

Investigatory Study of Long Term Doses of *Costus afer*, Snail Slime, and Their Combination with a Standard Pharmaceutical Drug on Blood Glucose Level of Alloxan Induced Swiss Albino Rat

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Abstract

The Plant, *Costus afer* Ker Gawl. belongs to the family of Costaceae and has various uses where they exist. Their use in folk medicine and phytomedicine is in the treatment and management of variety of human ailment, like diabetes mellitus, abdominal problems etc. The search for new antidiabetic therapies has become increasingly urgent due to the development of adverse effects and resistance by the chemically synthesized drugs on one hand and effectiveness with low cost of the plant materials on the other hand. The investigations carried out is to determine the long term effects of *Costus afer* leaf methanol extract, snail slime and the combined *Costus afer* and snail slime extracts on blood glucose levels of alloxan induced diabetic Swiss albino rats treated orally for 21 days on graded dose of (100 mg/kg and 300 mg/kg). From the determination, the snail slime showed positive effect on blood glucose lowering level but less effective when compared with similar dose of the *Costus afer* leaf methanol extract. The investigation indicated that there was 103 mg/dL and 87 mg/dL blood glucose reduction for the low dose of *Costus afer* and Snail slime respectively while the standard hypoglycemic drug (Glibenclamide, 5 mg/kg) used for comparison yielded a blood glucose level reduction of 103 mg/dL. Similarly, the high dose used in the study gave a blood glucose reduction of 99 mg/dL and 95 mg/dL for *Costus afer* leaf methanol extract and Snail

slime respectively. The results obtained when alloxan induced rats was treated with *C. afer* leaf methanol extract, Snail slime extract, and combined *C. afer* and snail slime extracts was analysed using Statistix 8.0 American version. The result showed a dose dependent fashion and the difference obtained from the compared results was statistically significant at $p < 0.05$. This result supports the views of other researchers that some herbal anti-diabetic remedies which reduce blood glucose levels were similar to those of synthetic oral hypoglycemic drugs like metformin and sulfonylurea etc [1]. Still to that, medicinal and pharmacological activities of medicinal plants are often attributed to the presence of the so called secondary plant metabolites. Hence this regenerative capacity of snail slime and the fact that diabetes is characterized by damage of the pancreatic beta cells, may give credit to the hypoglycaemic effect observed in *Costus afer* methanol leaf extract and snail slime for possible drug formulation for anti-diabetic remedy. Our findings may approve snail slime which is insoluble in both acid and alkaline medium, to act as a carrier of chemical and biological nanoparticles for medical and pharmaceutical use.

Keywords

Nanoparticles, Combined *Costus afer* and Snail Slime Extract, Hypoglycaemic, Glibenclamide, Phytomedicine, Snail Slime, *Costus afer*, Antidiabetic

1. Introduction

In recent time, the leaf of the plant *Costus afer* Ker Gawl. (Costaceae family) is traditionally well known for its anti-hyperglycaemic and insulin secretory activity apart from a number of other plant species known to possess diabetic control properties. *Costus afer* is shown in **Figure 1**. The plant is commonly called Ginger lily or Bush cane in English. The Igbo people call it “Okpete” or “Okpoto”, were as the Hausa of Northern Nigeria and Yoruba of Western Nigeria refer it as “Kakizawa” and “Tete-egun” [2], [3] respectively.

In Nigeria, Snail is called different names with respect to the geographical location as recorded in [4]. In Northern Nigeria, the Hausa tribe call it *Dodon Kodi* while in the Eastern Nigeria, that is, the Igbo community and Yoruba in the Western Nigeria call it *Ejula* and *Igbi* respectively. As it is known, Snail uses its slime to regenerate its shell and skin when broken and damaged. Looking at the fact that diabetes is characterized by damage of the pancreatic beta cells, and snail is capable of using its slime to regenerate its broken shell lead to this investigation of the activity of *C. afer* leaf extract, snail slime and their combination for possible anti-diabetic remedy through regeneration of the pancreatic beta cells.

Since time in memorial, people have used herbal medicine or phytomedicine for the purpose of treatment, control and management of varieties of ailments [5] [6] [7].



Figure 1. (a) The leaf of *Costus afer*; (b) Flower of *Costus afer*.

Back home in Nigeria and Africa, much is not known in this developmental trend of prehistoric herbal records of ancient African traditional medicine systems because African tradition systems of medicines are poorly recorded up till date [8] [9].

