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In Vivo Studies of Antibacterial Activity of *Gongronema latifolium* and Antibiotics on *Staphylococcus aureus* Infected Albino Rats

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ABSTRACT

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Many plants in Africa are medicinal. The use of medicinal natural plants predates the establishment of antibiotics and other modern drugs into the African continent. The antibacterial activity of *Gongronema latifolium* and a synthetic antibiotic (Rocephin) on a clinical isolate of *Staphylococcus aureus* was evaluated *in vivo* using albino Wister rats. Five (5) adult female rats with weight ranging from 120 – 180 kg were purchased, housed, fed and nurtured for five days. Four (4) of the rats were inoculated with 0.1 ml 4hour growing broth culture of *Staphylococcus aureus*. Blood was collected from the rats prior, during and after inoculation and treatment for haematological analysis and bacteraemia. Treatment was done by administering 0.5ml of Rocephin (equivalent to 0.125mg) to rat 1 and 0.5ml of purified ethanolic plant extract (equivalent to 0.5mg) to rat 2, A combination of both preparations as above was administered to rat 3. Rat 4 was not given any treatment. Results indicated that the extract of *G. latifolium* leaves possesses higher antibacterial activity than the antibiotic Rocephin and the combination therapy. This result has shown the relative potency of *G. latifolium* against *Staphylococcus aureus* bacteria *in vivo*.

Introduction

Gongronema latifolium is a climber tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine (Ugochukwu and Babady, 2002; Ugochukwu *et al.*, 2003). It's wide spread in tropical Africa and can be found from Senegal to Chad to DR Congo. It occurs in rain forest, deciduous and secondary forests and in mangrove and disturbed roadside forest, from sea level up to 900m altitude (Chattopadhyay,

1999). *G. latifolium* leaves are sharp-bitter and bland and are widely used as a leafy vegetable or spice for sauces, soups and salads (Anaso and Onochie, 1999). The plant leaves are very effective as an anti-diarrhoea and anti-tussive (Sofowara, 1982; Iwu, 1993). Although modern medicine may be available in developing countries, the use of herbs in treatment of diseases have often gain popularity for historical and cultural reasons, making traditional medicine an unavoidable

global discuss (Nwangwu *et al.*, 2009; Balogun *et al.*, 2016).

Staphylococcus aureus is a Gram positive, facultative anaerobic coccoid bacterium that is found in the respiratory tract and on skin (Todar, 2008; Enright *et al.*, 2002). This organism cause boils, pimples, impetigo and throat infections and can be spread when carriers handle food. *S. aureus* produces toxins at warm temperatures. *Staphylococci* infections are contagious until the infection has resolved. Direct contact with an infected sore or wound, or with personal care items such as razors, bandages are common routes of transmission. It is a common cause of skin infections such as abscesses and respiratory infections such as sinusitis. *S. aureus* is one of the most common causes of bacteraemia (Tong *et al.*, 2015). Bacteraemia is the presence of viable bacteria in the blood. Bacteria can enter the bloodstream as a severe complication of infections during surgery or due to catheters and other foreign bodies entering the arteries or veins. Bacteraemia can have several consequences as the immune response to the bacteria can cause sepsis and septic shock, which has relatively high mortality rate. It may even lead to haematogenous spread, causing infections away from the original site of infection. Blood is normally a sterile environment, so detection of bacteria in the blood is abnormal (Ochei and Kocktiatkar, 2000).

G. latifolium is a genus of milk weeds first described in 1844. The species are native to Africa, South and South-east Asia. There are over fifteen (15) species including *G. angolense*, *G. bracteolatum*, *G. curtisii*, *G. filipes*, *G. finlaysonii*, *G. gaudichaudii*, *G. latifolium*, *G. multibracteolatum*, *G. nepalense*, *G. obscurum*, *G. taylorii*, *G. thomsonii*, *G. venticosum*, *G. wallichii* and *G. wrayi* (The plant list, 2013).

