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ORIGINAL RESEARCH

Effects of the Oral Administration of Aqueous and Methanolic Leaf Extracts of *Chenopodium ambrosioides* L. (Amaranthaceae) on Blood Glucose Levels in Wistar Rats

Félicien Mushagalusa Kasali[1–3], Justin Ntokamunda Kadima[3], Jonans Tusiimire[2], Clement Olusoji Ajayi[1,2], Amon Ganafa Agaba[4]

[1]Pharm-Bio Technology and Traditional Medicine, Mbarara University of Science and Technology, Mbarara, Uganda; [2]Department of Pharmacy, Mbarara University of Science and Technology, Mbarara, Uganda; [3]Department of Pharmacy, Official University of Bukavu, Bukavu, Democratic Republic of the Congo; [4]Department of Pharmacology and Therapeutics, Mbarara University of Science and Technology, Mbarara, Uganda

Correspondence: Félicien Mushagalusa Kasali, Pharm-Bio Technology, and Traditional Medicine, Mbarara University of Science and Technology, Mbarara, Uganda, Tel +256 750919712, Email felicienkasali@gmail.com

Background: Diabetes mellitus is a metabolic disorder that poses a major global health threat. The current diabetes mellitus uses insulin and oral hypoglycemic agents, which have limitations, including adverse effects and secondary failures. Herbal medicine is being evaluated for its role in the pharmacotherapy of diabetes. This study was aimed to assess the anti-diabetic potential and short-term toxicity level of *Chenopodium ambrosioides* collected from Bukavu in Democratic Republic of Congo.

Methods: Leaves of *C. ambrosioides* were extracted by infusion and maceration with distilled water and 95% methanol, respectively. Hypoglycemic and antihyperglycemic potentials of the aqueous and methanolic were investigated in normoglycemic and intraperitoneal glucose-loaded rats at 100, 200, and 400 mg/kg body weight. An oral acute toxicity test was carried out on healthy female Wistar rats.

Results: Acute toxicity test showed the mean lethal dose (LD_{50}) for both aqueous and methanol extracts of *C. ambrosioides* to be more than 2000 mg/kg. The group treated with glibenclamide (5 mg/kg b.w) and aqueous extract of the plant (200 mg/kg b.w) showed a significant reduction ($p< 0.0001$ and $p< 0.05$) of fasting blood glucose by 46.91% and 16.72%, respectively, compared to control and all other treatment groups. In acute conditions, a single oral administration of the aqueous and methanolic extracts lowered fasting blood glucose in rats. Any manifestation and signs of toxicity and mortality have been recorded for 14 days of observation.

Conclusion: Leaf aqueous and methanolic extracts of *C. ambrosioides* appeared safe at 2000 mg/kg. The plant demonstrated some anti-diabetic potential in rats, explaining its use as an anti-diabetic remedy locally.

Keywords: *Chenopodium ambrosioides*, anti-diabetic activity, fasting blood glucose, IGTT, LD_{50}

Introduction

The diabetic population has quadrupled globally in the past three decades, and diabetes mellitus is the ninth foremost cause of death.[1] Diabetes is a common and chronic disease, and its long-term hyperglycemia will cause damage to several organs, tissues, and systems of the body. It may lead to complications that significantly impact patients, both economically and physically.[2] In 2015, the International Diabetes Federation (IDF) stated that the global population of adults with both diabetes mellitus (DM) was projected to increase from 415 million to 642 million by 2040, with DMT accounting for 75% of cases in low- and middle-income countries.[3]

The current management of diabetes mellitus uses chemical agents, which have limitations, including adverse effects and secondary failures. Therefore, to reduce their cost, burden, and adverse effects, the focus has been shifted to medicinal plants for safe and effective use. Herbal medications appear to offer readily available means of managing metabolic disorders by minimizing the risk of side effects and sometimes potentiating the treatment outcomes of modern drugs.[4] Disinterested in the

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consistent utilization of medicinal plants and their derivatives, the difficult challenge faced is the lack of scientific obviousness for their pharmacological mechanism and safety profile in vivo and in vitro.[5] Nowadays, so many medicinal plants are being evaluated for their role in the pharmacotherapy of diabetes.[6] According to the world, ethnobotanical 800 medicinal plants prevent diabetes mellitus. Clinically proven that only 450 medicinal plants possess anti-diabetic properties, from which 109 medicinal plants have a complete model of action.[7]

The species *Chenopodium ambrosioides* L. (Amaranthaceae), also known as Mexican tea, Jesuit's tea or blue bush, Indian goosefoot, Spanish-tea or wormseed in English, is also known as Mexican tea, Spanish-tea, or an annual or perennial shrub with a strong aromatic smell. It is widely distributed in West Africa.[8] The WHO pointed out that *C. ambrosioides* (synonym: *Dysphania ambrosioides*) is among the most used plants in traditional medicines worldwide and used to treat several diseases.[9] It is used in different countries to manage diabetes mellitus, especially in Guatemala and Morocco.[10] Plant decoction and maceration are orally administrated in DM in the Democratic Republic of Congo.[11–13] However, scientific shreds of evidence justifying this use are missing.

