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***Conyza aegyptiaca* (L.) Dryand ex. Aiton effect on blood
glucose levels in normal albinos rats through oral
glucose tolerance test**

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Abstract

Conyza aegyptiaca (L.) Dryand ex. Aiton (*C. aegyptiaca*) is used in West Africa for diabetes treatment without any scientific study and adequate therapeutic indications. Our previous study shows that *C. aegyptiaca* extract is nontoxic on larvae and prevent hepatic glucose liberation in rat. In the present study, we evaluated the acute oral toxicity of *C. aegyptiaca* aqueous and ethanolic extracts at a unique dose of 300 and 2000 mg/kg body weight (bw). We also determined its effect on blood glucose level by the Oral Glucose Tolerance Test (OGTT) on albinos Wistar rats. Acute oral toxicity showed no mortality and no toxic effect throughout the study. There were no significant changes in glycaemia, creatinine, lipids, AST, ALT and ions Ca²⁺, Cl⁻, Na⁺, K⁺, and Mg²⁺ levels when compared to control. OGTT showed that only the aqueous extract at the dose of 500 mg/kg bw normalized blood glucose level 75 min after OGTT induction compared to 60 min for Glybenclamide. Results suggest that *C. aegyptiaca* aerial part extract is safe and possesses anti-hyperglycemia potential. Further studies are needed to isolate and characterize the plant active component and to test it for diabetes treatment.

Keywords: *Conyza aegyptiaca*, acute toxicity, OGTT, biochemical parameters.

Introduction

The Oral Glucose Tolerance Test (OGTT) measures the body ability to use glucose that is the main body source of energy. OGTT has been the mainstay for diagnosing diabetes. It detects more efficiently early diabetes as

well as subjects with impaired glucose tolerance (Bartoli et al., 2011), since fasting hyperglycemia is too late a criterion for the early diagnostic of type 2 diabetes.

Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus (Anitha et al., 2012). Several medicinal plants have been found to be successfully in diabetes treatment and some of them have been tested and their active ingredients isolated (Grover et al., 2002; Shu, 1998). This has led researchers to intensity research of suitable treatment from plant origin for diabetes care.

Conyza aegyptiaca (L.) Dryand ex. Aiton, belonging to the Asteraceae family, is a well known plant used by the population of Togo and Benin (West Africa). Also called Ahlonme in Ewe (local dialect), *Conyza aegyptiaca* (*C. aegyptiaca*) is widely used to overcome malaria, sickle cell disease, sore throat (Akpagana et al., 1996). Recently, an ethnobotanic study found that this plant is also used for obesity and diabetes treatment by West African populations (Gbekley et al., 2015).

The antidiabetic effect attributed to medicinal plants is generally reflected in their efficacy on inhibition of intestinal absorption, stimulation of insulin secretion by the pancreas, inhibition of glucose production by the liver or stimulation of the storage of blood glucose by the liver and muscles (Hanan et al., 2014). Many plant extracts have been reported to have the properties to stimulate or regenerate cell for insulin secretion and are most effective for controlling diabetes by various mechanisms which may finally lead to improvement of carbohydrate metabolizing enzymes towards the reestablishment of normal blood glucose level (Ali et al., 2012). In previous study, we showed that *C. aegyptiaca* extracts prevent liver glucose liberation *in vitro* (Akakpo et al., 2016), thus favouring glucose stocking in liver. Here, we used OGTT test to evaluate the effect of *C. aegyptiaca* on blood glucose level *in vivo*.

Material and Methods

Plant material

The plant material was composed of the aerial parts of *C. aegyptiaca* bought at Palimè (Republic of Togo). The leaves and stem of the plant were shade-dried and ground into a fine powder. The powder was then extracted with water and ethanol.

Extraction

For the aqueous extraction, decoction was done on a heating plate during 30 minutes by addition of 1000 ml distilled water to 100g of powder. The mixture was filtered with Whitman paper and evaporated under rota vapor at 65-70°C. The ethanolic extraction was done by macerating 100 g of powder in ethanol diluted at 50°C with distilled water, during 24 hours mixed by homogenizer. After the filtration with Whitman paper, the product was evaporated with the rota vapor at 40-45°C. Extracts were conserved at 4°C.

Animal material

Male and female albinos Wistar rats weighting 150 to 200 g at the age of six to eight weeks were used for the study. Animals were housed in polypropylene cages and maintained under standard conditions with an alternated cycle of twelve hours light and twelve hours dark. They had free access to food and water. Room temperature was maintained at 25°C with a relative humidity of 35-60%.

