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Comparison of Extracts of *Cyphostemma Glaucophilla* on Total Protein and Membrane Stabilisation

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ABSTRACT

The chloroform, ethanol and water extracts of pulverised dried leaves of Cyphostemma glaucophilla were used in this study. Three sets of inbred Wistar albino rats of either sex aged 7-9months and weighing 100-150g were fed on Top feed rat diet and allowed free access to water before and during the experiment. For each extract, five groups A, B, C, D and E of five animals each were respectively served daily doses of saline [0.85% Nacl,5ml/kg],0.5,1.0,1.5 and 2.0mg/kg respectively extract for 14days using stomach tubes. Twenty four hours after the last administration, the animals were sacrificed by ether anaesthesia. Blood samples were drawn into heparinised tubes by cardiac puncture and centrifuged at 5000xg for 10minutes.The supernatant was used as plasma. The livers were dissected out, homogenised and the homogenate used to determine protein concentration. In experiment two, the effect of extract on erythrocyte membrane stability was investigated using Human Red Blood Cell [HRBC] by the method of Born and Cross [1963] as modified by Nwodo [1981]. Data of protein concentration were analysed by one way ANOVA and student independent t-test and presented as means ± SEM and values with $P < 0.05$ were regarded as significant. The extract induced increases of concentrations of plasma and liver proteins were dose dependent and significantly [$P < 0.05$] increased compared with the control group. The extract also protected against hypo tonicity induced haemolysis. The order of potency of the extracts were water extract > ethanol extract > chloroform extract and the differences amongst the effect was significant.

Key words: *Cyphostemma glaucophilla,* protein,
ANOVA: Analysis of variance, hypotonicity Induced Haemolysis

INTRODUCTION

Kwashiorkor is a protein deficiency disorder of children between the ages of 1-5 years. The major signs of the disorder include swollen and severely bloated abdomen resulting from decreased albumin in the blood, failure to thrive, moon face, various skin changes giving rise to a reddish discoloration of the hair and skin in black African children [Tang, 1999]. Oedema, fatty liver and hypo albumineamia [Murray *et al.,* 2000].

Gleaner [2004] attributed the oedema in kwashiorkor to the effect of malnutrition in reducing plasma protein resulting in a reduced oncotic pressure and therefore increased osmotic flux through the capillary wall.

Cyphostemma glaucophilla which belongs to the family of vitaceae is a useful medicinal plant commonly found by streams and rivers. The aqueous extract is used successfully in traditional medicine in Kogi and Kwara States of Nigeria for the treatment of kwashiorkor. The efficacy of *Cyphostemma glaucophilla* aqueous extract in enhancing the concentration of haematological indices such as packed cell volume, haemoglobin, red blood cell has been well defined [Ojogbane, 2008].

In this study, a comparative effect of different extracts viz [chloroform, ethanol and water] of *Cyphostemma glaucophilla w*as carried out to evaluate the effective solvent for the extraction of active components on protein concentrations and the effect of the extracts on membrane stabilization.

EXPERIMENTAL SECTION

Plant material

Cyphostemma glaucophilla leaves were collected from the bank of River Niger along Idah-Ibaji road in Kogi State of Nigeria. They were authenticated in the Botany department of University of Nigeria Nsukka by A.O Ozioko.They were washed to remove dirts, air dried and pulverised with a milling machine into a coarse powder.

Animals: The animals used in this study were Wistar albino rats (*Rattus norvegicus)* of either sex, aged between seven and nine weeks and weighing between 100 and 160g. They were purchased from the Faculty of Biological Sciences Animal House University of Nigerian, Nsukka, Nigeria.

Blood samples: Blood samples were collected via cardial puncture into heparinised centrifuge tubes and were spun at 5000xg for five minutes to separate the plasma from the particulate substances.

Liver homogenate: The liver excised from freshly killed rats and homogenized in a warring blender. The homogenate was used to determine the concentration of total proteins.

Reagents: The protein reagent employed in this study was obtained form Randox laboratories Ltd, Diamond Road, Crumlin co. Antrim, United Kingdom;

Preparation of water extract: A 100g Quantity of pulverized leaves was macerated in five volumes [w/v] of water for eighteen hours with two changes of the solvent. The filtrate through Whatman No. 4 filter paper was evaporated in a water bath to obtain the dried extract and the percentage yield was calculated.

Preparation of Ethanol and Chloroform Extracts: A 400g of pulverized dried leaves of C*yphostemma glaucophilla* was macerated twice in five volumes [w/v] of chloroform-ethanol mixture [2:1] for eighteen hours with two changes of solvent. The Whatman no 4 filtrate of the macerate was shaken with 0.2ml volume water in a separating funnel. The two emerging layer; upper ethanol layer and lower chloroform layer were separated and dried *in vacuo*. The percentage yields were calculated.

Determination of the Effect of the Extracts on Protein Concentrations: To determine the effect of the three extracts vis chloroform, ethanol and aqueous extracts on the amounts of proteins in plasma and liver, three sets of inbred Wistar albino rats were used. They were fed or Top feed rat diet and allowed free access to water before and during the study.