The Efik/Ibibio people in south-eastern Nigeria call the *G. latifolium* leaves ‘utasi’, the Igbos call it ‘utazi’ and the Yoruba people ‘arokeke’ or ‘madumaro’ (Ugochukwu and Babady, 2002). Various reports have indicated the use of *G. latifolium* in folklore medicine by different ethnic groups. The leaves of *G. latifolium* are used by the Ikales of Ondo state of Nigeria to treat malaria, nausea and anorexia (Morebise and Fafunso, 1998; Morebise *et al.*, 2006). The leaf extract is commonly used by the Efik and Qua tribes of Cross River state of Nigeria to treat malaria, diabetes, hypertension and constipation (Edet *et al.*, 2011).

Materials and methods

Handling and blood collection of rats

Rats were acclimatized to handling to reduce stress. Rats are picked up by placing the hand firmly over the back and the rib cage and restraining the head with the thumb and fore finger immediately behind the mandibles. Holding the rat upside down keeps it distracted and reduces the chances of biting. An adult rat has a circulating blood volume of about 15-35ml (5-7% of the body weight). Blood can be collected from several sites in the rat including tail vein, saphenous vein, retro-orbital sinus, vena cave or by cardiac puncture (Kassel *et al.*, 1953)

The tail of rats was warmed by placing in a bowl of warm water. The rats were restrained before bacterial inoculation, treatment and blood collection. Blood was collected from the tail vein by making a slit 5mm in the tail with a scalpel. The tail is stricken gently with thumb and finger to enhance flow of blood into the collection vial. At the end of the collection pressure is applied to the cut with gauze to ensure blood completely stopped flowing before returning the rat to the cage. Blood was collected on the first, third and last

day of the experiment for haematological analysis. After the housing period, rats were sacrificed on the last day of the experiment by chloroform suffocation (1ml / 4.5kg) and blood was collected from the heart into an EDTA container for haematological analysis. Treatment was done intramuscularly for a period of five (5) days.

Rocephin

Rocephin, a ceftriaxone, is a cephalosporin antibiotic that targets bacterial cell wall in its antibacterial action.

Haematological analysis

Haematology is the branch of medicine that deals with the study, diagnosis, treatment and prevention of blood related diseases. Haematology test include laboratory assessments of blood formulation and blood disorders. Haematological indices of the rats were studied to include the values of the cellular elements of blood like white blood cell counts, lymphocytes, platelets, red blood cells, granulocytes, haemoglobin, etc, using the Sysmex automated blood analyser.

Plant sample

The leaves of *G. latifolium* were purchased from vegetable sellers at Marian market in Calabar municipality, Cross River State. The fresh and matured leaves with no sign of external damage were identified by the Department of Botany, University of Calabar, Calabar. Extraction by Soxhlet method was done in the Department of Biochemistry, University of Calabar, and standardized accordingly. Five hundred grams of leaves were shade-dried, pulverized and packed into the extract column of the Soxhlet apparatus and coupled for extraction with absolute ethanol (250ml) for 24 hours. Finally, the flask containing a deposit and a little of the

ethanol was evaporated to dryness and about 9.5grams of bioactive component (extract) was collected.

Source of organism

Known clinical isolates of *S. aureus* were obtained from the bacteriological laboratory of the University of Calabar Teaching Hospital, Calabar, Cross River State.

Animal model

Five (5) adult female albino Wister rats of the species *Rattus norvegicus* ranging from 120-180grams in weight were obtained from the animal house of the Department of Biochemistry, University of Calabar. The rats were housed separately in five cages and acclimatized for five days under standard laboratory conditions (temperature $27\pm 2^{\circ}\text{C}$ and humidity $55\pm 5\%$) allowing free access to commercial rat feed and water, following due ethical considerations.

Test tubes with 5ml each of sterile peptone water were inoculated with loopful of *S. aureus* and incubated for 4hours. Blood samples of the five (5) rats were collected prior inoculation of 0.1ml of the bacterial broth culture into the animals, except rat 5 which served as negative-control. The plant extract (weighing 9.5g) were dissolved in 20 ml of sterile dimethylsulfoxide (DMSO) and used for treatment of the rats. Similarly, 1g of the synthetic antibiotic Rocephin powder was dissolved in 4ml of sterile injection water.