According to literature data, only one study on anti-diabetic investigation of leaf methanolic extract has been evidenced in low-dose streptozotocin-treated and high-fat diet-fed mice after 2-weeks of treatment. Preliminary results indicated that methanolic extracts at different doses showed a significant ($p< 0.05$) in blood glucose levels compared to the control group that received distilled water.[14] No studies exist regarding hypoglycemic and antihyperglycemic effects of extracts from *C. ambrosioides*. However, the present investigation highlights the impact of different doses (100, 200, and 400 mg/kg b.w) of leaf aqueous and methanolic extracts of *C. ambrosioides* using glucose tolerance test, which is an essential test of carbohydrate function. Moreover, the ability of the plant extracts in glucose-lowering in normoglycemic rats is also reported.

This study was aimed to investigate the short-term anti-diabetic potential and toxicity levels of *Chenopodium ambrosioides*.

Materials and Methods

Chemical and Reagents

Carboxymethyl cellulose (Shanghai Huaxuan, China), Chloroform LR (Griffchem^{TM}, India), Dextrose monohydrate (Loba Chemie, India), Glibenclamide (Nobel, Turkey), Glucose intravenous infusion 50% (Abacus Parenteral Drugs-Uganda, Uganda), Methanol 99.5% (Loba Chemie, India), n-butanol 99% (Loba Chemie, India), Physiological solution (Abacus Parenteral Drugs, Uganda), Picric acid 10% (BDH Limited Poole, England), and Sodium chloride Extra Pure (Loba Chemie, India).

Plant Collection

The fresh leaves of *C. ambrosioides* were collected in Bukavu city (22°29′23 S, 28°50′51 E), located in South-Kivu-DRC, between April and October 2019. Those leaves were identified by a botanist, Mr. Gentil IRAGI, from the Department of Biology of Center for Research in Natural Sciences/Lwiro (Centre de Recherche en Sciences Naturelles), and voucher specimens deposed in the herbarium under number LWI563359346.

Preparation of Plant Extracts

Aqueous Extract

The infusion was prepared as follows. 250 g powder of *C. ambrosioides* was mixed with 250 mL distilled water in 20 min. Then, 9000 mL of boiling distilled water was added to the sample and left to stand at room temperature for 30 minutes by occasional shaking and stirring. Then, the extract was filtered using cotton wool and concentrated to dryness under reduced pressure using a rotary evaporator (IKA® RV 10, Germany) at 40°C. The obtained infusion was frozen (−80° C) and lyophilized using Benchtop Freeze Dryer (FD-ICL, Japan).

Methanolic Extraction

With adaptations, cold maceration in methanol was used according to the general method.[15] The leaf part of C. ambrosioides was air-dried at room temperature and then manually grounded to a fine powder. Thus, 250g of the plant material was repeatedly macerated in 3000 mL of methanol for 48 hours by occasional shaking and stirring. After filtration, the methanolic solution was thoroughly filtered using cotton wool and concentrated to dryness, by a rotary evaporator (IKA® RV 10, Germany), under

reduced pressure at 40°C. The extraction yield was calculated as g of dry residue per 100 g of dry plant mass. The dry residue obtained from both aqueous and methanolic extracts was weighed (60.26 g: yield 24.10%) and (24.24 g: yield 9.69%). They were then kept in the dark container in the freezer at −20° C until further pharmacological investigations.

Experimental Animals

Healthy male and female Wistar rats were used for anti-diabetic study and acute toxicity, respectively. Animals were obtained the from the animal research facility of the Department of Pharmacology, Mbarara University of Science and Technology (MUST) and kept in separated cages, and allowed access to food (pellets) and water *ad libitum*. All animals were maintained under standard laboratory conditions in the Animal Research Facility of Faculty of Medicine of MUST (the temperature at 23.8 ± 1.9°C; relative humidity: 63.6 ± 6.6% and 12 h daylight/dark cycle).[16,17] Their feed was constituted by carbohydrates, proteins, calcium, vitamins, and water.[18]

Experimental Design and Sample Size Determination

The Experimental Design Assistant (EDA) tool generated a randomization sequence. To randomly assign 40 animals to different groups (five animals by a group with eight groups), a person who was not aware of the type of intervention each group assigned did the assignment to treatment groups. However, investigators were aware of the treatment groups during treatment and measurements since blinding was impossible because of the difference in physical aspect between the control and other treatment groups.[19]

