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Original Paper

Determining blood glucose lowering effects of leaf extracts of *Cleome gynandra*, *Amaranthus cruentus* and their mixture in Normoglycaemic and Hyperglycaemic (Alloxan-diabetic) rats.

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ABSTRACT

Background: Normal blood glucose is maintained in the range 3.5 – 8.0 mmol/L. However, abnormally high blood glucose is characteristic of Diabetes Mellitus (DM) due to defects in insulin secretion, insulin action or both.
Objective: The present study was conducted to assess blood glucose lowering effects of ethanolic leaf extracts of Cleome gynandra (CG), Amaranthus cruentus (AC) and their mixture (1:1 ratio) in Normoglycaemic and Alloxan-diabetic rats.
Methods: The study was an in vivo experimental study using the rat model. The crude drugs were extracted from the leaves of AC and CG by maceration and formulated into 3 dose levels (200, 400 and 600 mg/Kg BW). Normal saline (0.9%) and Glibenclamide (5 mg/kg BW) were used as control and reference drug respectively. The crude drugs were administered orally to normoglycaemic and alloxan diabetic rats to assess glucose lowering effect. Data was analyzed by Students *`t'* test and one way ANOVA in order to make quantitative conclusion of the study.
Results: Extracts of AC, CG and their mixture produced maximum reduction in FBG of 30%, 35.4% and 26.1% respectively in normal rats after 6 hours. Glibenclamide produced maximum hypoglycemic effect of 50% after 4 hours (2.8 ± 0.09 to 1.6± 0.05) and only 2 % reduction in FBG was observed in the control group. Similarly, in diabetic rats, blood glucose levels were significantly lowered as compared to the control groups ($p < 0.05$). Mean RBG was lower in the experimental compared to diabetic control group at day 10 (17.6±1.61 Vs. 28.8 ± 0.23 mmol/L, $p < 0.05$). The leaf extracts seem to act via extra-pancreatic mechanisms.
Conclusion: Ethanolic leaf extracts had glucose lowering effects in normal and diabetic rats. The Leaf extracts seem to act via extra pancreatic mechanisms.
Key words: Alloxan Diabetes, Ethanolic Leaf Extracts, Amaranthus cruentus (AC), Cleome gynandra (CG), Rattus Novergicus.

1. Introduction

Normal blood glucose in a healthy person ranges from 3.5 to 8.0 mmol/L. However, disturbances may occur that affect normal blood glucose levels such as in Diabetes Mellitus (DM). DM is associated with chronic hyperglycaemia caused by defects in insulin secretion, insulin action or both [1].

The prevalence of DM is increasing globally. It has been estimated that 171 million people had diabetes in year 2000 and by 2030 it will increase to 366 million [2]. Treatment of DM involves the use of hypoglycaemic agents such as insulin analog, sulfonylureas, biguanides, thiazolidinedione (PPARγ agonist), α-glycosidase inhibitors and incretin-based therapies.

However, even with effective treatment, management of DM without any side effects is a challenge to the medical system [3]. Patients still develop side effects such as severe hypoglycaemia, lactic acidosis, idiosyncratic liver cell injury, permanent neurological deficit, digestive discomfort, headache, dizziness, and even death. Medicinal plants are alternative treatment methods due to fewer side effects, less costly, accessible, and compatible with the human body (although not all) and the presence of many phytochemical compounds [4,5,6].

Amaranthus Cruentus (AC) and *Cleome Gynandra* (CG) are used to treat diabetes in many parts of Zambia. Local names for AC are *Bondwe* (Bemba), *Libowa* (lozi), Lengalelenga (Nyanja). While for CG, local names are *Lubanga (*Bemba*)*, *Shungwa (*Tonga*)*, *Suntha (*Nyanja*)* and *Sishungwa* (Lozi).

