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Comparative Study of Anti - Diabetic Effects of Ginger Lily (*Costus afer Ker Gawl*) Leaf and Snail (*Archachatinamarginata*) Slime on Alloxan Induced Swiss Albino Rat and their Absorptivity Characteristics

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Abstract: ***The study of anti-diabetic effects of Ginger Lily (Costus afer Ker Gawl) leaf which belongs to the family of Costaceae and Giant African Land Snail (Archachatinamarginata) in this study has shown that their methanol extract is capable of reducing the blood glucose level of alloxan induced diabetic Swiss albino rat when treated orally. From the study carried out both the Costus afer leaf and Snail slime showed a positive effect on lowering the blood glucose level of a Swiss albino rat as compared with the standard Pharmaceutical drug (Glibenclamide, 5 mg/kg). The morphological investigation suggested that the pores were grouped into three sizes; small, medium and large pores. It was observed that majority of the pores were small and seen at 0.03μm^2 for the plant extract, snail slime and combined extracts. The medium and large pores in the snail slime (0.58 μm^{2}and 54.87μm^2) are bigger than that in the plant extract (0.15μm^2 and 14.63μm$^{2)}$ and combined extracts (0.12 μm^2 and 3.16 μm^2) This suggests the availability of pores in the snail slime, on which the plant extract can be absorbed for the purpose of surface modification of the snail slime. The size of the medium and large pores in the combined extracts (CaLME/SSE) was lower than the respective pores in the plant extract and snail slime. This suggests high absorption of the plant extract into the pores of the snail slime. These also suggests that the surface of the snail slime was successfully modified by absorption of the plant extract into the pores present in the snail slime which results in the reduction of the sizes of the medium and large pores.***

Keywords: Costus afer, Morphological investigation, Snail slime, Combined extracts, Pores

1. Introduction

As the world population complementarily rises each year with population of people with diabetes therefore research on diabetic treatment is gaining more ground, this rise is expected to hit 439 million by 2030 (Shaw et al., 2010). The knowledge of rise in population of diabetic patient has led to a vast discovery of new medications as well as natural products extracted from herbal plants. Many active ingredients extracted from herbal plants possess therapeutic values such as hypoglycaemic activity and antioxidant action, with many others yet to be discovered. In this work *Costus afer* is studied as a novel medicinal plants with its blood glucose lowering effects tested, studied and confirmed as other plants like *Allium sativum (*Garlic), *Aloe vera, Cinnamomum tamala, Coccinia indica, Gymnema sylvestre (*Gurmar), *Murrayikoningii, Ocimum sanctum, Trigonellafoenum-graecum (*Fenugreek), *Pterocarpusmarsupium (*Indian Kino) *and Syzigiumcumini (*Bailey and Day, 1989; Grover *et al.*, 2002; Bnouham *et al.*, 2006) studied for the same purpose all over the world. The *Costus afer* Ker Gawl. plant is commonly called Ginger lily or Bush cane in English. Amongst the Igbo's it is known as *'Okpete*' or '*Okpoto*' were as in Hausa and Yoruba it is called '*Kakizawa*' and '*Tete-egun'* (Ukpabi *et al.*, 2012; Anaga *et al.*, 2004) respectively. It is as well called '*Mbritem'* in Efik (Iwu, 1983). Spiral Ginger is a common name given to plants of the family *Costaceae* which along with the family *Zingiberaceae* make up a 'super' family of plants generally known as the Gingers (Bukil, 1985). *Costus afer* Ker Gawl. (Family Costaceae) is a tall perennial semi woody herbaceous unbranched medicinal plant with leafy canes, commonly found in moist or shady forests and riverbanks of tropical West Africa including Nigeria (especially Eastern Nigeria), Ghana and Cameroon (Iwu, 1983). It may grow up to 3m high and belong to the family *Costaceae (*Bukil, 1985). *Costus afer* bears terminal inflorescence of white and yellow flowers and is also commonly found in the forest zones of most places including Senegal, South Africa and Guinea and in the most region of tropical Africa, particularly in higher rainfall areas (Bukil, 1985, Ukpabi *et al.*, 2012).

Plate 1.1: Costus afer plant

Plate 1.2: Costus afer leaves on laboratory table ready for drying

In this study, the group of snails commonly referred to as Giant African Land Snails which belong to Phylum Mollusca, Class: *Gastropoda*, Subclass: Heterobranchia, Order: Pulmonata, Infraorder: Stylommatophora, Family: *Achatinidae*, Subfamily: *Achatininae*was used due to its abundance (Pilsbry, 1919; Bequaert, 1950).According to Steve (2013), the composition of snail slime is thought to vary according to species and may subsequently vary in its formulation. He enumerated that mucus consist of a complex mix of proteoglycans, glycosaminoglycans, glycoprotein enzymes, hyaluronic acid, copper peptides, antimicrobial peptides, and metal ions.