Treatment

0.5 ml / kg (containing 0.125g of Rocephin) body weight was administered to rat 1; 0.5 ml / kg (containing 0.25 g of *G. latifolium* extract) body weight was administered to Rat 2 while a combination of both was given to Rat 3. Rat 4 served as the positive-control as

it was only inoculated with the broth culture and Rat 5 served as the negative-control as it was neither inoculated with the broth culture nor given any treatment.

Bacteraemia assay

Whole blood from test rats was inoculated into Tryptic Soy agar (Hardy) by pour plate method, incubated for 18-24 hours at 37⁰C, and observed for colonies after incubation.

Results and Discussion

Following intramuscular inoculation of 0.1 ml of broth culture of *S. aureus*, rats 1- 4 developed bacteremia. Haematological test results obtained after inoculation (Table 2) showed differences compared to the test results prior inoculation, indicating the apparent presence of bacterial infection and compromised immunity. There was a significant (P<0.05) decrease in the number of colony forming units in rats 1, 2 and 3 during the treatment process with rat 2 showing the greatest reduction in bacterial numbers as shown in Figure 1.

In conclusion, this study investigated the effect of antibacterial activity of ethanolic extract of *G. latifolium* and that of Rocephin in albino Wister rats. Observed reduction in colony counts of shed blood of infected rats treated with the plant extract suggests its efficacy in the treatment of staphylococcal-related infections.

From the result obtained, rat 2, which was treated with 0.25g of the plant extract, showed the most effective antibacterial response than rat 1, which was treated with 0.125g of Rocephin, and rat 3, which was treated with a combination therapy of 0.25g and 0.125g of plant extract and Rocephin

respectively. It was observed that bacteraemia reduced significantly in rat 2, much more than in rats 1 and rat 3, a phenomenon that corroborates the post inoculation spike in white blood cell levels in rat 2. Rat 4 which was not given any treatment, showed the highest number of colonies indicating a significant (P<0.05) increase in daily bacteremia levels.

Blood culture of Rat 5, which was not inoculated with bacteria nor given any treatment, showed no growth indicating a sterile blood. Very significant (P<0.05) activity of *G. latifolium* against *S. aureus* was observed in rat 2 as there was a gradual decrease in the number of colony forming units. Eja, *et al.* 2011, had established minimum inhibitory concentration of 21.3mg/ml of *G. latifolium* for *S. aureus* in a previous study. Haematological analysis of the infected rats showed a significant (P<0.05) decrease in the number of white blood cells following post inoculation with broth culture and a complementary increase when treatment was administered. This may imply that there was immune depression of the rats by bacterial inoculation followed by a complementary boost of immunity after treatment.

These results complement previous reports on the usefulness of *G. Latifolium* as a versatile antibacterial agent (Enyi-Idoh *et al.*, 2012; Farombi, 2003; Ugochukwu and Babady, 2003). Phytochemical presence agrees with earlier reports of the phytochemical composition of *G. latifolium* that has been shown to consist of about 0.5% flavonoids, 2% tannins, 0.66% saponins, 0.33% polyphenols, 1.97% alkaloids and 13.2% hydrogen cyanide (Enyi-Idoh *et al.*, 2012).

