

NISEB 2010021/11108

Anti-inflammatory effects of aqueous extract of *Cyphostemma glaucophilla* leaves

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(Received July 24, 2010; Accepted November 17, 2010)

ABSTRACT: The aqueous extract of *Cyphostemma glaucophilla* (AQEC) is used to treat kwashiorkor effectively in ethnomedicine. Inflammation is a major symptom of kwashiorkor. The AQEC was therefore investigated for anti-inflammatory effect by the method of Nwodo(1981). AQEC was found to inhibit rat paw Oedema formation induced by Agar suspension in a dose related manner. Similarly, it produced concentration dependent inhibitions of hypotonicity induced haemolysis of human red blood cell suspension, calcium chloride induced aggregation of human platelets and of calcium chloride evoked mitochondrial swelling. It is thought that the extract exerted anti-inflammatory effect by membrane stabilization.

Key words: *Cyphostemma glaucophilla*, anti-inflammatory, inhibitions of platelet aggregation, mitochondrial swelling, hypotonicity, haemolysis.

Introduction

Cyphostemma glaucophilla is a useful medicinal plant commonly found by streams and rivers, it is a perennial herb with prostrate branches rooting from the node. It is found in such places as Nigeria, Togo, Sudan, East Africa, Democratic Republic of Congo and Angola (Bukil, 1985).

The aqueous, leaf extract of *Cyphostemma glaucophilla* is used in alternative medicine for the treatment of kwashiorkor in infants in Kogi and Kwara states of Nigeria. In an interview of randomly selected mothers in herbal homes, they claimed that the children usually got healed in less than two weeks at a dose of four table spoons of the aqueous extract administered orally to the children 3-4 times daily.

Kwashiorkor occurs most commonly in areas where there is limited food supply and low level of education leading to inadequate knowledge of diet and feeding technique. It is prevalent in children fed exclusively on starchy diet such as corn, millet, and cereals which lack the essential amino acids lysine, glycine and Tryptophan (Cohen and Lehman, 2002).

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Inflammation is a major symptom of kwashiorkor other symptoms include swollen and severely bloated abdomen, oedema, failure to thrive, moon face, fatty liver and various skin changes which gives rise to a reddish discoloration of the hair and skin in black African children. The skin becomes dark, dry and split open when stretched revealing pale areas between the cracks (Tang, 1999).

Studies carried out by Gleaner (2004) attributed the swollen abdomen in kwashiorkor to two causes. First the appearance of ascities due to increased capillary permeability from increased production of cysteinyl leucotrienes (LTC₄ and LTE₄) as a result of generalized intracellular deficiency of glutathione. He also attributed it to the suppressant effect of malnutrition on plasma proteins resulting in a reduced oncotic pressure and therefore increased osmotic flux through the capillary wall and secondly due to a grossly enlarged fatty liver.

This study sought to determine the anti-inflammatory effects of the aqueous extract of *Cyphostemma glaucophilla*. This is warranted by the fact that inflammation is a major symptom of kwashiorkor.

Materials and methods

Plant material: The leaves of *Cyphostemma glaucophilla* were collected from the bank of River Niger along Idah - Ibaji road in Kogi state of Nigeria. They were authenticated by Mr. Ozioko, A. Department of Botany, University of Nigeria Nsukka, Nigeria. They were washed to remove dirt, air dried and pulverized with a milling machine into a coarse powder. A voucher specimen (K.S.U No 112) was deposited at the Department of Botany Herbarium Kogi State University Anyigba.

Extraction procedure: A known amount, 80g of the pulverized dried leaves of *Cyphostemma glaucophilla* was macerated in five volume (w/v) water for 18 hours. The Whatman No 4 filtrate of the extract was evaporated to dryness in a boiling water bath and an aliquot of the dry extract was suspended in normal saline and used for determining the following biological activities.

Determination of the effect of aqueous extract on agar induced oedema formation in rat paws.