For each extract 5 groups of 5 animals per group were respectively served daily doses of saline [0.85% NaCl, 5mg/kg], 0,5,1.0,1.5 and 2.0mglkg extract for 14 days using stomach tubes. Twenty four hours after the last administration, the animals were sacrificed by ether anesthesia. Blood sample were drawn into heparinised tubes by cardiac puncture and centrifuged at 5000xg for 10min. the supernatants were used as plasma. The livers were also dissected out immediately after drawing blood, homogenized in a warring blender and the homogenate used to determine protein concentrations.

Effect of Extracts on Erythrocyte Membrane Stability Experiment: Nine ml of adult human blood was used in the experiment. The precipitate obtained after centrifuging human blood at 3000rpm for 10minutes was re-suspended in 9ml of normal saline and used as the human red blood cells [HRBC] for the test.

RESULTS

TABLE1: Effect of extracts on total protein concentrations of plasma [mg/dl]

Group	Dose [mg/kg]	Aqueous extract	Ethanol extract	Chloroform extract
A	Normal saline (5ml/kg)	4.08± 0.02	6.00± 0.02	5.01± 0.02
B	0.5	6.02± 0.03	7.30±0.01	5.43± 0.03
C	1.0	6.59± 0.01	7.80± 0.01	5.52 ± 0.01
D	1.5	7.17±0.02	7.95±0.01	5.91±0.02
E	2.0	7.80±0.01	8.10±0.01	6.60±0.01

The reaction medium [2.1ml] containing 0.1ml of HRBC, 1.0ml normal saline and 1.0ml of water was used as control, and incubated at 37°c for 30minute and centrifuged at 3000rpm for

10minutes. The supernatant was drawn out and its absorbance at 418 was measured. Blanks containing Cy*phostemma glaucophilla* extract without HRBC were used for each tube.

Three different reaction media were further made in which various increasing concentrations of the leaf extracts were added. They were incubated at 37^0c for 30minutes and centrifuged at 3000rpm for 10 minute. The absorbance of the supernatant at 418nm was measured using Genesyl 20 spectrophotometer.

All the three extracts produced significant [p<0.05] dose dependent increase in the concentration of total proteins in rat plasma. However, the aqueous extract caused the total protein concentration to increase from a control value of 4.08±0.02mgldi by 1.94± 0.01mgldi in group B of rats which received the lowest dose of extract and 3.72± 0.01lmgdi in group E. that were administered four times group B dose. This treatment also increased the concentration of total protein in the ethanol extract treated rats by 1.30 ±0/01l mg/dl in group B and 2.10 ±0.01 in group E. The chloroform extract also produced graded increase in protein concentrations by 0.42 ±0.0l mg/dl in group B and 1.59± 0.01mg/dl in group E respectively.

TABLE2: Effect of extracts on plasma albumin concentration [mg/dl].

Group mg/kg	**Dose of extract [mg/kg]**	**Aqueous extract**	**Ethanol extract**	**Chloroform extract**
A	Normal saline 5ml/kg	3.02± 0.04	4.00± 0.02	3.12± 0.05
B	0.5	3.89± 0.02	4.00± 0.01	3.30 ±0.04
C	1.0	4.43 ± 0.02	4.16 ± 0.02	3.59 ± 0.02
D	1.5	4.71± 0.03	4.90 ± 0.02	3.90 ± 0.02
E	2.0	4.86 ±0.02	5.41 ± 0.01	4.10 ± 0.02

TABLE 3: Effect of extracts on the concentration of liver protein (mg/dl)

Group	Dose of extract mg/kg	Aqueous extract	Ethanol extract	Chloroform extract
A	Normal saline 5ml/kg	4.10± 0.02	5.03± 0.01	6.01± 0.00
B	0.5	6.05 ± 0.01	5.45± 0.02	7.34 ±0.02
C	1.0	6.60± 0.02	5.60 ± 0.01	7.83 ± 0.01
D	1.5	7.19± 0.01	7.98 ± 0.01	6.01 ± 0.02
E	2.0	7.90 ±0.01	8.40 ± 0.01	6.61 ± 0.01

The concentration of albumin in plasma was affected in a scalar manner. The aqueous extract produced significant [p<0.05] increase in albumin concentration from a control value of 3.02 ± 0.04mg/dl to 3.89 ± 0.02 mg/ld in group B and 4.86 ± 0.02 mg/dl in group E representing increases of 0.87 ±0.02mgld and 1.84± 0.02mg/dl respectively. Similarly the ethanol extract produced graded increases in the concentration of albumin by 0.12± 0.01mg/dl in group B and 1.41 ± 0.01 mg/dl in group E. There was also a significant [p<0.05] increase in protein

concentration in the chloroform extract treated animals by 0.08± 0.01 mg/dl in group B and 0.98± 0.03mg/dl in group E respectively.