Table.1 Treatment design

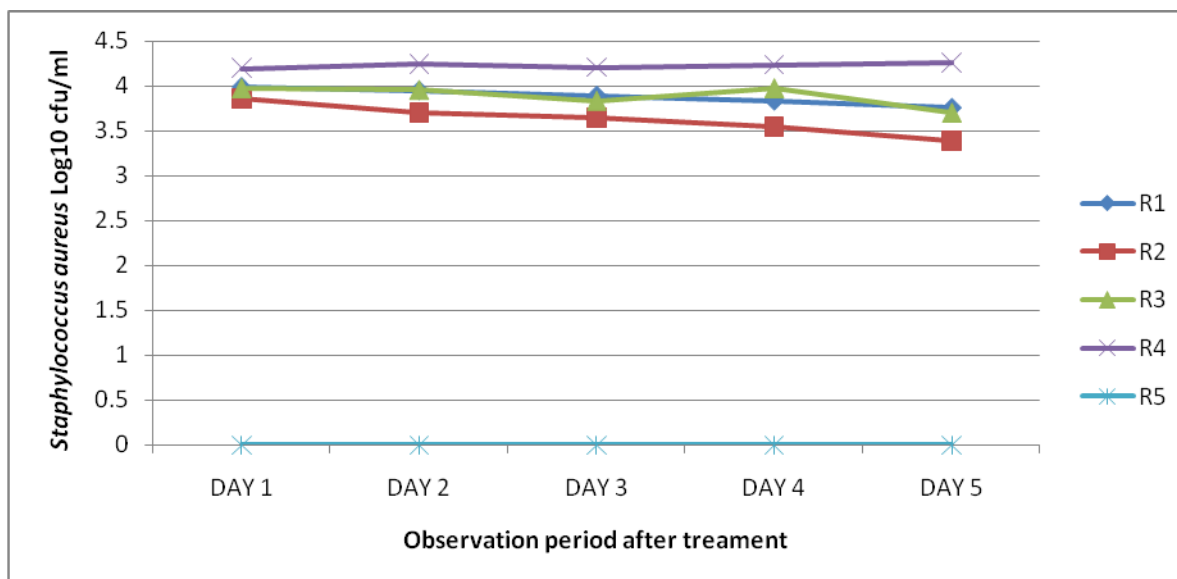
Rat	Treatment	Dosage
Rat 1	Rocephin antibiotic	0.125g
Rat 2	Plant extract	0.25g
Rat 3	Plant extract + Rocephin	0.25g + 0.125g
Rat 4	Inoculated but without treatment	
Rat 5	No inoculation and no treatment	