Acute toxicity test

Acute oral toxicity test was carried out according to the Organization for Economic Cooperation and Development (OECD) guidelines for Testing of Chemicals, number 423 (OECD, 2002). A total of 25 rats of 10 females and 15 males were used. The animals were divided into 5 groups of 2 females and 3 males each. Groups #1 and #2 received a single oral-dose of 300 and 2000 mg/kg body weight (bw) of *C. aegyptiaca* aqueous extracts respectively, groups #3 and #4 received a single oral-dose of 300 and 2000 mg/kg bw of *C. aegyptiaca* ethanolic extracts respectively and group #5 (control group) only distilled water at 10 ml/kg bw. Animals were kept fasting overnight prior to oral extracts gavages. Signs of toxicity and mortality were observed after the administration at the first, second, third and fourth hour and once daily for 14 days.

Physical parameters

Clinical observations (mortality, morbidity, healthy or reaction to treatment, behavior pattern, tremors, salivation, diarrhea, sleep disorder and coma) were made once a day.

Body weight

The body weights (bw) of each rat were measured three times a week. The differences of the bw were recorded.

Blood sample collection

Venous blood samples were collected from all rats after overnight fasting. Blood samples were collected on day 0 and day 14 from eye vein in a collection tube without anticoagulant (Vacutainer System; Becton Dickinson). They were properly labeled and placed directly on a rack into a cool-box containing icepacks. The blood was centrifuged within 30 to 60 min after sampling and the serum was aliquoted into 1.5 ml Eppendorf tubes and stored frozen at -70°C until laboratory analysis.

Measurement of Biochemical Parameters

Serum glucose was measured by Glucose Oxidase and Peroxidase (GOD-POD) method (ELITech Group, Puteaux, France) according to the manufacturer's

instructions. Aspartate amino transferase (AST) and alanine amino transferase (ALT) levels were measured using an automated blood analyser Hitachi 705 (Hitachi, Japan), with DiaSys (Diagnostic Systems GmbH, Germany) reagents. Serum levels of total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), creatinine (Creat.), potassium (K^+), chloride (Cl^-), sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) were measured using Elitech reagents (ELITech Group, Maizy, France).

Oral Glucose Tolerance Test

The standard method of Oral Glucose Tolerance Test (OGTT) for normal rats (Pareek et al., 2009) with some modifications (Sakine et al., 2011) was used to assess the effect of *C. aegyptiaca* extracts on glycaemia control alteration. The test was carried out in overnight fasted normal rats, which were equally divided into 7 groups of 5 rats (2 females and 3 males). All the rats, aged between 8 and 10 weeks and weighing 200g on average, were orally fed with extracts and drug using a gavage tube. The aqueous and the ethanolic extracts were administrated at the dose of 500 mg/kg bw (group #1 and group #3) and 1000 mg/kg bw (group #2 and group #4). Group #5 received drug Glibenclamide at a dose of 10 mg/kg bw, group #6 a solution of Dimethyl sulfoxide (DMSO) at 10% and group #7 (control) only a distilled water at 10 ml/kg bw. Fifteen (15) minutes after the last dose of the drug, all the rats were submitted to the OGTT. The capillary blood glucose levels were measured in all groups just before extracts and Glibenclamide administration (-15 min) and 15 min later (0 min). After this, all the rats of the groups 1-7 were given glucose suspension (3 g/kg bw) orally using gavage tube. Blood samples were collected from tail vein and glucose level was measured at 30, 60, 90, 120, 180 and 240 min after glucose administration with glucometer Diavue (BioCARE Corporation, Taoyuan city, Taiwan).

Statistical analysis

Data obtained from the experiments were expressed as mean \pm standard error of the mean (SEM) and evaluated by Student's t-test using the SigmaPlot statistical analysis software (Systat Software, Inc. San Jose, CA, USA). A level of $p < 0.05$ was set as significant.

Results

Acute toxicity

Physical parameters

No toxic effect was observed throughout the 14 days study period. During the first 30 min of gavages, some rats in the groups treated with the extracts exhibited fatigue signs and lacked appetite. These signs disappeared before the end of the first hour after gavages. Physical observation showed no signs of toxic effect such as changes on behavior pattern, tremors, salivation, diarrhea, sleep and coma in any group of rats throughout the study period. No mortality was observed in any group of the rats.