The present study had three objectives; (i) To assess glucose lowering effects of Ethanolic leaf extracts of A*maranthus cruentus* and *Cleome gynandra* in normal and Alloxan- treated diabetic rats. (ii) To assess glucose lowering effect of the mixture (1:1ratio) of Ethanolic leaf extracts of A*maranthus cruentus* and *Cleome gynandra i*n Normal and Alloxan-treated diabetic rats. (iii) To deduce possible mechanisms of action by which Ethanolic leaf extracts of A*maranthus cruentus* and *Cleome* lower blood glucose in Normal and Alloxan- treated diabetic rats.

2. Methodology

The study was an in vivo experiment using rats; it was conducted in the pharmacology laboratory of the University of Zambia, School of Medicine. The materials required were alloxan (Sigma Aldrich, St. Louis, MO, USA), ethanol (80%), standard rat cagesn, gavage Needles, Glibenclamide (Cipla), Animals (55 Albino Wistar Rats), glucometer (Roche Diagnostics), leaves of fresh *Amaranthus Cruentus* and *Cleome gynandra,* normal Saline, Lignocaine (Ranbaxy) and Chloroform.

Collection and identification of plants

Fresh leaves of AC and CG plants were collected from Lusaka, Zambia during the rainy season (November-December). The botanist from UNZA, Department of Biological Sciences assisted in identification of the plants.

Animal Preparations

The rat species used were male albino of wistar strain (*Rattus norvegicus*) weighing 120-300 grams. They were bred in the central animal house (University of Zambia School of Medicine) with normal blood sugar ranging 4.7 – 7.5 mmol/L. The animals were housed in standard rat cages under standard environmental conditions, with a 12 hours light/dark cycle maintained on a regular feed (vital feed) and water *ad libitum*. A standard Rat pellet feed was provided and comprised 45% fat, 35% protein and 30% carbohydrate as a percent of total Kilo calories. This feed was given throughout the study except a day prior to induction of Diabetes.

Methods of Extraction by Maceration

The crude drug was extracted from the leaves of AC and CG by maceration. The procedure was based on standard procedure widely used by researchers [7,8,9] and recommended by the International Centre for Science and High technology and United Nation Industrial Development Organization [10]. The leaves were washed, cut into pieces and shade dried at room temperature. The dried plant materials were subjected to size reduction by means of a grinder. The powdered materials of each plant were socked in 80 % ethanol separately in well labelled rubber corked bottle for 4 days with frequent agitation. After 4 days, the solution was filtered with a clean muslin cloth. The filtrate was evaporated to dryness in a rotary evaporator at 40 – 50°C. Then the amount of the test drug to be administered was calculated based on the body weight of the rats.

Induction of Diabetes

Alloxan monohydrate at dose 140 mg/kg BW was injected intraperitoneally to overnight fasted rat. It was administered rapidly and prevented from direct sunlight so as to prevent degradation of Alloxan to its toxic secondary metabolite [7,11,12,13]. FBG was then measured on the 2rd, 3rd and 5th day post-induction to ensure that the desired hyperglycaemia (FBG ≥ 11 mmol/L) was established and sustained [5]. Feeding of the Alloxan- diabetic animals continued with the standard diet and water *ad libitum*.

Acute studies in Normal Rats for 24 Hours

Administered various doses of crude drugs of AC and CG (200 mg/kg, 400 mg/kg and 600 mg/kg BW) using a gavage needle **(Table 1).** Then blood samples were collected from tail vein of each rat at 0, 1st, 2nd, 4th, 6th, 8th, 10th, 16th and 24th hours [14].

Acute studies in Alloxan Diabetic Rats for 24 Hours

The Alloxan-diabetic rats were fasted for 12 hours with access to water. The Ethanolic Leaf Extracts of AC, CG and their Mixture (200 mg/kg, 400 mg/kg and 600 mg/kg BW) using gavage needles were then administered **(Table 1.0**). Thereafter, mmeasured FBG at time intervals of 0, 1st, 2nd, 4th, 6th, 8th, 10th, 16th and 24th hours.

Sub-acute studies in Alloxan Diabetic Rats for 10 days

Treatments were given orally to Alloxan-diabetic rats once daily for 10 days. Then Random Blood glucose (RBG) was measured every alternate day for 10 days (Day 1, 3, 5, 7 and 10).