However, it was discovered and established through consultations with African traditional practitioners and testing that African medicine has therapeutic potentials [10]. The following plants *Acacia senegal* (Gum Arabic), *Agathosma betulina* (Buchu), *Aloe ferox* (Cape Aloes), *Aloe vera* (North African Origin), *Artemisia afra* (African wormwood), etc. has gone through such repeated testing [10].

Based on the discovered therapeutic potentials prior to, during and after the nineteenth century, plant medicines were administered mostly in their crude forms as herbal teas, alcoholic extracts, decoctions (boiled extract of roots or bark), syrup (extracts of herbs made with syrup or honey) or applied externally as ointments in form of balms and essential oils [11] [12].

During the late nineteenth and early twentieth centuries, scientists began isolating, purifying and identifying active ingredients from medicinal plant extracts [7] [12] [13]. This developmental changes led to the discovery of some of the most important drugs that are still widely used today in modern medicine [14], [15] [16]. For example, morphine isolated from opium poppy (*Papaver somniferum*) is a powerful pain reliever and narcotic, quinine isolated from *Cinchona* plant species is an effective anti-malarial drug

From the report of World Health Organization (WHO) [17], 60 % of the world's population depends on traditional medicine, whereas 80 % of the population in developing countries depend almost entirely on traditional medicine practices, for their primary health care needs [18] [19]. Although there is a decrease in the direct use of plant extracts in developed countries in the nineteenth and early twentieth centuries still medicinal plants play a role in health care system of many parts of the world [6] [20]. The long tradition of herbal medicine is still prominent to the present day Nigeria, India, South America, China and many other countries in Africa [6] [19] [20]. To testify for the existence and use of medicinal plants as reported by [6] [20], it is sold alongside other wares in

many village marketplaces of these countries. In Nigeria and other developing countries that practices the use of medicinal herbs, practioneers of herbal medicine often undergo a rigorous and extended training to learn the names, uses and preparation of native plants for many years [8].

The aim of the study is to investigate the anti-diabetic potentials of bioactive crude *Costus afer* methanol leaf extract, giant African land snail slime and their combination through the following objectives:

1) To investigate the acute *in vivo* effect of active crude *Costus afer* methanol leaf extract, snail slime and their combination on fasting blood glucose levels of normal and alloxan-induced diabetic white Albino rats.

2) To investigate the long term (21 days) *in vivo* effects of active crude *Costus afer* extract, snail slime, their combination and Glibenclamide tablets (commercial oral anti-hypoglycemic drug) on fasting blood glucose and body weight in normal and alloxan-induced diabetic white Albino rats.

2. Materials and Methods

2.1. Collection and Preparation of Plant and Animal Materials

Costus afer Ker Gawl was also collected from Duruogbuji-Umuewi village in Njaba L.G.A. of Imo State.

The method of [4] with modification was used for plant preparation whereby the plant leaves were washed, air dried on the laboratory tables and pulverized using Corona hand grinder. To obtain the methanol extracts, 200 g of plant materials was soaked in 1000 ml of methanol using cold maceration technique. In this process, the whole or coarsely powdered crude drug was placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of 2 days (*i.e.* 48 h) with frequent agitation. The mixture then was strained, the marc (the damp solid material) was pressed, and the combined liquids were clarified by filtration. As the residue was removed by filtration, the filtrate was concentrated (distilled, evaporated and vacuum dried) under reduced pressure by a rotary evaporator at 40°C.

2.2. Purchase, Preparation and Extraction of Snail Slime from Giant African Land Snail

The giant African Land Snails was purchased from Afor Awo-Omamma market in Oru-East Local Government Area of Imo State.

The method of [21] with modification was employed for the preparation and extraction of snail slime from the Giant African Land Snail. Giant African Land Snail and snail slime are shown in **Figure 2**. The Giant African Land Snails (*Achachatina marginata*) were washed with clean water to remove dirt and dust particles on the shells. The shells were knocked open at the apex. The inner content (*i.e.* fleshy body) of the snails was separated from the shells by a mechanical means involving the use of a spirally coiled rod inserted to remove the fleshy body. The mucus layer (slime) was gently scrapped off from the fleshy parts,