Oedema was induced with 2% Agar suspension as described by Ezekwesili and Nwodo (1998). Six groups of five rats each were fasted of food but not water for 18 hours. After that, the first group was administered orally with 5ml/kg normal saline. The second group received 100mg/kg phenyl butazone. The third, fourth and fifth groups were served with 2mg/kg, 4mg/kg and 8mg/kg body weight of extract respectively and allowed one hour rest. Then each animal was administered 0.2ml, 2% agar suspension in the sub plantar region of right hind paw. Immediately after that the pore volume of each rat right hind foot was measured by mercury displacement method. Thereafter pore volumes were measured at interval of 30 minute for six hours.

Determination of the effect on calcium induced platelet aggregation.

The effect of the extract on platelet aggregation activity was carried out by a modification of Born method of (Nwodo, 1981).

Nine milliliter samples were drawn by vein puncture from adult who had not taken any kind of drug for two weeks into plastic tubes each containing one milliliter 3.8% trisodium citrate. The blood samples were centrifuged for 10 minutes at 3000g. The supernatants were drawn with plastic draw pipettes and were used as the platelet rich plasma (PRP). Reaction medium final volume (3.0ml) containing 2.8ml normal saline and 0.2ml of PRP was used as the control next to 2.7ml of normal saline was added 0.2ml PRP. Induction of aggregation was by the addition of 0.1ml of 4 mM CaCl₂. The absorbance of the medium, was monitored at 520nm for 6 minutes at room temperature with Genesyl-20 spectrophotometer. Subsequently three different reaction media were made in which increasing concentrations of the extract were added and their absorbance monitored. The order of addition was as follows:

Table 1: Contents of reaction media.

Tubes	Normal saline (ml)	Extract (ml)	CaCl ₂ (ml)	PRP (ml)
1	2.7	-	0.1	0.2
2	2.2	0.5	0.1	0.2
3	1.7	1.0	0.1	0.2
4	1.7	2.0	0.1	0.2

Medium that did not contain PRP but *Cyphostemma glaucophilla* leave extract was used as blank for each tube.

Determination of the effect of *Cyphostemma glaucophilla* extract on hypotonicity induced haemolysis of HRBC.

The precipitate obtained from the supernatant (PRP) was used as the human red blood cells (HRBC). The (HRBC) was re-suspended in a volume of normal saline equal to that of the plasma and used for the determination.

The reaction medium final volume (2.1 ml) containing 0.1ml of HRBC, 1.0ml normal saline and 1.0ml of water used as the control, was incubated for 30 minutes at 37⁰ and centrifuged at 3000g for 10 minutes, supernatant was drawn and its absorbance at 418nm was measured using appropriate blank containing scalar concentrations of *Cyphostemma glaucophilla* leaf extract without HRBC. Three different reaction media were further made in which various increasing concentrations of the extract were added. They were incubated for 30 minutes at 37⁰C and centrifuged at 3000g for 10 minutes. The supernatants were drawn and their absorbance at 418nm was measured using General 20 spectrophotometer. Table 2 shows the content of the different tubes.

Table 2: Contents of reaction media.

Tubes	HRB C (ml)	NORMAL SALINE (ml)	WATER (ml)	EXTRACT (ml)	INCUBATE FOR 30 MINUTES At 37°C	CENTRIFUGE AT 3000g FOR 10 MINUTES.	OD 418nm	AT
1	0.1	0.9	1.0	0.1	√	√	√	
2	0.1	0.8	1.0	0.2	√	√	√	
3	0.1	0.6	1.0	0.4	√	√	√	

Absorbance readings were read at 0.5nm intervals

CaCl₂ induced mitochondrial swelling

Livers excised from freshly killed albino rats were rinsed, weighed and homogenized. Homogenized liver 10% w/v in 0.25M sucrose solution was centrifuged at 3000g x 30 minutes to remove particulate matter, then at 8000g x 30 minutes to remove up to the lysosomes and finally at 10000g x 30 minutes, to bring down the supernatant containing the mitochondria. Six test tubes were set up containing the mitochondrial suspension in 0.25M sucrose-normal saline and were treated as shown below (table 3). Their absorbance was measured at 520nm at intervals of one minute for six minutes. A graph showing the relationship of absorbance with time was plotted.