Acute Toxicity in Rats

According to the Organization for Economic Cooperation and Development (OECD) guidelines no 425, the toxicity test was carried out based on the limit dose test of the up and down procedure. Test Guidelines related to an Acute Oral Toxicity under a computer-guided Statistical Programme-AOT425statPgm, version 1.0,[20] at a limit dose of 2000 mg/kg body weight/oral route and default of Sigma at 0.5. The protocol of acute toxicity was followed as described,[21] with a few modifications. A systematic randomization technique was used. Two groups of three rats per group of healthy female young (without pregnancy and not nulliparous) were selected and individually marked out of a population of 25 Wistar rats (8–12 weeks old). The population sample was chosen such that the weight differences do not exceed ± 10% of the mean initial weight of the sample population. The rats were fasted for 6 hours (morning fast) before dosing on each occasion. Each was picked at a time, weighed, and dosed with a unique dose of 2000 mg/kg b.w of aqueous and methanolic extracts of *C. ambrosioides* dissolved in carboxymethylcellulose 1%, used as a vehicle. Feeding was given to rats using gastric feeding cannula. After the extract administration, each rat was observed for the first 5 min after oral administration for signs of possible regurgitation and then kept in a cage for observation. Each rat was watched for every 15 min in the first 2 h after dosing, then every 30 min for the successive 6 h and then daily for the successive 38 h for the short-term outcome and the remaining 14 days for the long-term possible lethal outcome. All animals were monitored as aforementioned with individual records being maintained for each rat. Behavioral manifestations of acute oral toxicity were also noted.

Hypoglycemia Evaluation

Morning fasted healthy male rats, aged 7–8 months old, were randomly divided into eight groups. The rats were treated with carboxymethylcellulose (1% prepared in saline solution), plant extracts, and glibenclamide. The blood glucose level of each animal was measured before treatment as a baseline and then at 30, 60, 90, and 120 min. Post-treatment.[22] The randomized rats involved in this present study were divided into the following groups:

Group 1-Control; rats received carboxymethylcellulose 1% (1 mL/100g b.w)
Group 2-Reference; rats, received glibenclamide (5 mg/kg b.w)
Group 3-Treatment 1; rats treated with AECA (100 mg/kg b.w)
Group 4-Treatment 2; rats treated with AECA (200 mg/kg b.w)
Group 5-Treatment 3; rats treated with AECA (400 mg/kg b.w)
Group 6-Treatment 4; rats treated with MECA (100 mg/kg b.w)
Group 7-Treatment 5; rats treated with MECA (200 mg/kg b.w)

Group 8-Treatment 6; rats treated with MECA (400 mg/kg b.w)

Glycemic change was calculated as a function of time (t) by applying the formula:

$$Glyceamic\ change = \frac{G0 - Gt}{G0}$$

Where $G0$ and Gt represent (zero time or 0 min) glycemic values before, and glycemic values at 30, 60, 90, and 120 minutes, oral administrations of the plant extracts, respectively.[23]

Assessment of Intraperitoneal Glucose Tolerance Test in Normal Rats

Healthy male rats, aged 7–8 months old, were randomly divided into eight groups (n = 5/group). Each rat's fasting blood glucose level was determined initially, after morning fasting with free access to water. Glucose (2g/kg b.w) was intraperitoneal administrated 30 minutes after oral administration of drugs. Blood glucose levels (BGL) were measured just before and 30, 60, 90, and 120 minutes after the oral administration of the test samples.[24,25] The animals were grouped and treated as earlier described in the hypoglycemic evaluation. The percentage reduction of glucose-induced hyperglycemia by the treatment and control groups was determined from the relation:[26]

$$Percentage\ reduction = \frac{Maximum\ glucose\_induced\_hyperglycemia - Glycemia\ at\ 120\ minutes}{Maximum\ glucose\_induced\_hyperglycema} X100$$

Determination of the Blood Glucose Levels

All rats were fasted for 6 hours (8:00 am to 2:00 pm) before determining BGL but allowed free access to water *ad libitum*. BGL (mg/dL) was determined using a glucometer (Accu-Chek®, South Africa) and tips by the enzymatic glucose oxidase method applied to blood. The blood samples were collected from the end of the tail at different times (T-0, T-30, T-60, T-90, and T-120 minutes).

Statistical Analysis

Values were represented as mean ± standard error of the mean of five experiments. Data were statistically performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using GraphPad Prism (version 8.0.1). The level of $p \leq 0.05$ was used as a criterion of statistical significance.

Results

Acute Oral Toxicity Effect

There was no sign of toxicity, and deaths of rats administered 2000 mg/kg body weight in female rats fed with aqueous and methanolic extracts of *C. ambrosioides* in the 2-week observation period outcome of the limit dose test Up and Down Procedure. In this condition, the LD_{50} from the AOT425Pgm program was estimated to be more than 2000 mg/kg body weight/oral route (Table 1). The aqueous and methanolic extracts were found to be safe at that dose. For this reason, those doses (100, 200, and 400 mg/kg) were selected for hypoglycemic and antihyperglycemic studies in normal and glucose-loaded rats, respectively. Any behavioral manifestation and mortality were observed in all treatment groups during the entire investigation (from oral drugs administration to 14 days).