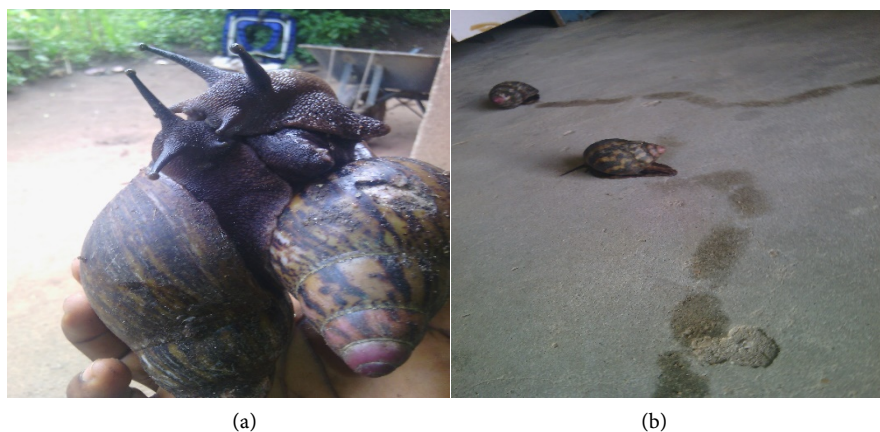


Figure 2. (a) Giant African Land Snail; (b) Trail of Snail (snail slime).

pooled together in a container and precipitated with chilled acetone. The precipitates were freeze dried (-4°C) to obtain greyish-brown flakes of the snail slime extract, which was then pulverized into fine powder bottled and stored in a refrigerator for further studies.

2.3. Formulation (Combination of *C. afer* and Snail Slime Extract) for Anti-Diabetic Drug

The method of [22] was employed for the drug formulation with slight modification. Equal ratio (1:1) of *Costus afer* and Snail Slime extracts was properly mixed using a magnetic stirrer. The drug formulation was achieved by allowing the *Costus afer* extract in the beaker stirred at 3000 rpm for 10 min by the magnetic stirrer before the drop wise addition of the Snail Slime onto the *Costus afer* extract using 10 ml syringe continuously. The mixture was further allowed to stir for 30 minutes for proper homogenization. The content was dried in an oven at 40°C overnight (12 h). The resulting formulation (drug) was dissolved in double distilled water and administered to the animals orally.

2.4. Procurement and Laboratory Maintenance of the Animal Model (Rats) Used for the Study

Albino rats of equal but different sexes with body weight ranging from (140 - 220g) were obtained from University of Jos, Nigeria, and used for the study. The animals were maintained in a laboratory temperature between 28°C to 32°C . The animals were fed standard rat pellets feed and filtered water *ad libitum*. The animals were kept in individual cages in an environmentally controlled room with a 12 h light/12h dark cycle. Animals described as fasted were deprived of food for 24 h but allowed free access to water. The animals were used according to the institutions' approved animal ethics guidelines for research and analysis.

2.5. Experimental Grouping of Animal

The experimental rats were divided into nine (9) groups of five (5) animals in each group. The rats that showed diabetic and healthy were randomly selected

and distributed into 9 groups of 5 animals each. Animals in the different groups received either distilled water, left untreated or graded doses (100 mg/kg and 300 mg/kg) of the extracts and standard hypoglycaemic drug (Glibenclamide). The extract was administered for a period of 21 days (3 weeks). Body weights of the animals were recorded every week. The animal grouping is as follows:

Group 1: Diabetic control (Untreated).

Group 2: Normal control (Distilled water).

Group 3: Snail Slime (SSE); 100 mg/kg b.w.

Group 4: Snail Slime (SSE); 300 mg/kg b.w.

Group 5: *Costus afer* Leaf Extract (CaLE); 100 mg/kg b.w.

Group 6: *Costus afer* Leaf Extract (CaLE); 300 mg/kg b.w.

Group 7: The combined extracts (CaLE/SSE); 100 mg/kg b.w.

Group 8: The combined extracts (CaLE/SSE) 300 mg/kg b.w.

Group 9: Glibenclamide (Standard Pharmaceutical drug); 5 mg/kg.

CaLE = *Costus afer* Leaf Extract, SSE = Snail Slime Extract, b/w = Body weight.