Table 3: Contents of reaction media.

Tubes	Mitochondrial suspension (ml)	CaCl ₂ (3mM) (ml)	Extract (ml)	Normal saline (ml)
1	0.2			1.8 control
2	0.2		0.2	1.6
3	0.2	0.1		1.7
4	0.2	0.1	0.2	1.5
5	0.2	0.1	0.3	1.4
6	0.2	0.1	0.4	1.3

Results

Fig 1 Show that sub plantar treatment of rats in their right hind paws with 2% Agar suspension induced oedema in all rats that were administered normal saline (control). The figure shows also that relative to control the animals that received phenyl butazone (100mg/kg) the paw volumes produced by agar treatment reduced significantly ($p < 0.05$).

When compared to controls the aqueous extract inhibited significantly and in a dose dependent manner the paw oedema formation induced by Agar treatment. The fig shows clearly that effect of above 2mg/kg the extract was more effective than 100mg/kg phenyl butazone.

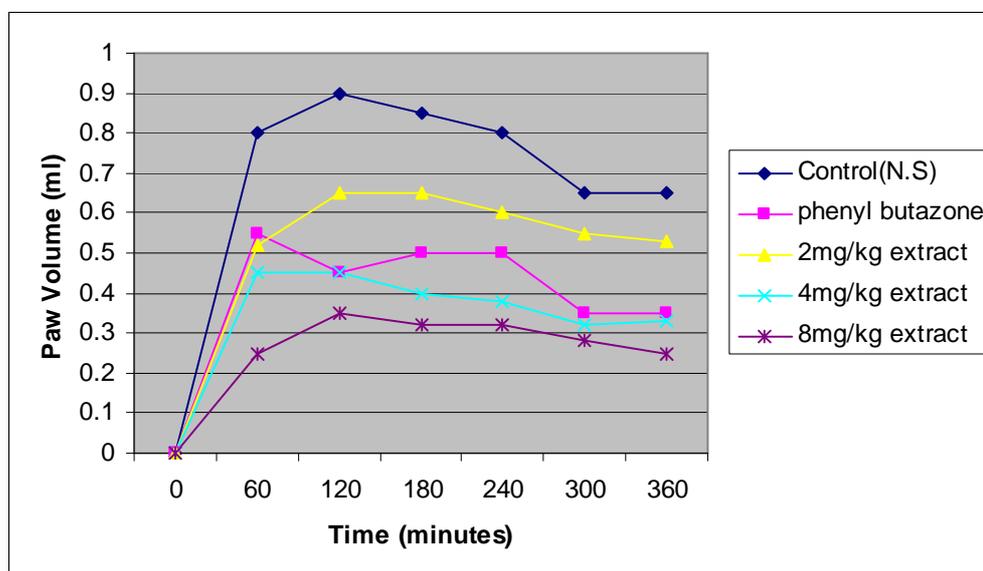


Fig1: Effect of the aqueous extract on Agar induced Rat Paw oedema.

Fig. 2 shows that there was a dose dependent decrease in absorbance which implies that it enhances viscosity of blood and does not cause occlusion which is an anti aggregator activity.