Studies have also shown that mucus/ snail slime contains peptides such as mucin which possess antibacterial activity against both Gram positive and Gram negative bacteria.

Plate 1.3: The Giant African Land Snail (*Achachatinamarginata*)

Plate 1.4: The trail of the snail (*Achachatinamarginata*) showing the dried slime

According to American Diabetes Association[ADA],2004, Diabetes Mellitus is a group of metabolic disease characterized by increased level of glucose in the blood (hyperglycaemia) resulting from defects in insulin secretion, insulin action or both.

Normally, a certain amount of glucose circulates in the blood. The major sources of this glucose are absorption of ingested food in the gastrointestinal tract and formation of glucose by the liver from food substances. In diabetes, the cells may stop responding to insulin or the pancreas may stop producing insulin entirely, this leads to hyperglycaemia, which may result in acute metabolic complications such as diabetic ketoacidosis (DKA) and hyperglycaemic hyperosmolar non-ketotic syndrome (HHNS). Diabetes mellitus is characterized by persistent hyperglycaemia from any of several causes, and is the most prominent disease related to failure of blood sugar regulation. Intake of alcohol causes an initial surge in blood sugar, and later tends to cause levels to fall. Also, certain drugs can increase or decrease glucose levels (Rosemary et al., 2006). Blood sugar levels outside the normal range may be an indicator of a medical condition. A persistently high level is referred to as hyperglycaemia; low levels are referred to as hypoglycaemia. Normal value ranges may vary slightly among different laboratories. A person's blood sugar level may be affected by many factors. When operating normally a body's homeostatic mechanism, restores the blood sugar level to a narrow range of about 4.4 to 6.1 mmol/L (79.2 to 110 mg/dL) as measured by a fasting blood glucose test) (http://journal.diabetes.org). The American Diabetes Association (2004) recommends a post – meal glucose level of less than 10 mmol/L (180 mg/dL) and fasting plasma glucose of 5 to 7.2 mmol/L (90-130 mg/dL) (ADA, 2006).

Table 1: The blood glucose level of some animals

Animals	Serum glucose in mg/dL	Reference
Cows	42 – 75	
Sheep	44 – 81	
Goats	48 – 76	
Rats	50 - 130	Eiler (2004)
Cats	61 – 124	
Dogs	62 – 108	Kahn (2005)
Horses	62 – 114	
Pigs	66 – 116	
Rabbits	75 – 155	
Ilamas	90 – 140	
Captured Mountain Goats	26 – 181	Rice et al., (2007)
Beluga Whales	94 – 115	Cornell et al., (1988)
White Rhinoceros	28 – 140	
Harp Seals	88 – 218	
Hooded Seals	135 – 283	Boily *et al.*, (2006)

Source: Eiler (2004)

2. Materials and Methods

2.1 Collection and Identification of Plant (Costus afer)

Fresh leaves of Costus afer were collected from Ohia Ime Orie Umuewi Village in Njaba L.G.A. of Imo State. The plant was carefully protected and parcelled to avoid the leaves drying abnormally and against laboratory practice. It was subsequently transported to Bali, Taraba State. The plant was identified and authenticated by Mr. Cletus

Ukwubile A., the taxonomist, Biology Unit of Science Laboratory Technology of Federal Polytechnic Bali.

2.2. Preparation of Plant Materials and Extraction

The plant leaves were washed; air dried under room temperature on the laboratory tables and pulverized using mechanical Corona hand grinder. To obtain the bioactive extracts of methanol from the plant leaves, three hundred (300 grams) powdered leaf samples were extracted using 1200 mLof methanol using cold maceration technique. The residue was removed by filtration, the filtrate was concentrated, that is, distilled, evaporated and vacuum dried under reduced pressure using rotary evaporator at 40^{o}C.

2.3Purchase of Giant African Land Snails *(Archachatinamarginata)*

The giant African land Snails was purchased from Eke Okwudor market in Njaba L.G.A. of Imo State. They were carefully packaged in a cage and transported to Bali, Taraba State, the experimental/research point (Agu, 2019).

2.4Preparation and Extraction of Snail Slime from Giant African Land Snail

As the Giant African Land Snail arrived to the research venue it was allowed to acclimatize for two weeks. That is, the Snail was allowed to move freely and fed appropriately in a confined environment for two week to remove every shock it sustained from the time of purchase and transportation to the research venue (Agu, 2019).