2.6. Induction of Experimental Diabetes

Diabetes Mellitus (DM) type 1 was induced in groups of 25 Albino rats by intraperitoneal administration of alloxan monohydrate (150 mg/kg body weight) after an overnight fast (with access to only water) of 12 h to make them more susceptible to developing diabetes [23] [24]. After 24 h, rats with diabetes mellitus was indicated by glycosuria and hyperglycemia with blood glucose range of 180 to 200 mg/dL body weight and bearing in mind to record the initial blood glucose level of all experimental rats at the start of the experiment. The animals were maintained in a diabetic state over a period of 21 days (3 weeks). Fasting blood glucose level was measured using glucometer (ONETOUCH-Ultra2 Blood Glucose Monitoring System).

2.7. Animal Treatment with Extract (*Costus afer* Plant and Snail Slime)

The plant and snail slime extracts were dissolved in distilled water according to the recommended graded doses of 100 mg/kg and 300 mg/kg of body weight for the experiment and given to the animals orally using an oral feeding needle (gavage). The blood glucose level was immediately monitored by One Touch Glucometer which is expressed in mg/dL of blood and the body weight determined at the same time. The blood sample were collected at on the following days (Day 0, Day 4, Day 7, Day 14 and Day 21) for all the nine groups of 5 animals each, by tail bleeding.

2.8. Induction of Experimental Diabetes

The fasting blood glucose levels test was performed in experimental and control rats in accordance to the method described by [4] [23] [25] with some modifications. This study was carried out using both male and female albino rats of body

weight from 140 - 220 g. They were grouped and kept with not more than 5 animals per cage under similar laboratory conditions. The male rats that has fasted for 18 h was induced with 100 mg/kg of freshly prepared alloxan monohydrate intraperitoneally while the female rat of similar fasting hours was induced with diabetes after intraperitoneally (i.p) administration of 90 mg/kg of alloxan monohydrate. After three days the blood samples of the rats were collected and tested (analyzed) for blood glucose level. All the rats that showed blood glucose level above 180 - 245 mg/dL were considered and selected as diabetic and used for further analysis. The entire fasting blood glucose test was carried out using Glucometer (ONETOUCH-Ultra2 model).

2.9. Determination of the Volume of Extract to Be Administered to the Animals

The volume of Plant and Snail Slime extracts administered to the animals was calculated using the method adopted by [26]:

$$\text{Volume of the extract (mL)} = \frac{\text{Weight of rat (kg)} \times \text{Dose rate (mg/kg)}}{\text{Extract concentration (mg/mL)}}$$

2.10. Long Term Anti-Diabetic effects of *C. afer* Leaf extract, Snail Slime, Combined *C. afer* and Snail Slime Extract and Standard Pharmaceutical Drug on Alloxan Induced Rats

Alloxan-induced and normal experimental rats were given a daily graded dose of 100 mg/kg body weight and 300 mg/kg body weight of *C. afer* leaf extract, snail slime, combined *C. afer* and Snail slime extract and standard pharmaceutical drug orally for three weeks. Control animals receive only water during the same period. Fasting blood glucose levels were determined by means of ONETOUCH-Ultra2 Glucometer (a diabetic fasting blood glucose monitoring system with blood glucose test strips) on day 0, day 4, day 7, day 14, and day 21 after initiation of treatment. Body weights of all groups of rats were measured on the same days that blood glucose levels were accessed.

3. Results and Discussion

The 21 Days Effect of *C. afer* Leaf Extract, Snail Slime and Combined *C. afer* and Snail Slime Extract With Standard Synthetic Hypoglycaemic Drug on Fasting Blood Glucose Levels of Alloxan Induced Rats

Table 1 and **Table 2** shows the summary of the result of the acute effect of low dose (100 mg/kg) and high dose (300 mg/kg) *C. afer* leaf extract, Snail Slime and combined *C. afer* and snail slime extract on blood glucose levels after oral daily administration for 21 days. The result obtained for the mean and standard deviation on five treated animal model per group (Mean \pm S_D; and n = 5) were shown on **Table 1** and **Table 2**.

From the investigation shown on **Table 1**, there was an indication of significant reduction of the fasting blood glucose level from an average of 228 mg/dL

Table 1. Shows the result of the effect of low dose (100 mg/kg) *C. afer*, Snail Slime, and, combined (*C. afer* and snail slime) extract on blood glucose level of alloxan induced Albino rats.

