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Effect of Aqueous Extract of *Cyphostemma Glaucophilla* on Protein Synthesis in *Rattus Norvegicus*

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ABSTRACT

The effects of aqueous extract of pulverised dried leaves of *Cyphostemma glaucophilla* was investigated in two sets of experiment. Thirty *Rattus norvegicus* of either sex, aged 7-9 months and weighing 100-130g were randomly assigned to five experimental group, A, B, C, D, and E of six animals each and were served single intraperitoneal daily doses of saline tetracycline was not significant [0.06±0.02mg/dL]. However, inhibition with Chloramphenicol and streptomycin was significant [4.60±0.02, 4.50±0. (0.85percent, Nacl.5ml/Kg),0.5,1.0,1.5,2.0mg/Kg of extract respectively for 14days. Twenty four hours after the last administration, the animals were sacrificed by ether anaesthesia and blood samples were drawn into heparinised tubes by cardiac puncture. The livers were excised. Protein concentrations of both plasma and liver homogenate were assayed. In experiment two, extract induced increase in protein concentration were inhibited with drugs that inhibit the different stages of protein synthesis. The concentrations of protein in plasma and liver homogenate were significantly [1.08±0.01, 1.35±0.02mg/dL] increased compared with those of the control and the inhibition of extract induced increase in protein concentration with [02mg/dl]. Result showed that extract most likely promote protein synthesis at elongation and termination stages. All the effects were dose dependent.

Key words: *Cyphostemma glaucophilla*, *Rattus norvegicus*, ip: Intraperitoneal and Inhibition of protein synthesis.

INTRODUCTION

The genus *Cyphostemma* belongs to the family of vitaceae which consist of a wide range of creeping plants with broadly ovate leaves. Even though various species now occur in different parts of the world, they originated from Africa and Madagascar.

Cyphostemma glaucophilla is a useful medicinal plant with creamy green flowers. It has unexpected corolla, subglobose leaflet and commonly found by streams and rivers. It is a perennial herb with prostrate branches rooting from the node and can be found in such places as Togo, Nigeria, East Africa, Democratic Republic of Congo and Angola [Bukik, 1985].

The leaf extract is used in alternative medicine in the treatment of kwashiorkor in children. The ground leaf paste is used by local orthopaedics in setting of fractured bones. The young shoots are also used as vegetables. [Singh and Mishra, 1984] recorded that the leaves of most plants in the family of vitaceae are often used as medicinal herbs because they contain some bioactive compounds such as vitamins, proteins, carbohydrates, phenols among others.

Protein energy malnutrition [PEM] which was first described in 1920s is observed most frequently in the developing countries [wardlaw, 2003]. It is applied to a group of disorders that includes marasmus and kwashiorkor and intermediate state of marasmic kwashiorkor. Beer and Berkow [1999] had recorded that geographically, more than seventy percent of PEM children live in Asia, twenty-six percent in Africa and four percent in America and the Caribbean. In some cases, their plight may well have begun even before birth with malnourished mothers.

Marasmus is derived from the Greek word 'marasmus' which means withering or wasting. It involves an inadequate intake of both proteins and calories characterized by emaciation. The term Kwashiorkor is taken from the Ga tribe of Ghana and it means "the sickness of weaning". It refers to an inadequate protein intake with reasonable calorie intake. The distinction between the two forms of PEM is based on the presence [Kwashiorkor] or absence [Marasmus] of Oedema. Marasmus represents an adaptation to starvation whereas kwashiorkor represents a dysadaptation to starvation [Bermek, 2004]

Kwashiorkor usually occurs between the ages of one and five years when breast feeding is discontinued, especially in areas of famine where there is limited food supply. Also contributing is low levels of education that leads to inadequate knowledge of diet and feeding techniques. It is prevalent in children fed almost exclusively on starchy diet like corn, millet, cereal that lack essential dietary factors such as lysine, glycine, tryptophan which are essential amino acids [Tang, 1999., Cohen and Lehman, 2002].

The major signs of kwashiorkor include swollen and severely bloated abdomen, oedema, failure to thrive, moon face, fatty liver and various skin changes. Pathological and biochemical changes include decreased concentrations of plasma proteins, reduced serum protein and albumin fractions, increased serum level of triglycerides, phospholipids and cholesterol, decreased levels of Amylase, lipase and trypsin. Also, Hemoglobin levels are low, especially if parasite infection is present. There is increase concentration of fat in the liver as a result of triacylglycerol deposit. However, the symptoms of kwashiorkor respond therapeutically to high protein diet containing

considerable quantity of meat and milk products [Grysbj, 2001 and WHO, 2000]. It is estimated that more than 65% of Nigerians cannot afford modern medicine and so, rely on traditional medicine which is based on curative plants [Odin *et al.*, 2003]. The effect of *Cyphostemma glaucophilla* aqueous extract in promoting the concentration of protein in plasma of rats has been studied and validated [Ojogbane, 2008]. This study sought to determine the mechanism by which the extract induces protein synthesis. Data were analysed by one way ANOVA and student independent t-test and presented as means \pm SEM. Values with $p < 0.05$ were regarded as significant.

