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ANTI-MICROBIAL STUDIES AND THE EFFECT OF THE AQUEOUS EXTRACT OF *CYPHOSTEMMA GLAUCOPHILLA* LEAVES ON THE CONCENTRATION OF TOTAL PLASMA PROTEINS AND ALBUMIN IN CORN-MEAL INDUCED KWASHIORKOR RATS

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ABSTRACT

C*yphostemma glaucophilla* is used in the treatment of kwashiorkor in ethno-medicine in Kogi and Kwara States of Nigeria. The aqueous extract of pulverised dried leaves of *C.glaucophilla,* administered at various concentrations on some micro organism were evaluated in cultured plates of nutrient Agar. Three different antibiotics; ampiclox, lincomycin and chloramphenicol were used as synthetic drugs to evaluate their comparative efficacy with the extract. Five Groups A, B, C, D and E of rats weighing 100-110g of either sex were served with rat feed and water (control, A). Kwashiorkor was induced in Groups B, C, D and E by given protein deficient diet(corn meal) for 14 days. After 14 days, the malnourished Groups were served oral daily doses of 0.5, 1.0, 1.5 and 2.0 mg/kg b.w of the extract for 14 days respectively while Group A recieved (0.85g NaCl; 5ml/kg). The blood samples were obtained via occular puncture into heparinised centrifuge tubes and spun at 1000rpm for five minutes to obtain the plasma on day 0 (start of treatment) and day 14 (end of treatment) for the determination of the concentration of total protein and albumin by standard methods. Data were analysed by one way ANOVA and student independent t-test and presented as mean ± standard deviation values of $p < 0.05$ were considered significant. *Cyphostemma glaucophilla* leaves extract exhibited anti-microbial properties comparable with the antibiotics and produced dose dependent significant ($p < 0.05$) increases in the concentration of plasma total protiens and albumin in kwashiorkor induced rats.

Key words*: Cyphostemma glaucophilla*, anti-microbial, antibiotics, kwashiorkor

INTRODUCTION

*C**yphostemma* species are native to Africa and some parts of Arabia, they are distributed throughout the desert habitat of Africa and some are endemic to Madagascar (Eggli, 2002). *C. glaucophilla* (Gurr) is used in the treatment of kwashiorkor in ethno-medicine around Kogi and Kwara States of Nigeria.

The aqueous extract of *C. glaucophilla* leaves has no adverse effect at oral doses of up to 3000mg/kg b.w (Ojogbane *et al.,* 2010). The extract induces increases in the concentration of liver and plasma total proteins especially albumin of healthy albino rats. In addition to its lipid lowering effect, the extract has anti-inflammatory and hepato-protective activity (Omale *et al*., 2009; Ojogbane *et al.,* 2010).

Bahn, (2013) had reported that atrophy of the intestinal epithelium in cases of malnutrition causes mal-absorption and accentuate malnutrition. A vicious cycle of diarrhoea-malnutrition-diarrhoea set in, which contribute to large majority of early childhood death either directly or indirectly. Diarrhoea is a common but potentially serious illness in early childhood. It has been shown(Ahmed *et al*., 2010) to have significant effect on nutrition. Study by (Arifin, 2009) identified diarrhoea as the major determining factor leading to malnutrition in developing countries, children with multiple episode of diarrhoea and particularly chronic diarrhoea, suffer most severely from protein energy malnutrition (Oshikoya *et al.,* 2010).
In children, attention are focused on adequate nutritional intake and selective judicious use of anti-microbials. This study is aimed at assessing the microbial activity of the aqueous extract and the effect of the extract on total proteins and albumin concentration in kwashiorkor induced rats.

MATERIALS AND METHODS

Animals:
The animals used in this study were wistar albino rats (100-110) of either sex which were purchased from the Animal House of the Department of Biochemistry, Kogi State University, Anyigba, Kogi State Nigeria in May, 2014.

Plant material:
Cyphostemma glaucophilla leaves were collected in May, 2014 along Idah-Ibaji road in Kogi State, Nigeria. The plant was authenticated by Mr. A. Ozioko of Department of Botany, University of Nigeria, Nsukka, Nigeria.

Micro organism:
The test organism used were *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* obtained from the Department of Microbiology, Nigerian Institute of Medical Research(NIMR) Yaba, Lagos, Nigeria.

Blood samples:
Blood samples collected via occular puncture into heparinised centrifuge tubes were spun at 1000 rpm for five minutes to seperate the plasma from the cells.

Reagents:
Protein and albumin reagent kits employed in this study were obtained from Randox Laboratories Limited, Diamond Road, Crumlin, Co. Antrim, United Kingdom.

Preparation of plant material and extraction:
Cyphostemma glaucophilla leaves were washed to remove dirts, air dried and pulverised with Creson high speed milling machine into a coarse powder. A 400g of pulverised dried leaves of *C. glaucophilla* was macerated in five volumes(w/v) of water for eighteen hours and then filtered. The filtrate was evaporated in a water bath at 100°C to get the dried residue and the percentage yield was calculated.