Hypoglycemic and Antihyperglycemic Effects

Table 1 Mean glycemia ± SME measured at different times before and after treatment in hypoglycemic test et glucose tolerance test.

Table 1 Glucose blood concentrations observed in hypoglycemic and glucose tolerance tests

Before administrating drugs to animals, the baseline fasting blood level was between 89.44 and 101.5 mg/dL. From the 30th minute, it was noted a significant decrease ($p< 0.05$) in the group treated with glibenclamide (5 mg/kg). This drop in blood glucose is just as effective ($p< 0.01$ and $p< 0.001$) compared to other treatment groups, particularly those that were receiving methanolic extracts. This decrease will remain significant compared to the different treatment groups,

Table 1 Glucose Blood Concentrations Observed in Hypoglycemic and Glucose Tolerance Test

Treatment	Before	After			
Hypoglycemic test					
	Baseline	30 min	60 min	90 min	120 min
CMC 1%	93.45 ± 2.19	100.0 ± 2.53	102.2 ± 3.11	101.1 ± 3.84	99.64 ± 1.33
Glibenclamide	89.45 ± 2.89	83.28 ± 4.16*[a]	68.73 ± 3.65****[a]	54.18 ± 3.17****[a]	52.73 ± 3.04****[a]
AECA 100	94.90 ± 3.17	102.2 ± 3.47*[b]	94.52 ± 2.99***[b]	94.18 ± 2.39****[b]	91.64 ± 1.86****[b]
AECA 200	96.36 ± 2.37	88.00 ± 4.04	86.54 ± 3.17*[b]	85.10 ± 2.77*[a]	82.92 ± 3.66*[a]
AECA 400	94.92 ± 2.10	97.44 ± 1.68	92.02 ± 3.06**[b]	96.00 ± 3.32****[b]	94.52 ± 1.15****[b]
MECA 100	91.66 ± 2.78	104.7 ± 2.90**[b]	90.54 ± 3.46**[b]	90.92 ± 1.90****[b]	93.46 ± 4.21****[b]
MECA 200	89.44 ± 3.69	104.0 ± 5.55**[b]	93.10 ± 5.34***[b]	99.30 ± 3.52****[b]	87.28 ± 3.63****[b]
MECA 400	101.5 ± 2.90	112.7 ± 3.30***[b]	108.4 ± 2.20****[b]	102.2 ± 2.32****[b]	99.98 ± 3.81****[b]
Antihyperglycemic test					
CMC 1%	92.63 ± 3.06	162.9 ± 1.78	148.7 ± 4.04	133.1 ± 4.75	122.9 ± 3.57
Glibenclamide	90.91 ± 3.59	156.4 ± 3.90	115.6 ± 4.69****[a]	89.45 ± 4.35****[a]	70.91 ± 3.59****[a]
AECA 100	78.91 ± 5.35	163.3 ± 3.78	127.3 ± 5.48*[a]	113.8 ± 4.62**[b]	105.1 ± 2.89****[b]
AECA 200	87.27 ± 3.25	162.9 ± 3.88	137.5 ± 4.09	111.6 ± 4.54*[a, b]	110.9 ± 2.98****[b]
AECA 400	89.82 ± 4.40	165.1 ± 4.57	128.0 ± 4.76*[a]	115.3 ± 4.72**[b]	110.5 ± 4.68****[b]
MECA 100	96.36 ± 3.77	187.3 ± 3.77**[a,b]	116.4 ± 3.30***[a]	116.4 ± 3.77**[b]	113.8 ± 5.03****[b]
MECA 200	86.46 ± 3.51	200.0 ± 3.51****[a,b]	150.9 ± 2.37	128.0 ± 1.87****[b]	126.9 ± 3.69 ****[b]
MECA 400	86.54 ± 3.23	196.0 ± 4.31****[a,b]	157.8 ± 3.91***[b]	115.6 ± 4.40**[b]	106.2 ± 4.86****[b]

Notes: Values given represent the mean (±S.E.M, n=5). *P< 0.05; **P< 0.01; ***P< 0.001; ****P< 0.0001. [a]p=compared to CMC1%; [b]p=compared to Glibenclamide.
Abbreviations: CMC 1%, carboxymethylcellulose; AECA, aqueous extract; MECA, methanolic extract.

up to the 120th minute (with a maximum reduction of 41% vs −6.62% from the control). However, a significant decrease (p< 0.05) in blood glucose was also noted in the group treated with AECA200 (Maximal reduction by 14% vs −6.62% from the control). Figure 1 indicates the effectiveness of glibenclamide from the thirtieth minute until the end of the evaluation. It is also observed that the glycemic values of AECA200 are lower than the control and other treatment groups, except at the 90th minute when the glycemia increases but manages to stabilize at the 120th minute (Figure 1).