Body weight

The body weight of control and treated rats were shown in Table 1. There were no significant changes in body weight of control and treated rats. No significant difference was seen in the body weight of the treated rats compared to the control rats.

Oral acute toxicity test

The blood samples were taken on the day before the administration of the extracts (D0) and on the 14th day (D14). Results of the biochemical parameters obtained on D14 were compared with D0 (Table 1).

Blood glucose and creatinine levels did not vary significantly at D14 versus D0, regardless of the type of extract and dose administered. Lipid profile also showed no significant changes at D14 compared to D0 except for TC level which was significantly ($p < 0.01$) higher at D14 compared to D0 at the dose of 2000 mg/kg bw of the ethanolic extracts (Table 1). The level of the enzyme AST remained unchanged regardless of the dose and the type of the extract on D14 compared to the control. In contrast, ALAT significantly increased ($p < 0.01$) on D14 versus D0 in the group of rats receiving the ethanolic extract at the dose of 300 mg/kg bw. The level of ALAT did not change significantly in the other groups of rats compared to control. The levels of potassium, chloride, sodium, calcium and magnesium did not vary significantly during the experiment, whatever the dose and type of extract used, compared to the control group.

Table 1: Serum biochemical parameters in rats treated for 14 days with *C. aegyptiaca* extracts

Extract dose Parameter	Control	Aq. Ext. 300 mg/kg	Eth. Ext. 300 mg/kg	Aq. Ext. 2000 mg/kg	Eth. Ext. 2000 mg/kg
Weight (g)	201.00 ± 9.54	192.00 ± 5.51	186.33 ± 7.69	195.67 ± 6.96	188.33 ± 4.17
Glucose (g/l)	0.76 ± 0.10	0.85 ± 0.02	0.83 ± 0.23	0.84 ± 3.41	0.82 ± 0.03
Creatinine (mg/l)	5.33 ± 0.10	6.00 ± 0.00	6.67 ± 0.43	6.33 ± 3.03	5.67 ± 0.10
TC (g/l)	3.17 ± 0.02	3.10 ± 0.06	3.16 ± 0.05	3.26 ± 10.6	3.35 ± 0.03
TG (g/l)	1.83 ± 0.10	1.59 ± 0.05	1.66 ± 0.02	1.61 ± 30.63	1.68 ± 0.03
HDL-C (g/l)	0.78 ± 0.31	0.84 ± 0.21	0.77 ± 0.46	0.85 ± 6.31	0.86 ± 0.26
LDL-C (g/l)	2.05 ± 0.08	2.14 ± 0.11	2.15 ± 0.46	2.41 ± 2.82	2.04 ± 0.15
ASAT (UI/L)	168,33 ± 0,03	158,67 ± 0,04	148,00 ± 0,21	176,67 ± 2,70	159,00 ± 0,29
ALAT (UI/L)	34,00 ± 0,16	36,67 ± 0,16	38,33 ± 0,22 ⁺	33,00 ± 2,24	34,00 ± 0,22
K ⁺ (mmol/L)	3,80 ± 0,12	3,73 ± 0,08	3,86 ± 0,03	4,10 ± 0,37	3,80 ± 0,05
Cl ⁻ (mmol/l)	99.67 ± 4.02	91.67 ± 3.08	99.33 ± 5.03	99.67 ± 6.24	96.00 ± 7.03
Na ⁺ (mmol/l)	138.33 ± 10.01	139.00 ± 7.01	138.67 ± 9.01	136.67 ± 12.51	141.00 ± 8.02
Ca ²⁺ (mmol/l)	88.31 ± 5.07	91.75 ± 3.01	90.26 ± 7.09	88.57 ± 4.81	86.87 ± 9.08
Mg ²⁺ (mmol/l)	16.69 ± 2.11	20.97 ± 1.27	18.23 ± 3.23	21.38 ± 7.06	24.10 ± 5.07

