, Mensah FO. Plasma levels of uric acid, urea and creatinine in diabetics who visit the clinical analysis laboratory (Can-lab) at Kwame Nkrumah University of science and technology, Kumasi, Ghana. J Clin Diagn Res. 2015;9: 5-9.

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