Ethical Consideration

The study protocol was observed and approved by University of Zambia Biomedical Research Ethics Committee (UNZABREC). Ethical clearance reference No. 011-09-16. Euthanasia procedure was performed according to the Institutional Animal Care and Use Committee [15,16].

Statistical Analysis

Data was expressed as mean ± standard deviation of 5 rats per group. One-way ANOVA supplemented with *Schaeffer's post-hoc* test was used. A paired student's *'t' test* was used to compare the effect of the Ethanolic Leaf Extracts to normal saline and the reference drug. Statistical significance was set at $p < 0.05$. Graph pad software version 6 was used for all data analysis.

Table 1: Leaf extracts Treatment options performed for acute studies in Normoglycaemic and Alloxan-diabetic rats (24 Hours)

Group # (n=5)	Treatment	
	Before Alloxan (normoglycaemic)	**Alloxan-treated rats (hyperglycaemic)**
1	Saline/distilled water.	Saline/distilled water.
2	Glibenclamide 5 mg/Kg body weight	Glibenclamide 5 mg/Kg body weight
3	200 mg/kg AC	200 mg/kg AC
4	400 mg/kg AC	400 mg/kg AC
5	600 mg/kg AC	600 mg/kg AC
6	200 mg/kg CG	200 mg/kg CG
7	400 mg/kg CG	400 mg/kg CG
8	600 mg/kg CG	600 mg/kg CG
9	200 mg/kg AC + CG mixture.	200 mg/kg AC + CG mixture.
10	400 mg/kg AC + CG mixture.	400 mg/kg AC + CG mixture.
11	600 mg/kg AC + CG mixture.	600 mg/kg AC + CG mixture.

CG = *Cleome gynandra*, AC = *Amaranthus cruentus*; AC + CG = mixture of AC and CG leaf extracts

3. Results

Preliminary Results

The percentage yield of extracts of *Amaranthus Cruentus* and *Cleome gynandra* were 21.5% and 16.3% respectively. Average weight of Rats was 181.7 ± 6.57 g. The three dose levels of Ethanolic Leaf extracts tested were 200, 400 and 600 mg/kg BW of which 600 mg/kg BW was the most effective.

Acute studies on Normal Rats

RBG and FBG levels in the normal rats (n = 55 rats) was in the range 4.7-7.5 mmol/L and 2.46 - 3.28 mmol/L respectively. The ethanolic leaf extracts of AC, CG and their mixture (at dose 600 mg/kg BW) showed hypoglycaemic action **(Figure 1).** The hypoglycaemic action of the Leaf extracts (AC, CG and Mix) was not statistically different from that of reference drug (Glibenclamide, $p > 0.05$) but significantly different from the control (normal saline, $p < 0.05$).

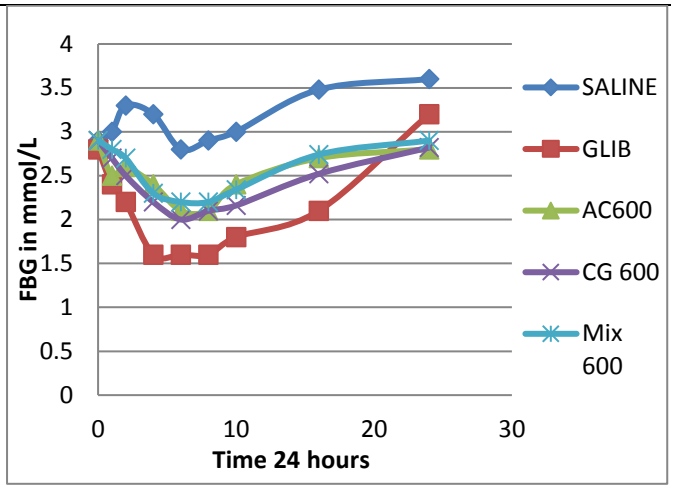

***Figure 1**: The effect of ethanolic leaf extract of AC, CG and MIX (600 mg/kg) on FBG levels in normolglycemic Rats. Each point is the mean ± SEM*

Acute studies on Alloxan Diabetic Rats

Blood glucose levels after induction of alloxan- diabetes was in the range 12.0 - 26 mmol/L. There was significant blood glucose reduction after administering of the Leaf extracts **(Figure 2).**The blood glucose lowering action of AC, CG and Mixtures at 600 mg/kg BW was not statistically significant compared to the control ($p < 0.05$).