Similarly, at the same dose, the methanolic extract experiences the highest glycemic peak at the 30th minute, followed by a considerable drop at the 60th minute. Subsequently, the blood glucose values stabilize until the end (Figure 1). The table above (Table 1) represents the antihyperglycemic potential of plant extracts, vehicles, and the standard drug in Wistar rats. After glucose loading, 30 minutes, the fasting blood level increased in all treatment groups. The glycemic pics have significantly increased (p< 0.01 and p< 0.0001) in the groups treated with methanolic extracts compared to reference and control groups. In the reference group, glibenclamide significantly (p< 0.0001) lowered the blood glucose from the 30th minute to the 120th minute. In comparison to the other treatment groups, glibenclamide was the most potent, especially at T-120 (significant decrease, p< 0.0001), where the maximal percentage reduction of glycemia was obtained (55.23%).

On the other hand, aqueous and methanolic extracts at the dose of 100 mg/kg significantly (p< 0.05) reduced blood glucose at the 60th minute, compared to the control group. Furthermore, the reduction was maintained until the 90th minute with the aqueous extract.

Figure 1 shows that the blood glucose level obtained from that aqueous extract reached the same level as glibenclamide at the 60th minute. Nonetheless, which value increased at the 90th and 120th minute, later. However, the methanolic extract produced the lowest glycemia among all treatment groups after glucose loading at the 90th and 120th minute (Figure 1). Overall, in all treatment groups, the glucose blood level was reduced from the 90th to 120th minute but was not significant compared to the control group, even less to the reference group. On the other side, in the hypoglycemic test, the reduction T120-T30 of the group treated with glibenclamide was more potent than other groups. However, methanolic extracts (MECA200, 400, and 100) and aqueous extracts (AECA100, 200, and 400) were influential. Nonetheless, MECA200 and glibenclamide presented the same pic in antihyperglycemic, followed by MECA100 and MECA 200. For aqueous extract, the best pic is that from ACE100.

Figure 2 shows the differences between treatment groups and control groups.

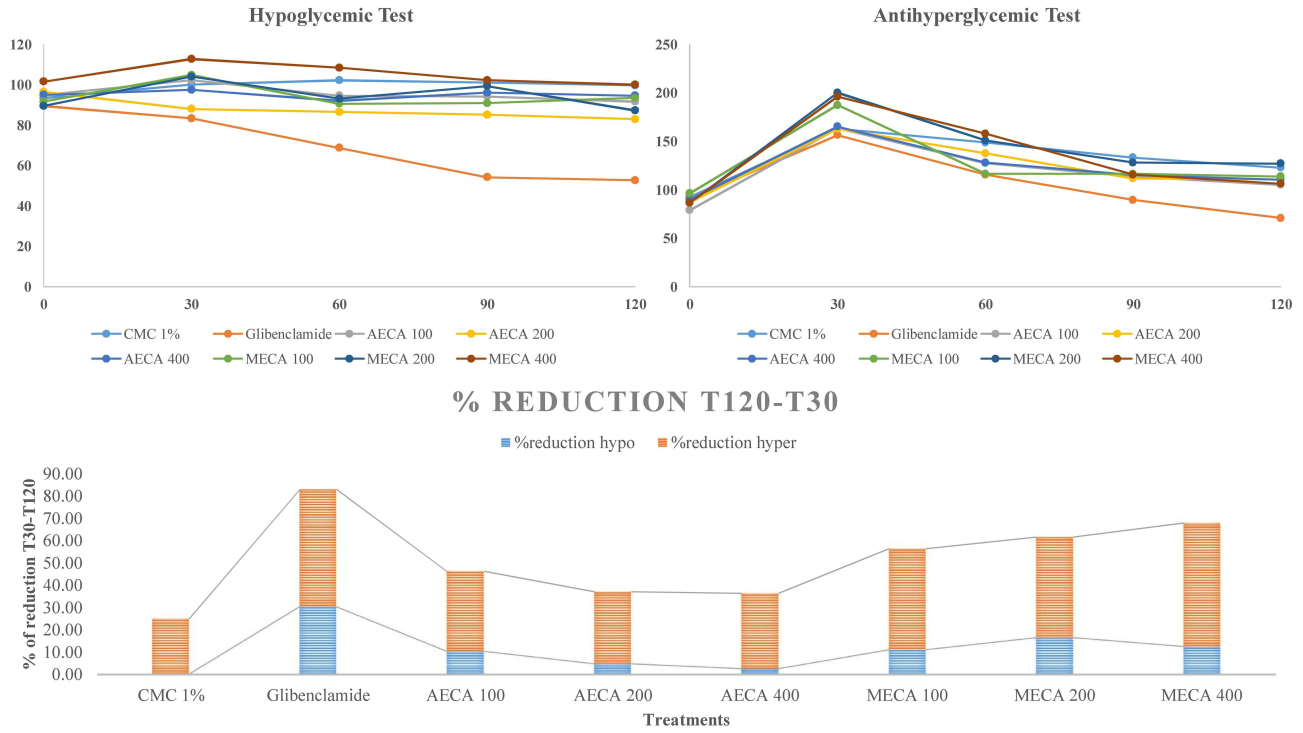

Figure 1 Graphical presentation of the evolution of blood glucose levels after oral administration of aqueous AECA (100, 200, and 400 mg/kg b.w), CMC1%, and glibenclamide (5mg/kg b.w) in both hypoglycemic test and antihyperglycemic test, and the effect of treatments on the glucose baseline level.