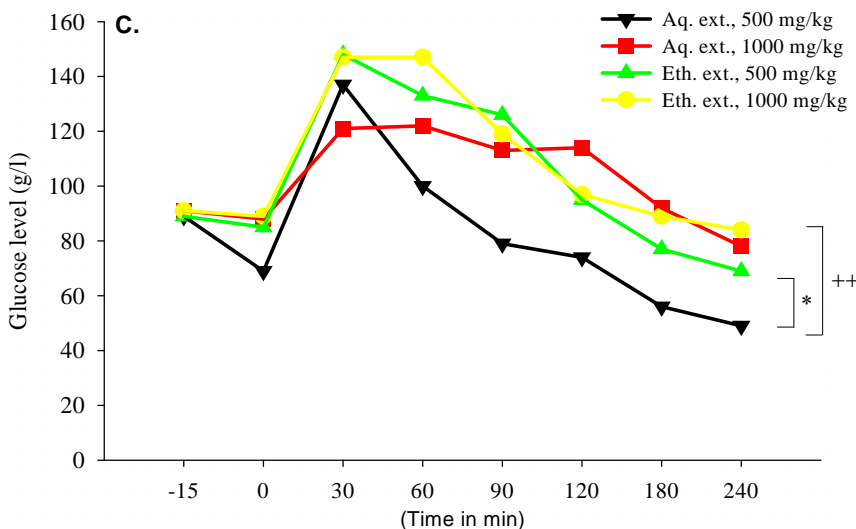
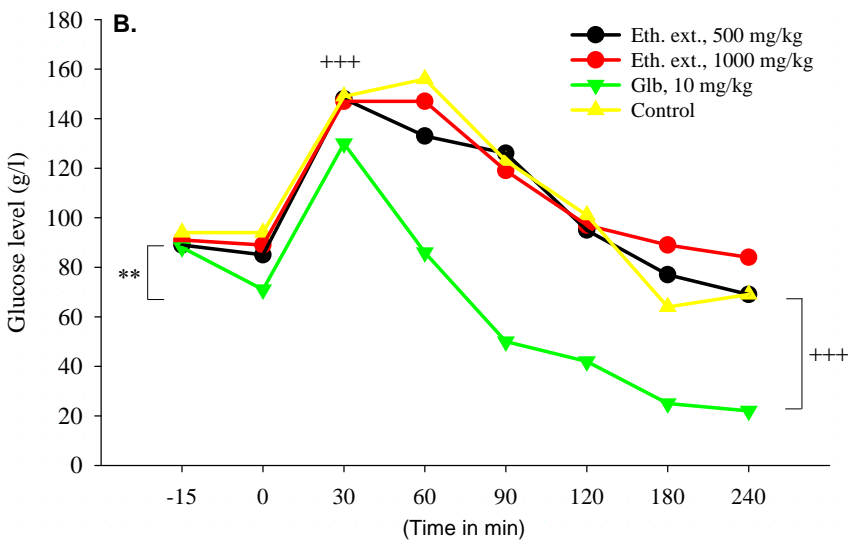
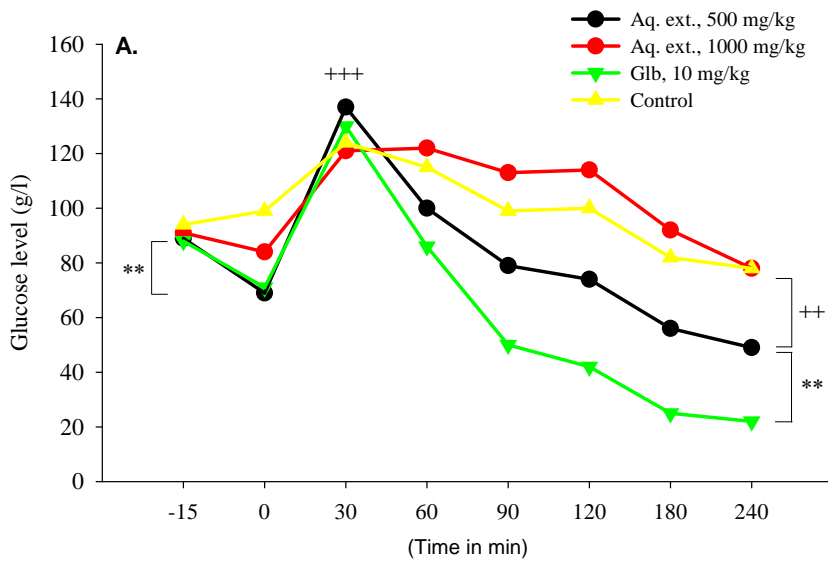
Values are mean ± SEM of 5 independent experiences (n = 5)

Aq. Ext.: Aqueous extract; Eth. Ext.: Ethanolic extract; ASAT: Aspartate Aminotransferase; ALAT: Alanine Aminotransférase; TC: Total cholesterol; TG: Triglycerides; HDL-cholesterol (HDL-C); LDL-cholesterol (LDL-C). (+p<0.01: D14 compared to D0).