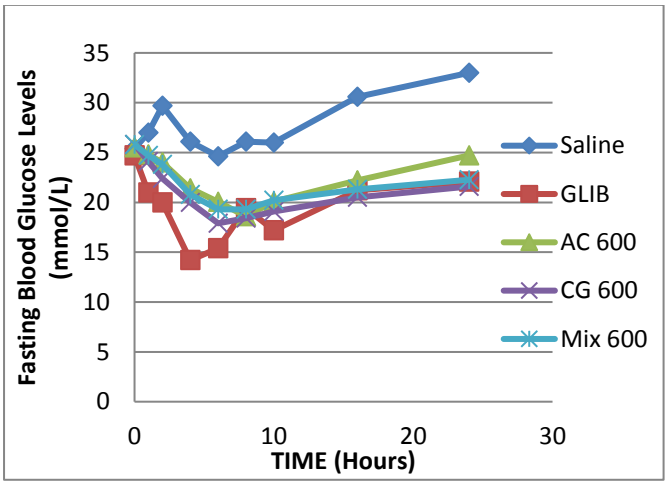

***Figure 2**: The effect of ethanolic leaf extract of AC, CG and MIX (600 mg/kg) on FBG levels in Alloxan- diabetic Rats. Each point is the mean ± SEM*

Acute studies in normal and Alloxan diabetic Rats-compared (Deducing possible mechanisms of action)

The average percentage change in FBG was compared over a 24 hour period between that of Normoglycaemic and Alloxan diabetic rats. There was no significant difference in the average percentage change in the two groups after administering the Ethanolic leaf extracts. However, the average percentage change in FBG between normal and alloxan- diabetic rats treated with Glibenclamide was

significantly different **(Figures 3, 4 and 5).**

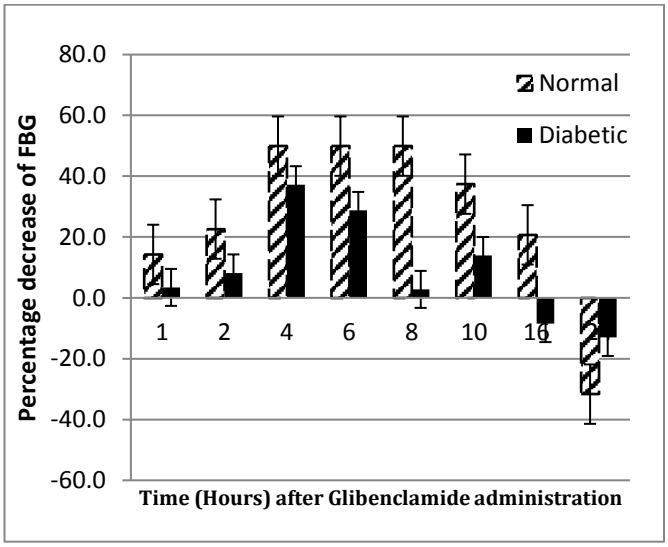

Figure 3: *The effect of Glibenclamide (5 mg/kg) on Blood Glucose levels in Normal and Alloxan diabetic Rats*