The method of Adikwu and Nnamani (2005) was employed for the preparation and extraction of snail slime from the Giant African Land Snail. The Giant African Land Snails (*Achachatinamarginata*) 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, pooled together in a container and precipitated with chilled acetone. The precipitates was freeze dried to obtain greyish – brown flakes of the snail slime extract, which was then grounded into fine powder, packaged and stored in a refrigerator until the time for the examination or studies.

2.5 Experimental Grouping of Animal with Drug administration and treatments

According to the work of Agu, 2019 the experimental rats were divided into nine (5) groups of five (5) animals in each group. The rats that showed diabetic and healthy were randomly selected and distributed into 5 groups of 5 animals each. Animals in the different groups received distilled water, left untreated, plant or animal dose of the extracts and standard hypoglycaemic drug (Glibenclamide). The extract was administered for a period of 30 days (4 weeks). Body weights of the animals were recorded every week.

The solutions of the extracts were dissolved in distilled water according to the recommended dose of 300mg/kg for the experiment and given to the animals orally. This was followed by monitoring the effect of blood glucose level for both the plant (C. afer) extract and animal (Snail Slime). The blood samples were collected at Initial (Day 0), First week (Day 7), Second week (Day 14), Third week (Day 21) and Fourth week (Day 30) for all the groups of 5 animals by tail bleeding and the fasting blood glucose level was calculated by Accu – Chek (Active) Glucometer which is expressed in mg/dL of blood.

2.6 Instrumentation analysis

The morphological study of the *Costus afer* extracts, snail slime and the combination of *Costus afer* and snail slime was carried out using both Scanning Electron Microscope (SEM) The pore size analysis which determines the pore sizes of the extracts in micro meter square (μ^2) was established using scanning electron microscope. The micrographs of the extracts were also determined to establish if there was absorption or modification of the surface of the snail slime extract. The scanning electron microscope model used was PHENOM WORLD with magnification of 5000.

3. Results

Table 3.1: The Effect of the 300mg/kg Dose of the Oral Administered Extract (C. afer and Snail Slime) on Blood Glucose Level after Daily Administration for 30 days

Days	Normal control	CaLME (300mg/kg)	SSE (300mg/kg)	CaLME/SSE (300mg/kg)	GC (5mg/kg)
Day 0	109.6	217	217.6	203.8	239
Day 7	108.8	187	197	183	190
Day 14	111	162.8	171.8	163.6	144.8
Day 21	110	128	147.8	139	122.6
Day 30	114	118	122.6	120	103

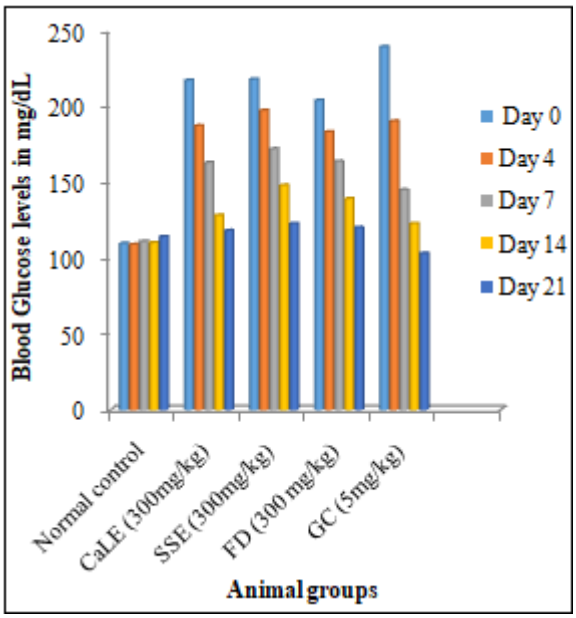

Figure 3.1: Effect of 300mg/kg Dose of C. afer, Snail Slime and their combination on Blood Glucose level after Daily Oral Administration for 30 days

Instrumental analysis used for morphological study of the *C.afer*, Snail slime and Combined extracts (CaLME/SSE).