Oral glucose tolerance test (OGTT)

Glucose level decreased significantly (p<0.005) in rats 15 min after administration of aqueous extract at the dose of 500 mg/kg bw (fig. 1A) and Glybenclamide (fig. 1A, 1B) compared to non treated rats. Thirty (30) min after OGTT induction, the level of glucose increased significantly (p<0.001) in all animal groups compared to its initial level. Glucose time course variation showed gradual decrease over time in rats receiving Glybenclamide (10 mg/kg bw) and *C. aegyptiaca* aqueous extract (500 mg/kg bw) (fig. 1). Glybenclamide induced significant hypoglycemia 90 min post OGTT induction while *C. aegyptiaca* aqueous extract (500 mg/kg bw) did not show any such behaviours (fig. 1A). Glucose minimum level was reached at 240 min after OGTT induction (fig. 1A). The level of glucose was significantly (p<0.005) lower in

Glybenclamide (10 mg/kg bw) than in aqueous extract (500 mg/kg bw) (fig. 1A). At 240 min post OGTT test, glucose level was similar in rats receiving *C. aegyptiaca* aqueous extract at the dose of 1000 mg/kg and in control groups (fig. 1A). But that level of glucose was significantly (p<0.001) higher compared to the group of rats that received Aqueous extract (500 mg/kg bw) and Glybenclamide (fig 1A). Glucose level was similar in rats receiving *C. aegyptiaca* ethanolic extracts at the dose of 500 mg/kg bw, 1000 mg/kg bw and in non treated rats (control) (fig. 1B). Glucose level decreased significantly (p<0.05) faster in rat treated with aqueous extract at the dose of 500 mg/kg bw compared to ethanolic extracts at the same concentration (fig. 1C). *C. aegyptiaca* aqueous extract and ethanolic extract at the dose of 1000 mg/kg bw showed similar result to that in non treated rats (control) (fig. 1C).



Oral Glucose Tolerance Test (OGTT)

Thirty five (35) rats, aged between 8 and 10 weeks and weighing 200g on average, are divided into seven (7) groups of five rats each. Blood glucose was determined by glucometer in each rat at the beginning of the experiment after overnight fasting. Products were administrated to the rats by oral gavages. Group of control received only vehicle, distilled water for aqueous extracts (Aq. ext.) (A) and DMSO at 10% for ethanolic extracts (Eth. Ext.) (B). The other groups of rats received respectively Aq. Ext. of *C. aegyptiaca* at 500 mg/kg body weight (bw), Aq. ext. of *C. aegyptiaca* at 1000 mg/kg bw (A), Eth. ext. of *C. aegyptiaca* at 500 mg/kg body weight (bw), Eth. ext. of *C. aegyptiaca* at 1000 mg/kg bw, Glibenclamide (Glb) 10 mg/kg bw (B). Following 15 min post extract administration, all the animals were fed with D-glucose (3 g/kg bw) for OGTT test. Blood samples were collected from tail vein prior to dosing and then at 0, 30, 60, 90, 120, 180 and 240 min after glucose administration. Data from aqueous and ethanolic extracts were similar (C). Blood glucose was measured by Glucose Oxidase and Peroxidase (GOD-POD) method (ELITech Group, Puteaux, France). The curves are representative of 5 independent experiments. (*P<0.05; **p<0.005; +P<0.01; ++p<0.001; +++p<0.0001)

Discussion

Conyza aegyptiaca (L.) Dryand ex. Aiton is widely used to treat malaria, sickle cell disease, sore throat (Akpagana et al., 1996), obesity and diabetes (Gbekley et al., 2015) by West African populations. Medicinal plants utilization is based on long-term clinical experience and the lack of scientific validation and toxicity evaluation has attenuated their usage for diseases treatment. In order to determine the dose and toxicological effects of *C. aegyptiaca* in rats, acute toxicity study was conducted according to OECD guidelines 423 document for acute oral toxicity testing (OECD, 2002). In the present study, during acute toxicity evaluation, none of the animals died at the doses administered indicating that the LD50 of *C. aegyptiaca* was much higher than 2000 mg/kg bw. We found no significant changes in food and water consumption. Results revealed that the animals in the different treatment groups did not exhibit significant changes in the body weight when compared to control group. These finding suggests that *C. aegyptiaca* had no effect on the normal growth of rats. Thus, the aqueous and ethanolic extracts of *C. aegyptiaca* can be considered to be nontoxic at acute administration since the extracts were well tolerated and there was no observed adverse effect.