Group	Dose (mg/kg)	Differences in Body weight (g)	Blood glucose levels (mg/dL)				
			Day 0	Day 4	Day 7	Day 14	Day 21
Normal Control		+0.3	110 ± 8	109 ± 6	111 ± 3	110 ± 6	114 ± 7
CaLE	100	-0.4	228 ± 12	212 ± 7	185 ± 11	142 ± 6	125 ± 7
SSE	100	-0.3	220 ± 11	204 ± 11	183 ± 13	160 ± 7	133 ± 2
CaLESSE	100	-0.4	220 ± 7	202 ± 3	180 ± 4	145 ± 4	124 ± 4
GC	5	-0.4	239 ± 13	190 ± 123	145 ± 9	123 ± 5	103 ± 2

S_D = standard deviation; Mean and n = number of the animals per group; - = reduction in body weight, + = increase in body weight, CaLe = *Costus afer* Leaf Extract, SSE = Snail Slime Extract, GC = Glibenclamide.

Table 2. Shows the result of the effect of high dose (300 mg/kg) *C. afer*, Snail Slime, and, combined *C. afer* and snail slime extract on blood glucose level of alloxan induced Albino rats.

Group	Dose (mg/kg)	Difference in Body weight (g)	Blood glucose levels (mg/dL)				
			Day 0	Day 4	Day 7	Day 14	Day 21
Normal Control		+0.3	110 ± 8	109 ± 6	111 ± 3	110 ± 6	114 ± 7
CaLE	300	-0.9	217 ± 15	187 ± 10	163 ± 5	128 ± 4	118 ± 2
SSE	300	-0.5	218 ± 4	197 ± 8	172 ± 4	148 ± 3	123 ± 3
CaLESSE	300	-0.9	204 ± 9	183 ± 8	164 ± 3	139 ± 4	120 ± 2
GC	5	-0.4	239 ± 13	190 ± 13	145 ± 9	123 ± 5	103 ± 2

S_D = standard deviation; Mean and n = number of the animals per group; - = reduction in body weight, + = increase in body weight.

for the low dose of 100 mg/kg body weight of the animal on treatment with *Costus afer* methanol leaf extract to 125 mg/dL after 21 days oral administration. There was no observable change indicated with regards to the body weight of the animals. The change in the average body weight before the experiment and after the experiment was 0.4 g (that is a reduction in weight which was not significant after 21 days of oral administration of the extract).

From the investigation shown on **Table 2**, the effect of high dose of 300 mg/kg *Costus afer* methanol leaf extract on fasting blood glucose level for 21 days oral administration yielded an average significant reduction of 99 mg/dL (i.e. from 217 mg/dL to 118 mg/dL). The body weight reduction gave an average of 0.9 mg/dL which was not significant. However the high dose of the *Costus afer* methanol leaf extract on blood glucose level for 21 days oral administration shows more effect of hypoglycemia activity than the lower dose.

Similarly, **Table 1** shows the effect of low dose of 100 mg/kg snail slime (SSE) on blood glucose level was investigated on five rats for 21 days oral administration, indicated a reduction in blood glucose level from day 0 to day 21 with an average reduction of 87 mg/dL associated with non-significant body weight reduction of 0.3 g. The effect for high dose of 300 mg/kg of snail slime on blood

glucose level after 21 days of oral administration gave an average of 95 mg/dL blood glucose reduction and a body weight reduction of 0.5 g as shown on **Table 2**.

For the combined *C. afer* and snail slime extract as shown on **Table 1** and **Table 2**, indicated that a low dose of 100 mg/kg had an average blood glucose level reduction of 95 mg/dL and a body weight reduction of 0.4 g whereas the high dose of combined *C. afer* and snail slime extract (300 mg/kg) gave a blood glucose level and body weight reduction of 84 mg/kg and 0.9 g respectively.

To buttress the investigation, a 5 mg/kg standard hypoglycaemic drug (Glibenclamide) was also administered on the animals at similar conditions to compare the effect of the plant material (*C. afer*), snail slime and the combined *C. afer* and snail slime extract on blood glucose level of the animal after 21 days oral administration. The result indicated that there was a significant reduction on blood glucose level to a tune of 136 mg/dL while the body weight of the animals was reduced to 0.4 g.

The following plots indicated the blood glucose level effects of the extract, slime and combined *C. afer* and snail slime with the standard pharmaceutical drug (Glibenclamide, 5 mg/kg) respectively over 21 days oral administration. In each of the presentation the normal control and the standard pharmaceutical drug was constantly used to compare the plant extract, the snail slime and the *C. afer* and snail slime extract in each case for effect of the blood glucose reduction.