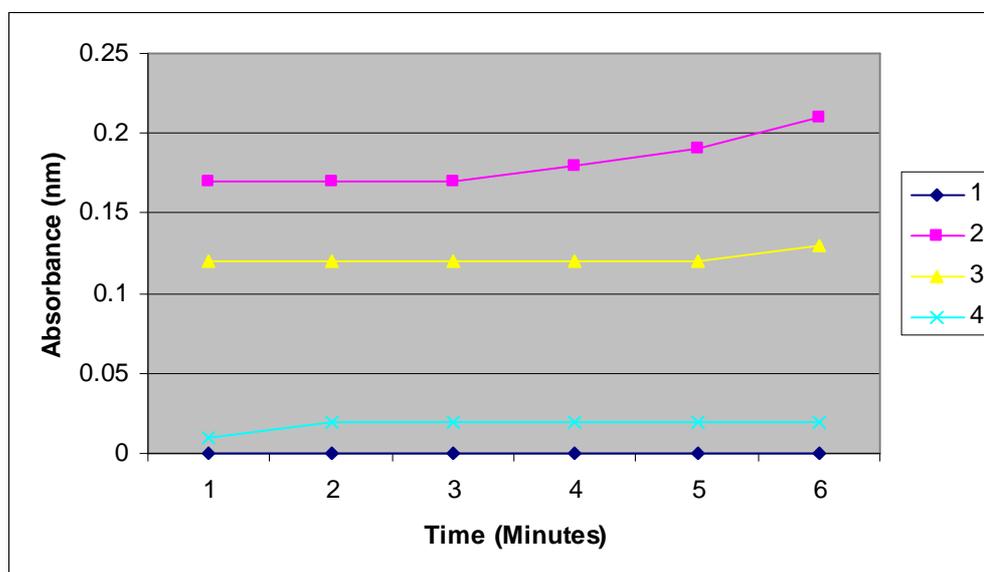


Fig2: Effect of extract on calcium chloride induced platelet aggregate activity

Table 1: Effect on Hypotonicity Induced Hemolysis.

Tubes	Concentration of extract(mg/kg)	Absorbance at 418nm	percentage haemolysis	Percentage inhibition
X1	0.1ml water	0.41	100	0
X2	0.2mg/ml	0.36	87.5	12.5
X3	0.4mg/ml	0.26	62.2	37.5
X4	0.1ml N.S	0.01	0	100

The erythrocytes were fairly stable in normal saline, the absorbance at 418nm was 0.01. Incubation of the erythrocyte in water a low Osmotic medium caused the absorption of the supernatant solution at 418nm to increase to 0.41. The value 0.41 was the maximum lysis of the red blood cells. Extract decreased the absorption of the supernatant after incubation. Concentrations of extract at 0.2mg/ml inhibited lysis by 12.5% and 0.4mg/ml extract inhibited lysis by 37.5%. The extent of haemolysis was dose dependently reduced.

Fig 3 shows that the rat mitochondrial suspension was fairly stable within the duration of study (six minutes). It also reveals that the extract did not alter this stability. However, 3mM calcium chloride decreased the absorption hence, caused swelling of the mitochondria. It was fascinating to note that the extract produced a dose related inhibition of the rate of calcium chloride induced swelling of the mitochondria.