Acute exposure of the animals to *C. aegyptiaca* extracts, even at higher dosage, showed no significant changes in biochemical parameters such glycaemia, creatinine, lipid profile, AST, ALT and ions Ca^{2+} , Cl^- , Na^+ , K^+ , and Mg^{2+} levels when compared to the control animals and to pathological values (Wolford et al., 1986). The finding that biochemical parameter creatinine value was normal in rat receiving *C. aegyptiaca* suggests that the extract do not produce any sort of disturbance in the renal function, as it has been reported for various plant extracts (Yakubu et al., 2003). Then the kidney may not be affected by the extracts in terms of maintaining the electrolyte balance in the blood. Transaminases are good indices of liver and heart damage (Martin et al., 1981). The normal enzyme activity of AST and ALAT suggests that the function of the liver and the heart is not affected by the oral supplementation of extracts. *C. aegyptiaca* crude extract was reported to reduce heart amplitude and frequency activity in a study using heart perfusion method (Kpegba et al. 2011). Alterations in the concentration of lipid parameters can give useful information on lipid metabolism and predisposition of the heart to atherosclerosis and its associated coronary heart diseases (Yakubu et al., 2008). The extracts had no effect on the serum cholesterol concentration in our study. This suggests that *C. aegyptiaca* had no significant influence on cholesterol biosynthesis.

In previous study, we showed that *C. aegyptiaca* extracts prevent hepatic glucose liberation in vitro (Akakpo et al., 2016). Here, we used OGTT to show that *C. aegyptiaca* extracts accelerate blood glucose cleanup. The OGTT is a widely used test to evaluate apparent insulin release and insulin resistance in various clinical settings (Stuvoll et al., 2000). We used two different concentrations of 500 mg/kg and 1000 mg/kg bw to access the effect of both aqueous and ethanolic extracts of *C. aegyptiaca* on circulating glucose levels. The results showed that only aqueous extract at the dose of 500 mg/kg bw performed well when compared to the standard drug Glibenclamide. It displayed similar pattern of blood glucose levels variation during OGTT with the standard drug Glibenclamide. The aqueous extract at the dose of 500 mg/kg bw restored normal glucose level 75 min after OGTT induction or 45 min after the pic of blood glucose level. By comparison, Glibenclamide normalized blood glucose level 60 min post OGTT and 30 min after the higher level of blood glucose was reached. More importantly, *C. aegyptiaca* at the dose 500 mg/kg bw did not induce significant hypoglycemia in opposite to Glibenclamide which provoked hypoglycemia at 90 min post OGTT induction. Aqueous extract at the dose of 1000 mg/kg bw and ethanolic extract at the dose of 500 mg/kg bw and 1000 mg/kg bw did not show any significant difference compared to the control. In previous study, we reported that the aqueous extract of *C. aegyptiaca* at the dose of 500 mg/kg bw prevents liver glucose liberation (Akakpo et al., 2016). Results showed here supported our earlier finding and confirmed that aqueous extract at 500 mg/kg bw is the best dose and type presentation of *C. aegyptiaca* extract for its role on blood glucose control. In our previous study however, *C. aegyptiaca* ethanolic extracts performed better than the aqueous extracts in preventing liver glucose release (Akakpo et al., 2016). It is reported that medicinal plants used to treat diabetes act through mechanisms similar to conventional molecules. Such mechanism includes the inhibition of intestinal absorption, the stimulation of insulin secretion by the pancreas, inhibition glucose production by the liver or the stimulation of glucose catabolism and its storage in liver and muscles (Hanan et al., 2014; Nair et al., 2013; Gao et al., 2008). Further studies on isolation and exact mechanisms of action of specific compounds from *C. aegyptiaca* are to be carried out in the future.

Conclusion

Results present here showed that the acute toxicity study of *C. aegyptiaca* plant extracts at the dose of 300 and 2000 mg/kg bw, administered orally to albinos Wistar rats, did not caused any death or acute adverse effect on blood biochemical parameters. The OGTT study showed that *C. aegyptiaca* aqueous extract, but not ethanolic extract, reduced blood glucose to normal level without causing hypoglycemia. Thus, *C. aegyptiaca* aerial parts extract is safe and has anti-hyperglycemia potential. Further studies are needed to isolate and characterize the active component present in this plant and to test it for diabetes treatment.

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