From the investigation shown on **Figure 3**, there was an indication of reduction of the fasting blood glucose level from an average of 228 mg/dL for the low dose of 100 mg/kg body weight of the animal on treatment with *C. afer* methanol leaf extract to 125 mg/dL after 21 days oral administration. There was no observable change indicated with regards to the body weight of the animals. The change in the average body weight before the experiment and after the experiment was 0.4 g (that is a reduction in weight which was not significant after 21 days of oral administration of the extract).

From the investigation shown on **Figure 4**, the effect of high dose of 300 mg/kg *C. afer* methanol leaf extract on fasting blood glucose level for 21 days oral administration yielded an average reduction of 99 mg/dL (i.e. from 217 mg/dL to 118 mg/dL). The body weight reduction gave an average of 0.9 mg/dL which was not significant. However the high dose of the *C. afer* methanol leaf extract on blood glucose level for 21 days oral administration shows more effect of hypoglycemic activity than the lower dose.

Similarly, as shown on **Figure 5**, the effect of low dose of 100 mg/kg snail slime (SSE) on blood glucose level was investigated on five rats for 21 days oral administration. There was an indication of significant reduction in blood glucose level from day 0 to day 21 with an average reduction of 87 mg/dL associated with non-significant body weight reduction of 0.3 g.

As shown on **Figure 6**, the effect for high dose of 300 mg/kg of snail slime on blood glucose level after 21 days of oral administration gave an average of 95 mg/dL blood glucose reduction and a body weight reduction of 0.5 g.

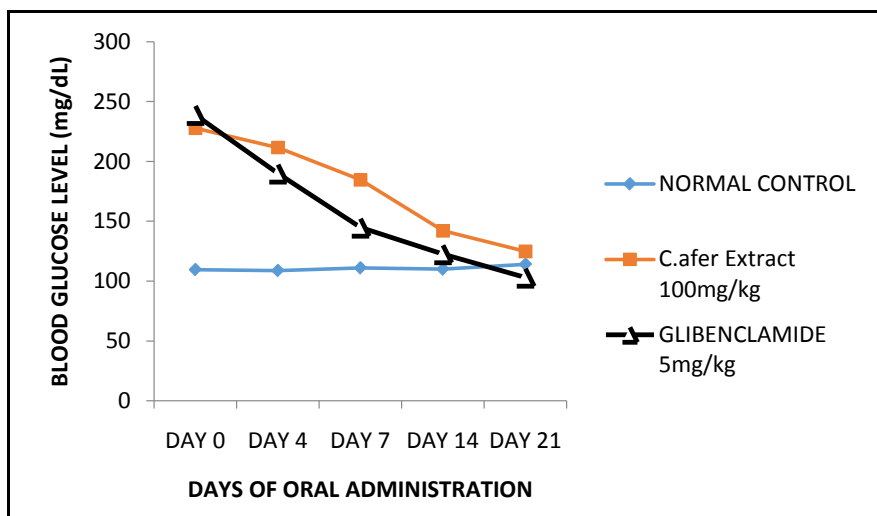


Figure 3. Effect of *C. afer* (100 mg/kg bw) and Glibenclamide (5 mg/kg) on Swiss albino rats.

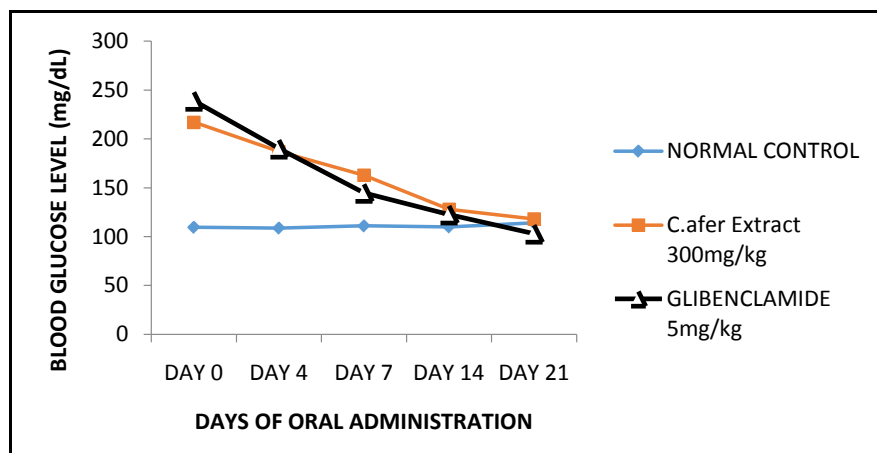


Figure 4. Effects of *C. afer* (300 mg/kg bw) and Glibenclamide (5 mg/kg) on Swiss Albino Rats.