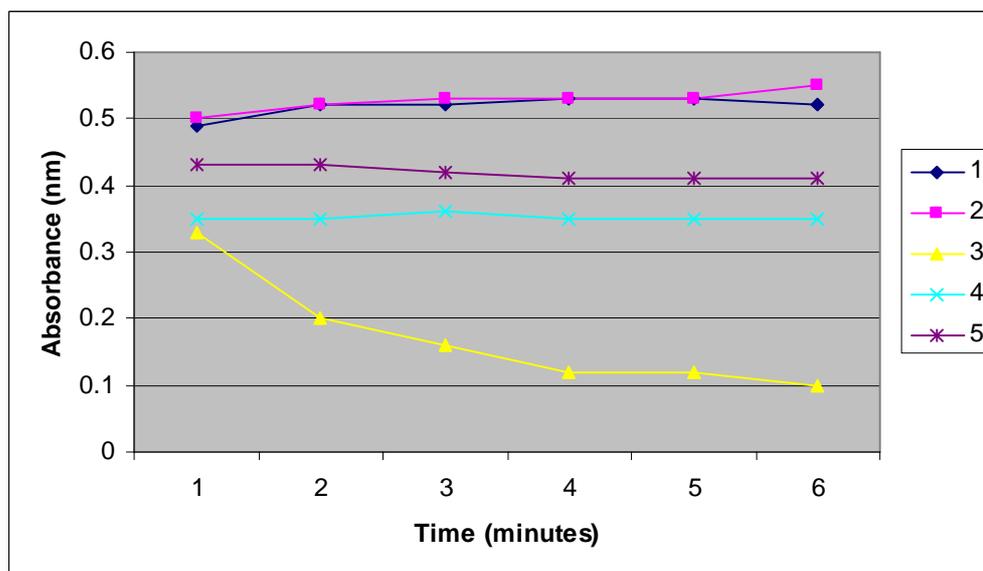


Fig 3: Effect of extract on calcium chloride induced mitochondrial swelling

Discussion

In most experiments, experimentally induced paw oedema in rats was achieved with carrageenin. In this study the same result was produced by Agar; thus substantiating the finding of Ezekwesili and Nwodo(1998) that agar, a chemical analogue of carrageenin induces oedema in rats.

The findings that the aqueous extract of *Cyphostemma glaucophilla* inhibited the oedemogenic effect of agar in rat paws showed that the leaves of *Cyphostemma glaucophilla* contain some anti-inflammatory agents and consequently possess anti-inflammatory activity. The extract was found to produce comparable effect as the standard anti-inflammatory drug at about x1/50 dose. Evidently, the effect produced by 4mg/kg extract was significantly ($p < 0.05$) more than that produced by 100mg/kg phenyl butazone.

The anti-aggregatory activity of the extract when it was investigated on its effect on platelet aggregatory activity (fig 2) even when the platelet rich plasma was challenged with calcium chloride supports its interference with calcium utilization (Jansco, 1991). The extract is therefore able to inactivate the action of platelet activating factor which is a potent molecular signal that is released from leukocytes called basophils and stimulates platelets aggregation. Platelet activating factor also exerts a variety of effects on organs like the smooth muscle, heart and plays an important role in inflammation and allergic responses.

In Table 1, the decrease in absorbance at 418nm while the effect of the extract on hypotonicity induced haemolysis was carried out at different increasing concentrations suggest that it stabilizes erythrocyte membrane confirming its anti-inflammatory property, so it can inhibit the activities of phospholipase A₂, non-steroidal anti-inflammatory drugs; steroidal inflammatory drugs of mobilizing the substrate arachidonic acid from phospholipids fatty acids for the production of inflammatory mediators (Eze and Nwodo, 2001).

In Fig 3, Mitochondria responds to metabolic changes such as energy status by induction of permeability transition pore (PTP) leading to cell damage (virolainen *et al.*, 2002). Accumulation of Ca²⁺ ions into the mitochondrial matrix induces the opening of permeability transition pore, leading to loss of inner membrane potential; this results in swelling of the mitochondrial matrix (Smaili *et al.*, 2000). The observation showing that the aqueous extract of *Cyphostemma glaucophilla* inhibited CaCl₂ induced swelling of the mitochondria in a dose dependent manner indicate that the extract stabilizes mitochondrial membrane. (Virolainen *et al.*, 2002) had also reported that the integrity of the mitochondrial membrane is

maintained by dissipation of inner membrane potential; this could be a possible mechanism by which *Cyphostemma glaucophilla* aqueous extract inhibited CaCl₂ induced swelling.

Cyphostemma glaucophilla leaf extract has been demonstrated by this study to have potent anti-inflammatory effects. It could achieve this effect by membrane stabilisation. As inflammation, specifically oedema is a symptom of kwashiorkor it is evident that the use of the leaves extract in the treatment of kwashiorkor is empirical.

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