Table.2 Haematological test results for rats

Inoculations	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Normal range
Pre-inoculation	6.44x10 ⁹ /l	6.45 x10 ⁹ /l	8.10 x10 ⁹ /l	8.32 x10 ⁹ /l	7.53 x10 ⁹ /l	WBC 5.00–10.00/l
Inoculation	3.96 x10 ⁹ /l	4.46 x10 ⁹ /l	5.32 x10 ⁹ /l	7.25 x10 ⁹ /l	7.53 x10 ⁹ /l	
Post inoculation	4.86 x10 ⁹ /l	17.54 x10 ⁹ /l	10.97 x10 ⁹ /l	6.61 x10 ⁹ /l	7.53 x10 ⁹ /l	
Pre-inoculation	4.37 x10 ⁹ /l	6.28 x10 ⁹ /l	4.42 x10 ⁹ /l	5.99 x10 ⁹ /l	3.71 x10 ⁹ /l	LYM 1.30–4.00/l
Inoculation	2.15 x10 ⁹ /l	2.24 x10 ⁹ /l	2.18 x10 ⁹ /l	4.11 x10 ⁹ /l	3.71 x10 ⁹ /l	
post inoculation	3.44 x10 ⁹ /l	8.71 x10 ⁹ /l	6.2710 ⁹ /l	3.22 x10 ⁹ /l	3.71 x10 ⁹ /l	
Pre-inoculation	1.89 x10 ⁹ /l	1.29 x10 ⁹ /l	0.82 x10 ⁹ /l	1.80 x10 ⁹ /l	1.06 x10 ⁹ /l	MID 0.15 –0.70/l
Inoculation	0.66 x10 ⁹ /l	0.44 x10 ⁹ /l	0.21 x10 ⁹ /l	1.35 x10 ⁹ /l	1.06 x10 ⁹ /l	
post inoculation	1.05 x10 ⁹ /l	2.26 x10 ⁹ /l	1.34 x10 ⁹ /l	0.87 x10 ⁹ /l	1.06 x10 ⁹ /l	
Pre-inoculation	3.38 x10 ⁹ /l	3.02 x10 ⁹ /l	2.08 x10 ⁹ /l	4.42 x10 ⁹ /l	2.77 x10 ⁹ /l	GRA 2.50 –7.50/l
Inoculation	1.73 x10 ⁹ /l	1.60 x10 ⁹ /l	1.32 x10 ⁹ /l	2.98 x10 ⁹ /l	2.77 x10 ⁹ /l	
post inoculation	2.11 x10 ⁹ /l	6.57 x10 ⁹ /l	3.36 x10 ⁹ /l	1.96 x10 ⁹ /l	2.77 x10 ⁹ /l	
Pre-inoculation	5.62x10 ¹² ₁	5.83 x10 ¹² ₁	6.22 x10 ¹² ₁	8.22 x10 ¹² ₁	7.18 x10 ¹² ₁	RBC 4.00 -5.00/l
Inoculation	0.28 x10 ¹² ₁	3.11 x10 ¹² ₁	2.48 x10 ¹² ₁	6.90 x10 ¹² ₁	7.18 x10 ¹² ₁	
post inoculation	00.77 x10 ¹² ₁	7.23 x10 ¹² ₁	8.00 x10 ¹² ₁	4.38 x10 ¹² ₁	7.18 x10 ¹² ₁	
Pre-inoculation	3.00 g/dl	6.81 g/dl	12.2 g/dl	16.1 g/dl	14.1 g/dl	Hgb 12.0 -16.0g/dl
Inoculation	1.3 g/dl	4.7 g/dl	5.73 g/dl	121.16 g/dl	14.1 g/dl	
post inoculation	1.4 g/dl	12.8 g/dl	14.4 g/dl	9.9 g/dl	14.1 g/dl	
Pre-inoculation	8.83%	20.45%	36.6%	48.2%	41.43%	HCT 36.0 -48.0/l
Inoculation	3.82%	14.0%	14.3%	36.5%	41.43%	
post inoculation	4.12%	37.45%	42.19%	29.8%	41.43%	
Pre-inoculation	305 x10 ⁹ /l	648 x10 ⁹ /l	520 x10 ⁹ /l	720 x10 ⁹ /l	383 x10 ⁹ /l	PLT 150 -400/l
Inoculation	150 x10 ⁹ /l	399 x10 ⁹ /l	380 x10 ⁹ /l	690 x10 ⁹ /l	383 x10 ⁹ /l	
post inoculation	108 x10 ⁹ /l	789 x10 ⁹ /l	665 x10 ⁹ /l	530 x10 ⁹ /l	383 x10 ⁹ /l	

Key: WBC = white blood cell, LYM = lymphocytes, MID = minimum inhibitory dilution, GRA = granulocytes, RBC = red blood cells, Hgb = haemoglobin, HCT = hematocrit, PLT = platelet

Fig.1 Bacteremia plate count



Hence, it can be concluded that the presence of phytochemical compounds in this plant could be responsible for the in vivo antibacterial activity of the plant extract against the test bacteria. The plant extracts which are known to have substantial quantities of saponins, flavonoids and alkaloids makes it a useful and credible supplement for antibiotics (Enyi-Idoh *et al.*, 2012).

There was significant post inoculation increase in blood parameters in rat 2 as compared to rat1 and a commensurate decrease of bacteraemia in the same period. In view of the huge collection of bioactivity reports on *G. latifolium*, there is need to focus research on the isolation and characterisation of the individual active compounds and their use for bioactivity studies. A possibility of using *G. latifolium* leaf extracts as approved medicinal formulations for staphylococcal-related infections should be explored. Exploitation of the qualities of this plant promises to offer solutions to some prevailing clinical and nutritional conditions, hence an extensive research on its pharmacodynamics and proper standardization is necessary in

exploiting their therapeutic uses to combat various diseases. The results of this work have justified the potency of *G. latifolium* as an antibacterial agent.

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