Pore Size Analysis

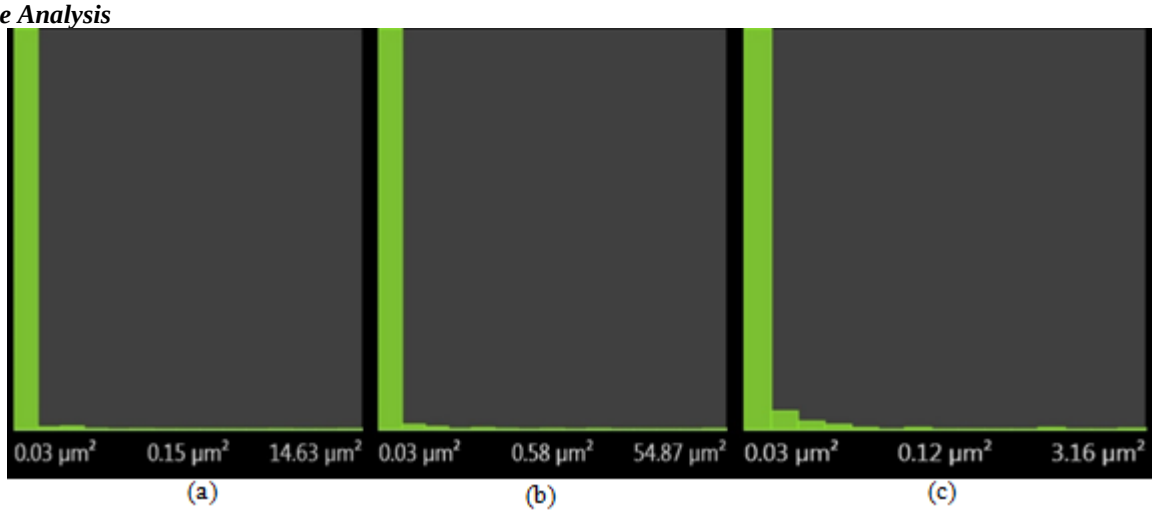

Figure 3.2: Pore Size Analysis for (a) Plant Extract (b) Snail Slime (c) Combined extracts (CaLME/SSE)

4. Discussion

The doses of 300 mg/kg for snail slime, *C.afer* extract and their combination showed significant hypoglycemic effect on alloxan monohydrate induced diabetic rats for 30 days of oral administration or treatment. The significant blood glucose reduction for the snail slime, Costus afer extract and combination of the extracts were 95mg/dL, 99 mg/dL and 83.8 mg/dL respectively. Oral administration of 5 mg/kg glibenclamide also produced significant reduction on induced diabetic rats than the *C.afer* extract, snail slime and the combined extracts for the 30 days of test. On the other hand there were no signs of any side effect of the plant extract (*C. afer*), snail slime and combined extracts on the animals used which is in line with the result obtained from the toxicology determination of Agu et al., 2015. During each of the treatment for snail slime, Costus afer and their combined extracts from day one to the thirtieth day it was observed that a regular gradual decline or reduction in blood glucose level occurred.

The pores are grouped into three sizes; small, medium and large pores. It was observed that majority of the pores are small as shown in the bar chart. The medium and large pores in the snail slime are bigger than that in the plant extract and combined extracts (CaLME/SSE). This suggests the availability of pores in the snail slime, on which the plant extract can be absorbed for the purpose of surface modification of the snail slime. It was observed from (Figure 3.2 c) that there was significant reduction in the sizes of the medium and large pores present in the combined extracts (CaLME/SSE) when compared with those in the snail slime (Figure3.2 b). The size of the medium and large pores in the combined extracts (CaLME/SSE) (Figure 3.2 c) was lower than the respective pores in the plant extract and snail slime. This suggests high absorption of the plant extract into the pores of the snail slime. The presence of a noticeable second bar next to the bar of small pores shows increase in the number of small pores. These also suggests that the surface of the snail slime was successfully modified by absorption of the plant extract into the pores present in the snail slime which results in the reduction of the sizes of the medium and large pores. Onwuka et al., (2016) reported similar findings on the surface modification of *delonixregia* pods using acetylation.

5. Conclusion

The doses of 300 mg/kg for snail slime, *Costus afer* extract and their combination showed significant hypoglycemic effect on alloxan monohydrate induced diabetic rats for 30 days of oral administration or treatment. The significant blood glucose reduction for the snail slime, Costus afer extract and combination of the extracts were 95mg/dL, 99 mg/dL and 83.8 mg/dL respectively. Oral administration of 5 mg/kg glibenclamide also produced significant reduction on induced diabetic rats than the *Costus afer* extract, snail slime and the combined extracts for the 30 days of test.

From the preliminary investigation and examination of the snail slime in both acid and alkaline medium it was observed that the snail slime had different degrees of solubility ranging from not soluble to slightly soluble. This may go a long way to present a snail slime as a carrier of chemical and biological materials for use as nanoparticles in medical and pharmaceutical industry as it may possess the ability to release the drug intermittently due to the presence of pores. Most importantly, since Snail uses its slime to regenerate its shell and skin when 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 hypoglycemic effect observed in *C. afer* leaf methanol extract and snail slime extract for anti-diabetic remedy

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