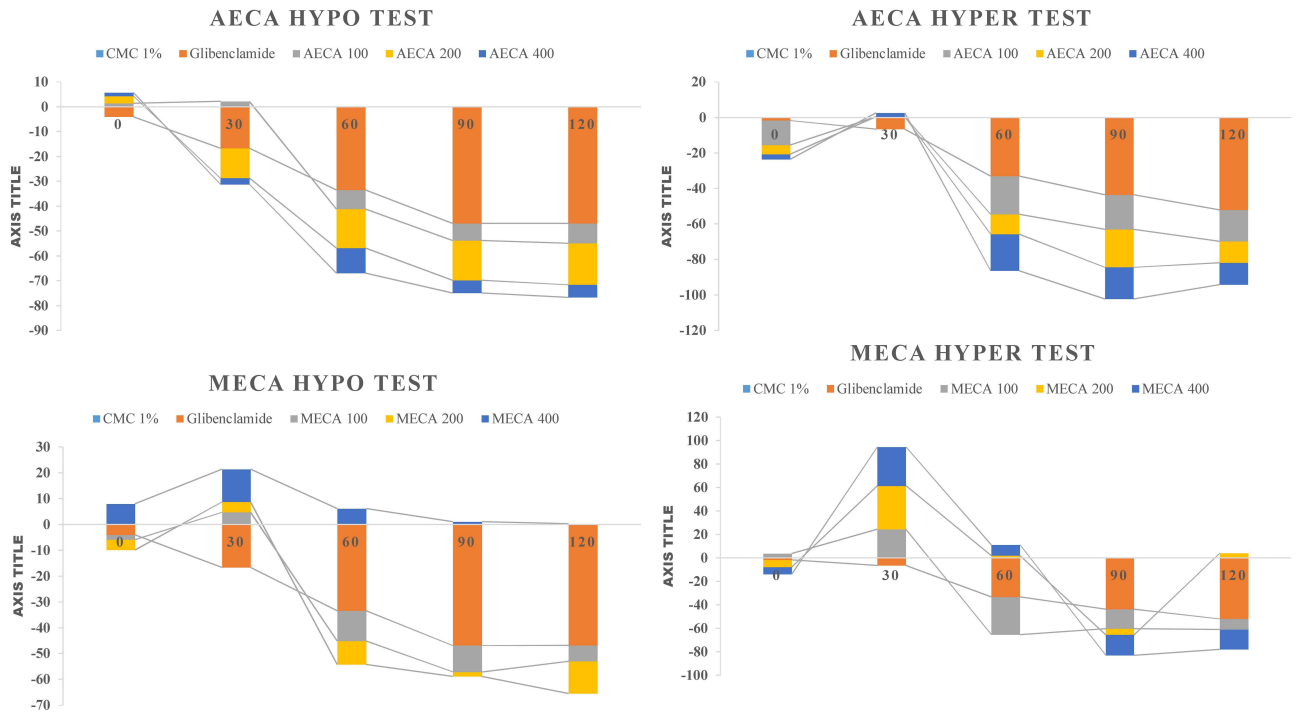

Figure 2 Differences between treatment groups and control groups.

The baseline values are put to zero. The differences are positive or negative accordingly. Positive and negative values represent the increase and decrease in BGL compared to the control group. In hypoglycemic evaluations, the highest negative values are observed (High percentage of reduction) at T-120, followed by T-90, T-60, and T-30. Between different groups, BGL

decreased significantly ($p< 0.0001$) in the standard (reference) group from T-30, T-60, T-90, and T-120, with percentages of reduction of −16.72, 33.47, 46.92, and 49.91, respectively. In the treatment group, AECA200 produced a reduction in BGL also from T-30 to T-120. Hence, BGL was reduced by 12% (T-30), 15.66% (T-60), 16% (T-90) and 16.72 (T-120). On the other hand, MECA100 exhibited a decrease of 11.66% and 10.18% at T-60 and T-90. At T-30, his BGL was high (+4.7%), and it was progressively reduced at T-60 (−11.66%). However, an increase in BGL in all groups treated with the methanolic extracts, especially MECA400 treated group, where that value decreases slowly until T-120 (null value). In the antihyperglycemic study, as previously values in glibenclamide-treated (reference) group decreased ($p< 0.0001$, after glucose loading to the end of the study. Thus, the percentages of reduction were 6.5%, 33.1%, 43.65% and 51.99% at T-30, T-60, T-90 and T-120, respectively. Values in BGL decreased variably in treatment groups. In methanolic extract treated, BGL lowered significantly ($p< 0.05$) at T-30, with percentages of reduction of 24.4% (MECA100), 37.1% (MECA200), and 33.1% (MECA400). Moreover, the values decreased significantly ($p< 0.05$) at 21.4%, 11.2% and 32.3%, in AECA100, AECA200 and MEPA100, respectively. At T-90, only the AECA200-treated group reduced ($p< 0.05$). At last, although not significant statistically, AECA100 produced the best percentage of reduction (17.8%) among treatment groups followed by MECA400 (16.7%), AECA400 (12.4%), and AECA200 (12%).