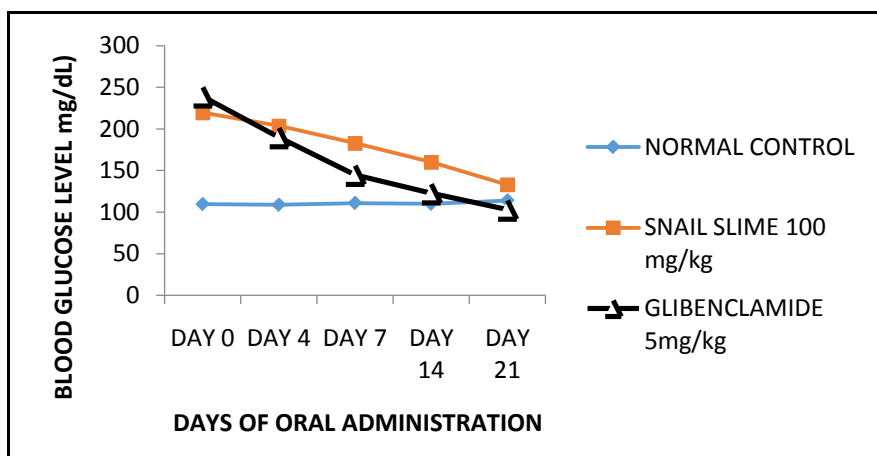


Figure 5. Effects of Snail Slime (100 mg/kg bw) and Glibenclamide (5 mg/kg) on Swiss Albino Rats.

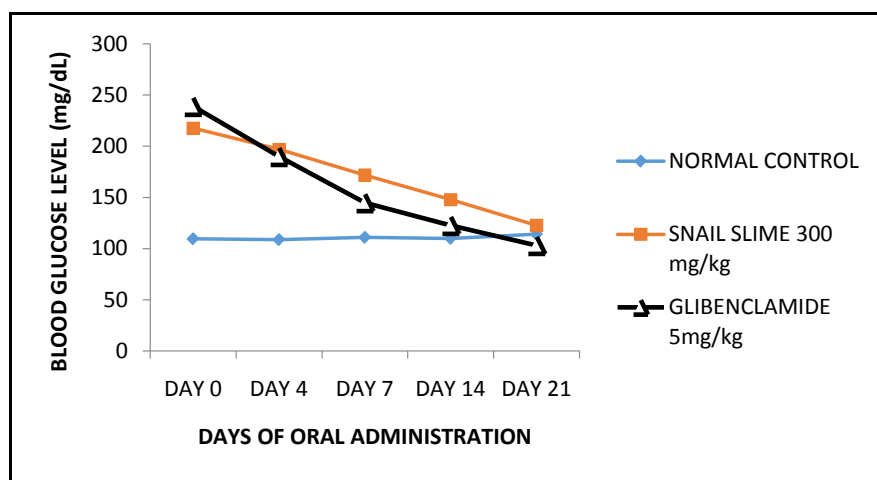


Figure 6. Effects of Snail Slime (300 mg/kg bw) and Glibenclamide (5 mg/kg) on Swiss Albino Rats.

For the combined *C. afer* methanol leaf extract, and snail slime extracts on **Figure 7**, it was observed that a low dose of 100 mg/kg had an average blood glucose level reduction of 95 mg/dL and a body weight reduction of 0.4 g whereas the high dose of combined *C. afer* methanol leaf extract and snail slime at 300 mg/kg gave a blood glucose level and body weight reduction of 84 mg/kg and 0.9 g respectively as shown on **Figure 8**.

To buttress the discussion on the investigation, a 5 mg/kg standard hypoglycaemic drug (Glibenclamide) was also used on the animals to compare the effect of the plant material (*C. afer*), snail slime and the combined *C. afer* and snail slime extracts on blood glucose level of the animal after 21 days oral administration. The result indicated that there was a reduction on both blood glucose level and body weight of the animals as 136 mg/dL and 0.4 g respectively. Similar result was obtained in the work of [27] where significant reduction of fasting blood glucose level was observed for doses of 100, 200, and 400 mg/kg body weight at 44.91, 38.70 and 53.63% respectively in comparison to the reduction obtained with glibenclamide (5 g) used as standard drug.