Induction of kwashiorkor in albino rats:
Albino rats of either sex, weighing 100-110g were assigned into five Groups A, B. C, D and E of five rats each. Group A was the control and received rat feed and clean water, Groups B, C, D and E took protein deficient diet (corn meal) and clean water for 14days respectively. Signs of malnourishment such as falling hairs, weakness, pale bodies were noticeable. Blood samples were obtained via occular pressure into heparinised centrifuge tubes(day 0) for the determination of the concentration of proteins and albumin. The malnourished rats were then served with oral daily doses of 0.5, 1.0, 1.5 and 2.0 mg/kg b.w of the extract while the control Group A, received (0.85g NaCl; 5ml/kg) respectively for 14days. Blood samples collected via occular puncture, 24 hours after the last administration were collected into heparinised centrifuge tubes and spun at

1000 rpm for five minutes to obtain the plasma for the determination of the concentration of protein and albumin (end of experiment) by the Biuret reaction and BCG method of Spencer and Price, (1977).

The anti-microbial activity and minimum inhibition concentration of the extract on the microorganism was by the method of (Perez *et al*., 1990).

RESULTS

At a concentration of 5mg/ml, all the five microorganisms were sensitive to ampiclox, lincomycin and the extract used as shown on Table1. The extract was not as effective as standard antibiotics like ampiclox and lincomycin, however, the zone of inhibition produced by the extract on *Bacillus subtilis* is comparable to that produced by ampiclox and lincomycin.

At the concentration of 10 mg/ml presented in Table 2 all the micro organisms were sensitive to the extract, chloramphenicol, ampiclox and lincomycin. The order of increasing sensitivity is ampiclox > lincomycin > extract > chloramphenicol.

In Table-3 all the micro organisms were sensitive to the extract and the antibiotics treatment at 20mg/ml. The zones of inhibition produced by the extract on *Bacillus subtilis* is comparable to that produced by ampiclox and lincomycin. However, the other organisms *K.pneumonia, P.aeruginosa, E.coli, and S.aureus* were more sensitive to ampiclox and lincomycin than the extract and chloramphenicol respectively.

In Table-4, the minimum inhibition of *Cyphostemma glaucophilla* on *K. pneumonia* was 20mg/ml while for chloramphenicol, ampiclox and lincomycin the minimum inihibtory concentration of 10mg/ml was observed for each. *Cyphostemma glaucophilla, c*hloramphenicol, ampiclox and lincomycin had MIC of 20mg/ml on P. aeruginosa while *Cyphostemma glaucophilla and c*hloramphenicol has MIC of 10mg/ml on *E. coli, a*mpiclox and lincomycin both had MIC of 20mg/ml. The extract had an MIC of 5mg/ml on *B. subtilis*, chloramphenicol and lincomycin both had an MIC of 10mg/ml and ampiclox had MIC of 20mg/ml respectively. Chloramphenicol,

Table 1: Effect of the extract and antibiotics at 5 mg/ml on the growth of organisms

Test Organism	Zones of Inhibition (mm)			
	Cyphostemma glaucophilla	Chloramphenicol	Ampiclox	Lincomycin
Klebsiella pneumonia	5	0	12	10
Pseudomonas aeruginosa	4	0	11	6
Escherichia coli	5	0	10	12
Bacillus subtilus	8	0	9	8
Staphylococcus aureus	5	0	9	6

Table 2: Effect of the extract and antibiotics at 10mg/ml on the growth of organisms

Test Organism	Zones of Inhibition (mm)			
	Cyphostemma glaucophilla	Chloramphenicol	Ampiclox	Lincomycin
Klebsiella pneumonia	4	1	10	9
Pseudomonas aeruginosa	2	2	10	5
Escherichia coli	2	1	9	10
Bacillus subtilus	8	1	8	7
Staphylococcus aureaus	2	1.5	7	5

Table 3: Effect of the extract and antibiotics at 20mg/ml on the growth of organisms

Test Organism	Zones of Inhibition (mm)			
	Cyphostemma glaucophilla	Chloramphenicol	Ampiclox	Lincomycin
Klebsiella pneumonia	1	1	10	9
Pseudomonas aeruginosa	0.5	2	9	5
Escherichia coli	2.0	1	7	8
Bacillus subtilis	8.0	1	7	8
Staphylococcus aureaus	1.0	2	7	5

Table 4: Minimum inhibition capacity (MIC; mg/ml) of the extract and some antibiotics on some selected micro organism.

Test Organism	*Cyphostemma glaucophilla*	chloranphenicol	Ampiclox	Lincomycin
Klebsiella pneumonia	20	10	10	10
Pseudomonas aeruginosa	20	20	20	20
Escherichia coli	10	10	20	20
Bacillus subtilis	5	10	20	10
Staphylococcus aureaus	20	10	10	10

Table 5: Extract induced increases in the concentration of proteins and albumin (mg/dl).