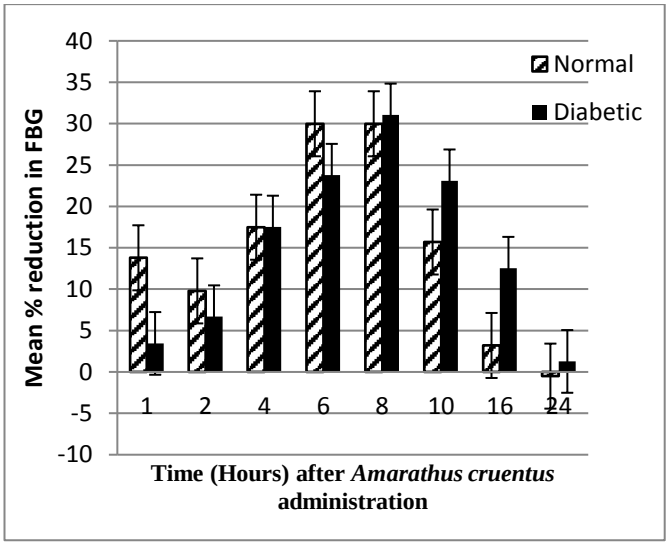

Figure 4*: The effect of ethanolic leaf extracts of Amaranthus cruentus (600 mg/kg) on Blood Glucose levels in Normal and Alloxan diabetic Rats*

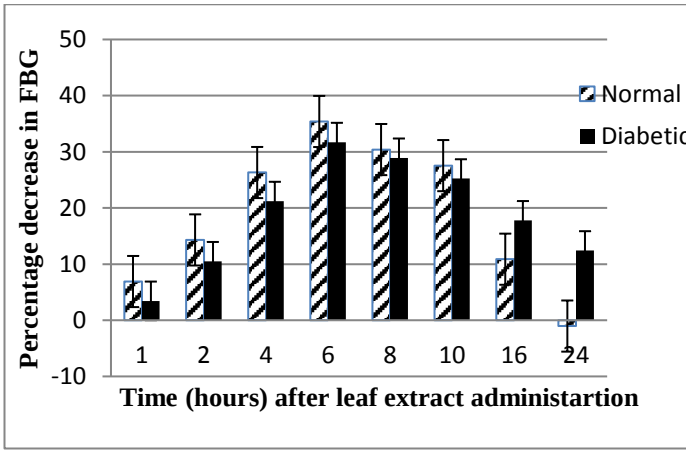

Figure 5*: The effect of ethanolic leaf extracts of C.gynandra (600 mg/kg) on Blood Glucose levels in Normal and Alloxan diabetic Rats*

Sub-Acute studies in Alloxan-diabetic Rats for 10 days

Daily administering of the leaf extracts to the Diabetic rats showed reduction in blood glucose and arresting of hyperglycaemia. The Glucose lowering pattern after administering AC 600, CG 600 and MIX 600 mg/kg BW was not significantly different from that of the control (p <0.05) as shown in **Figure 6.**

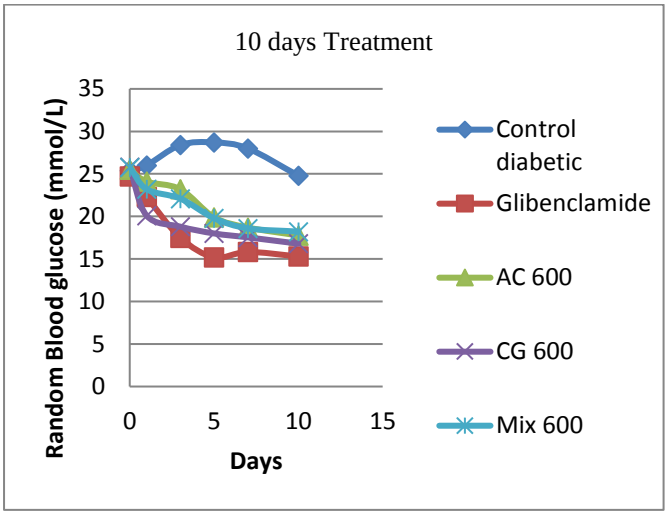

Figure 6*: 10 days Glucose lowering effect of leaf Extracts of AC, CG and Mixture on diabetic rats. . Each point is mean ± SEM*

4.0 Discussion

The First objective was to determine glucose lowering effect of ethanolic leaf extracts of A*maranthus cruentus* and *Cleome gynandra i*n Normoglycaemic and hyperglycemic (Alloxan-treated diabetic) rats.