Among animals treated with the aqueous extract, there was a significant [p<0.05] dose dependent increase in the concentration of liver protein by 2.04± 0.01mg/dl in group B and by 3.80± 0.01mg/dl in group E respectively. Group C and D which were administered doses of 1.0 of 1.0mg/kg and 1.5mg/kg body weight of extract also showed increases in concentration by 2.50 ± 0.01mgld and 3.09 ± 0.01mgld from the control value. Like the aqueous extract, the ethanol extract produced significant [p<0.05] increase in liver protein concentration of treated rats in group B by 1.33 ± 0.01mg/dl and by 2.39 ± 0.01mg/dl in group E. Similarly, the chloroform extract elicited an increase of 0.42± 0.01mg/dl in group B and 1.58 ± 0.01mg/dl in group E respectively.

TABLE 4A: Effect of aqueous extract on hypotonicity induced haemolysis

Tube	Concentration of extract	Absorbance at 418nm	$\frac{X_n - X_4}{X_1 - X_4}$ percentage heamolysis	100– percentage heamolysis= percentage inhibition
X_1	0.1 ml water	0.41	100	0
X_2	0.2mg/ml extract	0.36	87.5	12.5
X_3	0.4mg/ml extract	0.26	62.5	37.5
X_4	0.1ml normal saline	0.01	0	100

TABLE4B: Effect of ethanol extract on hypotonicity induced haemolysis

Tube	Concentration of extract	Absorbance at 418nm	$\frac{X_n - X_4}{X_1 - X_4}$ X 100 percentage heamolysis	100–percentage heamolysis percentage inhibition
X_1	0.1 ml water	0.41	100	0
X_2	0.2mg/ml extract	0.38	92.5	7.5
X_3	0.4mg/ml extract	0.32	77.5	22.5
X_4	0.1ml normal saline	0.01	0	100

Table 4A shows that the erythrocytes were fairly stable in normal saline, the absorbance at 418m 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 cells. The aqueous extract decreased the absorption [418nm] of the supernatant after

incubation. Water extract Concentration at 0.2mg/ml inhibited lysis by 12.5% , also 0.4mg/ml extract inhibited lysis by 37.5%. The extent of reduction of haemolysis was dose dependent.

The ethanol extract also elicited a dose dependent inhibition of lysis. At 0.2mg/ml it inhibited the lysis of the red cell by 7.5% and 22.5% at a concentration of 0.4ml extract respectively.

TABLE4C: Effect of chloroform extract on hypotonicity induced haemolysis

Tube	Concentration of extract	Absorbance at 418nm	$\frac{X_n - X_4}{X_1 - X_4} X100$ percentage haemolysis	100–percentage haemolysis= percentage inhibition
X_1	0.1 ml water	0.41	100	0
X_2	0.2mg/ml extract	0.40	97.5	2.5
X_3	0.4mg/ml extract	0.35	85	15
X_4	0.1ml normal saline	0.01	0	100

The chloroform extract also inhibited lysis of red cells by 2.5% at the concentration of 0.2mg/ml and 15% at 0.4mg/ml it inhibited hypotonicity induces haemolysis respectively.

DISCUSSION

All the three extracts [Viz aqueous, ethanol and chloroform extracts] of *cyphostemma glaucophilla* induced significant [p<l0.05] dose dependent increases in the concentration of total protein both in the plasma [Table 1] and in the liver. [Table3]. In comparing the effect of the extracts weight for weight, the water extract was more effective. It elicited twice the effect of ethanol extract and almost thrice the effect of the chloroform extract. Wardlaw, [2003] reported that kwashiorkor can be reversed by supplying diet ample in protein. *Cyphostemma glaucophilla* could be a possible remedy to kwashiorkor. It revolutionaries kwashiorkor treatment in that the disorder can now be tackled from two point that are diet based that is, balanced diet and diet containing *Cyphostemma glaucophilla* leaves as vegetables. There was also a dose dependent significant [p<0.05] graded increase in the concentration of albumin [Table 3] for all the extracts. However, the major cause of oedema in kwashiorkor is a deficiency of albumin [Cohen and Lehman 2002]. Extracts induced increase in albumin concentration is probably one of the mechanisms by which the leaves of *Cyphostemma glaucophilla* is invaluable among the Igalas of Nigeria. Similarly the effect of aqueous extract was more.

As shown on table 4 [A, B and C] the optical density of the supernatant in water relative normal saline [5ml/kg] was high. In the presence of the extracts, there were dose dependent decreases in the optical density showing decreased amount of haemoglobin released into the medium. This shows that the extracts protected against haemolysis caused by water a hypotonic solution. Protection against hypotonicity induced haemolysis is related to membrane stabilization which is an anti-inflammatory index. The order of potency of the extracts is aqueous extract> ethanol

extract> chloroform extract. Kwashiorkor which is usually characterized by inflammation could be treated with this extracts.

CONCLUSION

The leaf active components seem to be extracted more with water into the aqueous extract. The results also justify the popular use of *Cyphostemma glaucophilla.* Pointing to the potential benefit of the plant aqueous extract in alternative medicine for the treatment of kwashiorkor in children.

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