Groups	Dose (Mg/Kg) for kwashiorkor Induced rat before treatment	Total protein (Mg/dl) for kwashiorkor Induced rat before treatment	Total protein (Mg/dl) for kwashiorkor Induced rat after treatment	Albumin concentration for kwashiorkor induced rat before treatment.	Albumin concentration for kwashiorkor induced rat after treatment.
A	Control	4.51±0.01	4.58±0.01	3.02±0.01	3.12± 0.03
B	0.5	3.33±0.03	6.07±0.04	3.18 ±0.03	3.30±0.02
C	1.0	3.02±0.04	6.59±0.01	3.14±0.01	3.59±0.04
D	1.5	3.82±0.02	7.17±0.03	3.27±0.04	3.90±0.01
E	2.0	3.82±0.01	7.60±0.02	3.21±0.01	4.01±0.03

ampiclox and lincomycin had MIC of 10mg/ml on *S. aureus* while *Cyphostemma glaucophilla* had MIC of 20mg/ml respectively.

In Table 5, Extract treatment at 0.5mg/kg b.w induced significant ($p<0.05$) increases in the concentration of proteins by 2.7mg/dl, from the control value of 4.58±0.01mg/dl. Scalar doses of extract at 1.0, 1.5 and 2.0mg/kg b.w caused significant ($p< 0.05$) dose dependent increases of 3.57, 3.45 and 3.93 respectively from the control value of 4.58 ± 0.01 mg/dl in Group-A. Similarly, the concentration of albumin was consequently affected. There were significant ($p<0.05$) dose dependent increases of 0.18, 0.47, 0.78 and 0.89 from the control value of 3.12 ±0.03 respectively. There were also significant increases in the concentration of total proteins and albumin between the treated and untreated animals.

DISCUSSION

The extract was shown to be effective against *K. pneumonia, P. aeruginosa, E. coli* (Gram negative bacteria), *B. subtilis and S. aureus* (Gram positive bacteria) at 5 mg/ml, even though, the zones of inhibition produced by ampiclox and lincomycin against these organisms were higher except for *B. subtilis* where the zone of inhibition produced by the extract is comparable to that of ampiclox and lincomycin. None of the organisms was sensitive to chloramphenicol at 5 mg/ml. This suggest that the extract may contain component which may be effective in treating supperative infections and superficial skin lesions (Cosgrove *et al*., 2009) as boils caused by *S. aureus. K. pneumonia* causes pneumonia and wound infection especially in immunocompromised children as in kwashiorkor. Other pathogenic conditions which could be caused by *P. aeruginosa* and *E. coli* could be mitigated by the extract. However, ampiclox and lincomycin will be more effective in such treatments than the extract.

The microorganisms such as *K. pneumonia, P. aeruginosa, E. coli, B. subtilis* and *S. aureus* were sensitive to the extract and the antibiotics chloramphenicol, ampiclox, and lincomycin but the order of increasing sensitivity and potency is ampiclox>lincomycin>extract>chloramphenicol. Except for chloramphenicol, the effect of the extract on the microorganisms is not as effective as in ampiclox and lincomycin. It is only comparative with ampiclox and lincomycin against *B. subtilis.* The organisms were sensitive to chloramphenicol at the concentration of 10 mg/ml.

Result in Table 4, showed that the extract concentration of 5 mg/ml caused visible growth against *B. subtilis.* Even though the sensitivity of the extract against *B. subtilis* is comparable with ampiclox and lincomycin the antibiotics was able to cause the same effect at 10 mg/ml.

The demonstration of antimicrobial activity against both Gram negative and Gram positive organisms (Hong *et al.*, 2009) is an indication that the extract possesses some antimicrobial agent which could be used as a potential source for the production of drugs with broad spectrum of activity even though the comparative standard antibiotics used were more effective than the extract in some of the organisms.

In this investigation, the extract produced significant ($p<0.05$) increase in the concentration of plasma total proteins and a graded increase in albumin concentration. This result further confirms the result of earlier studies on the leaf extract on healthy rats by (Ojogbane and Nwodo, 2010). The extract induced elevation of plasma proteins concentrations which might alleviate the protein deficiency in kwashiorkor. Also, the hallmark of kwashiorkor, oedema beleived to be caused by albumin deficiency (Heird, 2008). Due to the fact that albumin is used for the maintenance of colloid osmotic pressure (Guyton and Hall, 2006). A decrease in albumin (less than 5g/dl) results in the lowering of plasma colloid osmotic pressure in a way that it can no longer counteract the effect of the hydrostatic pressure of blood. This results in an increased outward movement of fluid from the capillary wall and decreased inward movement of fluid from the interstitial space causing oedema. The impaired immune response and high risk of infections which are consequent on reduced synthesis of protein (Ahmed *et al.*, 2009) can be tampered with an increase in plasma protein concentrations (Slater-jeffenes *et al*., 2011) as shown for *C glaucophilla* leaf extract. These findings substantiate and provide scientific evidence for the use of *Cyphostemma glaucophilla*aqueous leaves extract in traditional medicine for the treatment of kwashiorkor.

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