4. Conclusions

Since prehistoric time people have tried to find medications to ameliorate and cure different illnesses. At every successive century from the development of humankind with the trend of civilizations, the healing properties of many medicinal plants were identified, noted, and conveyed to our generation as detected on *C. afer* leaf and snail slime extracts in this investigation. The results of the effect of *C. afer* leaf methanol extract, snail slime and combined *C. afer* and snail slime extracts on blood glucose level revealed that at high dose of 300 mg/kg body weight of the animal there was average reduction of blood glucose level without any significant side effect as compared to the low dose of 100 mg/kg body weight of the animal for the same extracts after 21 days oral administration.

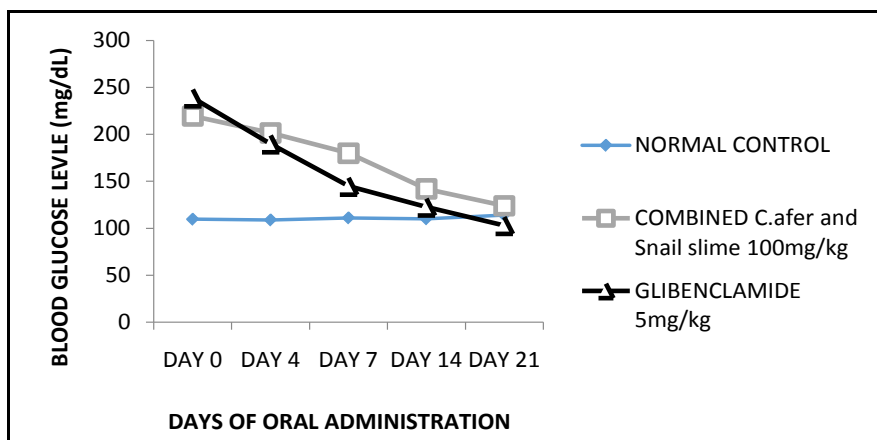


Figure 7. Effects of combined extract (CaLME/SSE) (100 mg/kg bw) and Glibenclamide (5 mg/kg) on Swiss Albino Rats.

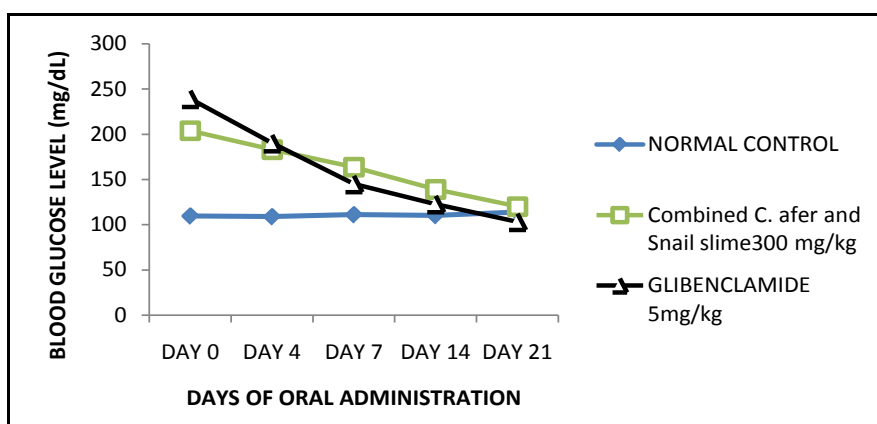


Figure 8. Effects of Combined extracts (CaLME/SSE) (300 mg/kg bw) and Glibenclamide (5 mg/kg) on Swiss Albino Rats.

The results obtained when alloxan induced rats was treated with *C. afer* leaf methanol extract, Snail slime extract, and combined *C. afer* and snail slime extracts was analysed using Statistix 8.0 American version. The results showed a dose dependent fashion and the difference obtained from the compared results was statistically significant at $p < 0.05$.

Conclusively, this investigation may achieve the formation of new strategies for the development of novel antidiabetic drug to treat serious condition like diabetes which represents a global public health problem. Based on the idea that snail uses its slime to regenerate its shell and skin when broken or damaged hence the regenerative capacity of snail slime and the fact that diabetes is characterized by damage of the pancreatic beta cells, may give credit to the reduction of blood glucose level observed in the use of *C. afer* methanol leaf extract and snail slime for possible for anti-diabetic treatment.

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