When administered to normoglycaemic and Alloxan diabetic Rats, the glucose lowering effects of *AC, CG* and *Mix* (600 mg/kg BW) was significantly different from that of the control. Both acute and sub-acute studies showed that the leaf extract lowers blood glucose (**Figures 1 and 2).** Species of *Amaranthus* and *Cleome* have been known to have hypoglycemic effects e.g. *Amaranthus tricolor, Amaranthus esculentus* at 400 mg/kg BW was comparable to Glibenclamide of dose 10 mg/kg BW to streptozotocin-induced diabetic rats [14,17,18]. Similarly, *Cleome gynandra* caused significant reduction in serum glucose and was comparable with Tolbutamide drug at a dose of 40 mg/kg [5]. Phytochemical compounds such as alkaloids, anthocyanin, coumarins, flavonoids, triterpenoids, steroids and many others found in *A.cruentus* and *C. gynandra* contributes to a variety of mechanisms responsible for hypoglycaemic activities [6,23,24].

Second objective was to determine glucose lowering effect of the mixture (1:1) of Ethanolic leaf extracts of *Amaranthus cruentus* and *Cleome gynandra* on Alloxan-treated diabetic rats. Mixing of the leaf extracts improved the efficacy of the Leaf extract **(Figures 1 and 2).** The most effective among the leaf extracts was CG 600 followed by Mix 600 and lastly AC

600 (mg/kg BW).The effective dose of the Mixture (Mix 600 mg/kg) was not statistically different from Glibenclamide ($p > 0.05$) but was significantly different from the control ($p < 0.05$).

Although there is paucity of data regarding the combination of *A. Cruentus* and *C. Gynandra,* there is enormous amount of evidence that shows enhanced performance following herbal mixing. Herbal mixtures are an important aspect of natural healing due to the fact that they increase levels of active components, formation of new phytochemical structures and reduced side effects [19,20,21,22].

Third and last objective was to deduce possible mechanism of action by which ethanolic leaf extracts of A*maranthus cruentus* and *Cleome gynandra* lowers blood glucose in Normoglycaemic and Alloxan diabetic Rats. This was done by comparing the glucose lowering effects of the Leaf extracts between the normal and diabetic rats.

The Normal healthy rats had intact pancreatic activity and the diabetic rats had reduced pancreatic activities. Therefore, comparison gave some information regarding mechanism of action of the leaf extracts (**Figure 3, 4 and 5).** Since, the ethanolic leaf extracts of AC, CG and Mixture had glucose lowering action in both Normoglycaemic and Alloxan Diabetic Rats, ethanolic Leaf extracts seem to reduce blood glucose in alloxan diabetic rats by extra pancreatic mechanisms (or peripheral actions). Reducing glucose absorption from the gut, facilitation of glucose entry into muscle by a non-insulin responsive mechanism, inhibition of gluconeogenesis in the liver, suppression of oxidative glucose metabolism and enhanced anaerobic glycolysis are possible extra-pancreatic mechanism [6,13,16]. Presence of a wide spectrum of phytochemical compounds in the Leaf extracts (e.g. Alkaloids, anthocyanin, Coumarins, flavonoids, triterpenoids, steroids and many more) contributes to a variety of mechanisms responsible for hypoglycemic activities [6,24, 25].

Conclusion

The Ethanolic leaf extracts of A*maranthus cruentus* (600 mg/kg BW) and *Cleome gynandra* (600 mg/kg BW) lowers blood *i*n Normoglycaemic and hyperglycemic (Alloxan-treated diabetic) rats and were comparable to that of Glibenclamide (5 mg/kg BW). Furthermore, mixing of the leaf extracts (1: 1 ratio) enhanced the glucose lowering activities). Since the ethanolic leaf extracts reduced blood glucose in both groups (i.e. normal and diabetic rats) the leaf extracts seem to act via extra pancreatic mechanisms on the diabetic group.

Recommendations

The present study recommends the isolation and testing of toxicity of active compounds found in *Amaranthus cruentus and Cleome Gynandra* responsible for hypoglycaemia. Furthermore to study in great detail synergism of the two plants. Thereafter, herbal formulation useful in the management of Diabetes can be developed.

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