Discussion

Research investigation of plant extracts in animals is the most reliable means of detecting significant pharmacological and toxic potentials of bio-compounds for evaluating risks to human health. It is also crucial for ensuring and enhancing human and animal health.[27]

Acute oral toxicity investigation of leaf aqueous and methanolic extracts of *C. ambrosioides* indicated no mortality, and any signs of intoxication of rats occurred, at a limit dose of 2000 mg/kg b.w. Based on the classification of substances according to their LD_{50} values, substances with LD_{50} of 1000 mg/kg body weight/oral route are regarded as safe or of low toxicity. Clarke and Clarke (1977) cited by Adeneye et al. 2006.[20] Thus, this plant has low acute toxicity when administered orally. Following our results, an acute toxicity evaluation of different doses (300, 1000, and 2000 mg/kg) of aqueous leaf extract has not shown any clinical signs of toxicity and no macroscopic lesions in Wistar rats for 24 hours of monitoring.[28] In Swiss mice, a daily administration of different doses of hydro-alcoholic extract of leaves (5, 50, and 500 mg/kg) for 15 days has not exhibited any death and alterations in the treatment groups.[29]

Despite the wide use of *C. ambrosioides* worldwide and the promissory biological effects, native people have reported his toxicity. The leaves were mixed with milk before the ingestion to reduce their toxicity.[29] The plant is widely used for insecticidal properties, and its essential oils have been reputed to possess various biological activities. The toxicity of essential oils does not depend on high concentrations. All essential oils are toxic at high doses, particularly when taken orally.[30] Previous studies established the toxicity profile of essential oils or/and their components from *C. ambrosioides*.[10] For instance, the essential oil of *C. ambrosioides* can cause several effects, including irritation of the mucous membrane of the gastrointestinal tract, liver, and kidneys. Alterations in the CNS, paresthesis, incoordination, headaches and facial flushing have been reported. In addition, his overdoses could cause fatalities in both men and rats.[31]

Although *C. ambrosioides* is traditionally used in treating diabetes mellitus in some countries (Democratic Republic of Congo, Guatemala, and Morocco), acute anti-diabetic studies in vivo are lacking. The aqueous extract showed a hypoglycemic effect in normal rats at 200 mg/kg b.w. It also exhibited a more hypoglycemic effect two hours after and produced the highest percentage reduction among all other extracts. It maintained blood glucose level lowest at the end of 2 hours evaluation compared to other extracts. The reference group presented the highest pic of reduction followed successively by methanolic extracts (MECA200, 400, and 100) than aqueous extracts (AECA100, 200, and 400), considering reduction from T120 to T30 taken together (Figure 1). A previous study indicated that 20 µg/mL of *C. ambrosioides* root hexane extract showed an anti-diabetic potential by the high level of α- amylase inhibition.[32] Plant extracts may decrease fasting blood glucose concentration in normoglycemic rats by mechanisms of action involved in stimulating the residual pancreatic mechanism, increasing the peripheral utilization of glucose, converting glucose to glycogen, and promoting glycogen storage in the liver and skeletal muscles.[33] A similar funding has been found with *Camellia sinensis*. The plant extract reduced BGL in normoglycemic fasted and non-fasted rats. His activity was less potent than tolbutamide taken as a reference drug. Moreover, it was concluded that most of the medications used

in allopathic medicine (synthesized medicines), characterized chemically, are highly productive but induce several intolerable side effects.[34] However, in the sub-acute study of 2 weeks, different doses of leaf methanolic extracts (100, 200, and 400 mg/kg) significantly reduced blood glucose levels in low-dose streptozotocin-treated high-fat diet-fed diabetic mice. They were found to be dose-dependent.[14] According to our results in Table 1, the methanolic extract also produced a dose-dependent reduction of 39.22, 44.65, and 45.83% (100, 200, and 400 mg/kg, respectively) response in GTT conditions. However, only 100 and 400 mg/kg produced a significant lowering of the blood glucose at the 60th minute compared to the control group. From the 30th minute, the glycemia had significantly increased with the administration of that extract in all animals compared to control, reference, and other treatment groups.