EXPERIMENTAL SECTION

Plant materials:

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. Washed to remove dirt, air dried and pulverized with a milling machine into a coarse powder

Animals:

The animals used in this study were Wister Albino rats of either sex, aged between seven and nine weeks and weighing 110 -130g. They were purchased from the Faculty of Biological Sciences Animal House University of Nigeria, Nsukka, Nigeria.

Blood samples:

Blood samples collected via cardiac puncture into heparinised centrifuge tubes, were spun at 5000xg for five minutes, to separate the plasma from the particular substances.

Drugs:

Tetracycline and chloramphenicol capsules were products of Beecham, streptomycin was obtained from Pfizer.

Liver homogenate:

The liver excised from freshly killed rats in 0.25M sucrose was homogenized in a warring blender .

Reagent:

The protein reagent kit employed in this study was obtained from Randox laboratories ltd, Diamond Road, Cumlin co. Antrim, United Kingdom.

Preparation of water extract:

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

Assay on plasma and liver protein:

At the end of the experimental period, rats in all the groups were anaesthetized, dissected and bleed via cardiac puncture. The blood samples were collected into heparinised tubes and centrifuged at 5000rpm for 10 minutes the supernatant were used as plasma. The livers were also

dissected out immediately, Homogenized in a waring blender and the homogenate used for the assay. Total protein was determined by the method of Wooten [1959], Albumin by modified BCG method of Mcpherson and Everad [1972].

Determination of the effects of some drugs that inhibits protein synthesis.

Three different antibiotics were used to investigate the stage of protein synthesis involved in the process. They were:

- (a) Tetracycline, an inhibitor of initiation.
- (b) Chloramphenicol, an inhibitor of elongation.
- (c) Streptomycin, an inhibitor of termination [Connell *et al.*, 2003 and kraut, 2003].

Three sets of animals were separated into five groups of five animals each and assigned randomly to different groups A, B, C, D, and E.

Group A, the controls received [ip] 5ml/kg normal saline, group B was administered 8.0mg/kg of the inhibitor [Tetracycline or Chloramphenicol or streptomycin] Group C was administered 4.0mg of the inhibitor as in Group B, Group D received 2.0mg of the inhibitor while Group E was administered saline and 2.0mg/kg body weight of aqueous extract for 14 days.

The drugs were given 30 minute, before the 2.0mg/kg of extract was administered to each group except group A which was given a second saline dose.

Twenty-four hours after the last administration, the animals were anaesthetized with chloroform and the liver excised and homogenized. The homogenate was used to determine the concentration of protein.

RESULTS.

Results obtain from the experiment are presented below

Table 1: Water Extract Induced Increases In Protein Concentration

GROUP	DOSE [mg/kg]	CONCENTRATION IN mg/dL		
		TOTAL PLASMA PROTEIN	ALBUMINS	LIVER HOMOGENATE PROTEIN
A	Normal saline 5ml/kg	4.05± 0.01	2.07± 0.01	4.35± 0.01
B	0.5	4.95± 0.02 *	3.15± 0.01 *	5.60 ± 0.02 *
C	1.0	5.9 ± 0.01 *	3.90 ± 0.02 *	6.35 ± 0.03 *
D	1.5	6.60 ± 0.02 *	4.50 ± 0.01 *	7.15 ± 0.02 *
E	2.0	8.00 ± 0.02 *	5.65 ± 0.02 *	8.50 ± 0.02 *

* P<0.05 i.e. statistically significantly different from controls.

As shown in table 1, the extract produced a significant dose dependent increase in the concentration of total plasma proteins from a control value of 4.05 ± 0.01 mg/dL by a difference of 0.90mg/dL in plasma of rats which were administered the lowest dose of water extract and by 3.95mg/dL in group E that received 4 times that dose.

Similarly, the control group of rats that received normal saline had a plasma albumin concentration of 3.13 ± 0.04 mg/dL. Among the group of rats treated with 0.5mg/kg aqueous extract, the plasma albumin concentration increased by 1.08mg/dL. Scalar doses of extract produced progressive increases in the amounts of albumin in their plasma. At 2.0mg/kg [$\times 4$ the initial dose administered] the plasma albumin level of the group of rats increased by 2.43mg/dL. This increase was significant when compared to the initial increase obtained with the lowest dose. In addition, it was significant when compared to those treated with saline only. Extract produced a significant increase in liver proteins by 1.25mg/dL from a control value of 4.35 ± 0.01 mg/dL of rats which were administered the lowest dose in group B and by 4.15mg/dL in group E which received the highest dose of extract. Similar dose dependent increases were observed in groups C and D.