Our results revealed that the pic glycemic was observed 30 minutes after glucose loading in all groups treated with the methanolic extracts. In response to a GTT (Figure 2), the peak glucose levels are reflected in statistically significant differences at 15 minutes post-glucose loading. Previously, it was established that intraperitoneal glucose administration leads to an increased glucose excursion and a largely absent insulin excursion compared to oral administration, which leads to the incretin potency potentiating glucose-induced insulin release.[35,36] The effectiveness of glibenclamide and aqueous extracts may be linked to insulin release due to the excessive glucose concentration in the blood or the stimulation of peripheral glucose utilization. Several possible mechanisms could explain the homeostatic response to an ingested glucose, including stimulating tissue glucose disposal, inhibiting endogenous glucose production, and regulating intestinal glucose entry (excluded in IGTT). Accordingly, glucose handling during the glucose tolerance test depends on some factors: insulin secretion and action and the ability of blood glucose to regulate glucose uptake.[37] From T-60 to T-120, blood glucose was lowered variably in groups that received a single oral administration of plant extracts.

Interestingly, at the end of the study, all treatment groups, except those treated with MECA200, recorded glucose-lowering, with the percentages of reduction varying between 17.8% and 12%. Moreover, in the groups treated with the methanolic extract, after the glycemic peak observed at the 30th minute, the glycemia fell again from the 60th minute, with their lowest glycemic values at the 90th minute. Considering the reduction in an antihyperglycemic test from the beginning to the end of the study (Figure 1), methanolic extracts are close to glibenclamide and more than aqueous extracts. However, MECA400 is the most powerful among all extracts. The variable reduction in different groups could undoubtedly be linked to one of the aforementioned mechanisms. The decline recorded in the glibenclamide treated group remains significantly higher than in all treatment groups.

Our results showed that glibenclamide treatment indicated a more pronounced blood glucose-lowering effect in normoglycemic rats than in intraperitoneal glucose-loaded rats. In hypoglycemic and antihyperglycemic evaluations, glibenclamide significantly lowered the blood glucose level in all time intervals. His hypoglycemic effect was high, marked by reducing fasting blood glucose from 89.45 to 52.73 mg/dL after 2 hours of evaluation. After two hours of assessment, the percentage reduction of glucose in normoglycemic mice treated with glibenclamide products (Five tablets marketed in Addis Ababa, Ethiopia) was found to be at 41.24 ± 13.52 mg/dL.[38] Glibenclamide is a sulfonylurea derivative that causes hypoglycemia by stimulating pancreatic beta-cells to release more insulin and inhibit glucagon secretion. It inhibits ATP-sensitive potassium channels in beta cells, which cause cell membrane depolarization by opening the voltage-dependent calcium channel. Therefore, the intracellular calcium level in the beta-cell increases and results in the stimulation of insulin release. This sulfonylurea class of drugs is widely used to treat DMT2.[39–41] The results from this study indicated treatment with plant extracts was associated with an effective improvement of glucose intolerance. However, the mechanisms of action that justify the potency of the plant extracts induced hypoglycemia were not investigated in the present study.

The strength of the present study is that for the first time, the effects of the different doses of the aqueous and methanolic extract of *C. ambrosioides* leaf extracts regarding to his hypoglycemic and antihyperglycemic and their preliminary toxicity in short-term evaluation is reported in the animal model. However, the study's limitations are limited to animal species (only one has been used) and experimental design, including short-term evaluation, sample size (two plant extracts), and one outcome (BGL).

Conclusion

In conclusion, the plant extracts possess anti-diabetic potential in normoglycemic and intraperitoneal glucose-loaded rats. The suppression of the peak in the tolerance test suggests that the methanolic extract may contain some anti-diabetic compounds. Moreover, the aqueous and methanol leaf extract of *Chenopodium ambrosioides*, at the dose of 2000 mg/kg, does not cause any apparent toxicity in rats in short-term evaluation. Both the hypoglycemic and antihyperglycemic approaches used in rats outline the anti-diabetic effect of *C. ambrosioides* leaves extract. This study has opened the door for more in-depth pharmacological and phytochemical investigations. Therefore, further inquiries can be undertaken in long-term exposure (30 to 90 days) of plant extracts and isolated compounds in diabetic models, including high fat diet-fed, streptozotocin-and-alloxanized diabetic models. Moreover, the molecular level (enzyme and dipeptidyl peptidase inhibitions, glucose transport GLUT4 translocation, peroxisome proliferator-activated receptor, modulation on Krebs cycle enzymes, etc.) are need to be established of elucidation of the mechanisms of action.

Ethics Statement

The experiments procedures were performed following the standard ethical guidelines on the protection of animals used for scientific purposes as described in the European Community guidelines; EEC Directive 2010/63/EU revising Directive 86/609/EEC, adopted on 22 September 2010.[42] The protocol was submitted and then approved by Mbarara University of Science and Technology Research Ethical Committee (MUST-REC) with registration number MUST-REC 25/01-19 and by the Uganda National Council for Science and Technology (UNCST) with the number of registration NS440ES.

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Disclosure

The authors report no conflicts of interest in this work.

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