Table 2: Effects of Water Extract Induced Protein Synthesis

Group	Dose of extract mg/kg	Dose of tetracycline	Concentration of total protein mg/dl	δ in protein from group a	Δ in protein compared with e
A	Normal saline 5.0ml/kg	—	6.24 ± 0.01		
B	2.0	8.0	$8.04 \pm 0.01^*$	1.80	0.26
C	2.0	4.0	$8.10 \pm 0.01^*$	1.86	0.20
D	2.0	2.0	$8.15 \pm 0.01^*$	1.91	0.15
E	2.0	—	$8.30 \pm 0.01^*$	2.06	

Values with * in a column are statistically significant [$p > 0.05$].

Table 3: effects of chloramphenicol treatment on extract induced protein synthesis in rats

Group	Dose of extract [mg/kg]	Dose of chloramphenicol [mg/kg]	Total protein [mg/dl]	Δ in protein from group a	Δ in protein compared with e
A	Normal saline 5ml/kg	—	6.20 ± 0.02		
B	2.0	8.0	5.60 ± 0.02	- 0.60 *	2.60 *
C	2.0	4.0	5.70 ± 0.01	- 0.50 *	2.50 *
D	2.0	2.0	6.20 ± 0.01	0	2.0 *
E	2.0	—	8.20 ± 0.02	2.0	-

Among rats that were treated with normal saline [5mg/kg], the protein concentration was 6.24 ± 0.01 mg/dL. When the animal was treated with 2.0mg/kg extract, the protein concentration increased significantly [$p > 0.05$] by 2.06 ± 0.20 mg/dL. The protein concentration of rats that received scalar doses [2-8mg/kg] of tetracycline did not change significantly. The decreases shown on table 2 are not statistically significant [$p > 0.05$].

Daily treatment of rats for 14 days with 2.0mg/kg of the extract caused the plasma protein concentration to increase by 33% from 6.20- 8.20mg/dL. Table 3 shows that treatment with graded doses of Chloramphenicol and a constant amount of the extract [2.0mg/kg] produced significant [$p > 0.05$] scalar decrease in plasma protein concentration after 14 days.

Table 4: Effect Of Streptomycin Treatment On Extract Induced Protein Synthesis

In rats treated p.o with a 2.0mg/kg extract and scalar amounts of streptomycin [2-8mg/kg] for 14 days, the plasma protein concentrations decreased significantly in a dose related fashion.

GROUP	DOSE OF EXTRACT [mg/kg]	DOSE OF CHLORAMPHENICOL [mg/kg]	TOTAL PROTEIN [mg/dL]	Δ IN PROTEIN FROM GROUP A	Δ IN PROTEIN COMPARED WITH E
A	normal saline 5ml/kg	–	6.20 ± 0.01		
B	2.0	8.0	3.50 ± 0.01	-3.30 *	4.50 *
C	2.0	4.0	3.90 ± 0.01	-3.70 *	4.10 *
D	2.0	2.0	4.30 ± 0.01	-2.10 *	4.70 *
E	2.0	–	8.00 ± 0.01	1.80 *	

DISCUSSION

The extract was able to induce significant [$P > 0.05$] dose dependent increases in the concentration of total proteins both in the plasma and in the liver homogenates. Similarly, it produced graded significant increases in the concentration of albumin in the test groups when compared with the control. This observation validates the use of the water extract of *Cyphostemma glaucophilla* by the Igalas of Nigeria in the treatment of kwashiorkor. Asha et al. [2002] had recorded that impaired synthesis of VLDL-apo-B-100 in kwashiorkor is due to the shortage of amino acids because of the chronically inadequate dietary protein intake in such patients. The increase in total protein as is evident in [table 1] is an indication that the plant extract enhances the protein concentrations of both plasma and liver of the body. The significant increase in albumin concentration is also a major finding because albumin is used for the maintenance of colloid osmotic pressure at the capillary membrane to prevent plasma fluid from leaking into the intestinal cells. One of the causes of oedema is a deficiency in albumin [Cohen and Lehman, 2002] Albumin also carries fatty acid which form the membrane around and inside the cell. The fatty acids are rich sources of energy which may be broken down inside the cell to

yield energy [both free and stored]. These could help in reviving the kwashiorkor infant from weakness.

Insensitivity of extracts to the effect of tetracycline [table 2] an inhibitor of initiation stage of protein synthesis [Conell, 2003] is evident that the extract does not affect protein synthesis at this stage. The significant [p> 0.05] dose dependent inhibition of protein synthesis when chloramphenicol [table 3] and streptomycin [table 4] an inhibitor of elongation and termination stages of protein synthesis [Kraut, 2003] was administered indicate that the extract most likely promotes protein synthesis at two points on the protein synthesis pathway but the effect on elongation is greater than the effect on termination.

CONCLUSION

By effects on elongation, the extract probably influences either the elongation factors or guanine triphosphate [GTP] hydrolysis. Knowing that elongation of protein synthesis is powered by GTP hydrolysis.



It then becomes necessary to study the involvement of the nucleotides in the extract action.

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