

Adaptogenic Potentials of *Cyphostemma glaucophilla* Aqueous Leaf Extract

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ABSTRACT

Cyphostemma glaucophilla aqueous extract is used in Kogi and Kwara States of Nigeria to treat Kwashiorkor and to boost immunity. As part of the effort to evaluate its effect on stress, it was screened for adaptogenic potentials. This investigation was conducted using albino rats to determine the ability of this plant to increase non specific resistance against physical, biological and chemical stressors. Showed that, the extract significantly ($p < 0.05$) and dose dependently protected the rats from cold immobilization induced stress ulcers; the extract inhibited ulceration by 21.30% at a dose of 100 mg kg⁻¹ and 56.09% at a dose of 500 mg kg⁻¹ when compared with the control treatments (0.85% NaCl; 5 mL kg⁻¹). The extract protected the animals from bacterial induced mortality and morbidity and significantly ($p < 0.05$) reduced infection-induced leucocytosis in the rats and also alleviated the hepatic degenerative changes associated with ciprofloxacin. Findings have shown remarkable ability of the extract to increase non specific resistance to physical, biological and chemical stressors and could qualify as an adaptogen.

Keywords: Adaptogen, *Cyphostemma Glaucophilla*, Resistance, Cold Immobilization, Leucocytosis, Ciprofloxacin

1. INTRODUCTION

Wiegant *et al.* (2010) had defined stress as a non specific response of the body known to alter the physiological homeostasis of the organism resulting in various neuronal, endocrine and visceral disfunction.

In 1943, the people's commissar's council of the soviet socialist republics charged their scientist with the task of finding tonic substances to strengthen the health workers in the Russian defense industry during World War II (Panossian and Wikman, 2010). Thus began the effort to find remedial substances that would increase protective state of resistance during condition of stress. Panossian and Wikman (2009) showed that ingestion of certain plant extracts could improve stress markers in laboratory animals. However, between 1950 and 1960, these plant remedies were termed adaptogens and three

criteria were set to describe their remedial action (Olsson *et al.*, 2009). An adaptogen should be innocuous and cause minimal disturbance to the normal physiological function of an organism, its action should be non specific and it should have a normalizing action irrespective of the direction of the preceding pathological changes.

Further investigation of adaptogenic compounds led to the observation that the steroidal compound synthesized by plants for its defense called phytechdysteroid, B-sitosterol meets the three criteria of an adaptogen. These natural substances of plant origin is now commonly termed phytoadaptogen (Maslov *et al.*, 2010) certain plants like Bryonia and Alba tablets are now registered in America as herbal medicinal product in Sweden (Balandin, 2010) because they have demonstrated to fulfill the criteria of an adaptogen and therefore qualify as phytoadaptogen.

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Cyphostemma glaucophilla belongs to the family, Vitaceae. They are caudiform and use to belong to the genus cissus (Oshikoya *et al.*, 2010). The aqueous leaf extract is used to treat successfully diverse ailments ranging from systemic disease such as hypertension and diabetes to malnutritional disorders such as kwashiorkor in the Igalá, Yoruba and Epira speaking areas of Kogi State of Nigeria. Healthy individual usually takes it at will with the impression that it boosts their body immunity.

The extract has no adverse effect at the limit per oral dose of 3000 mg kg⁻¹ body weight administration to albino rats (Ojogbane *et al.*, 2011a). The leaf extract induces increase in liver total proteins and plasma proteins especially albumin (Ojogbane *et al.*, 2011b). In addition to its lipid lowering and membrane stabilization effects (Ojogbane *et al.*, 2011a; Ojogbane and Nwodo, 2010), the extract has anti-inflammatory and antioxidant activity (Menezes, 2009; Ojogbane *et al.*, 2011b) and it is hepato-protective (Ojogbane *et al.*, 2011a; Maslov *et al.*, 2010). Also the aqueous leaf extract is more potent than the ethanol and chloroform extract and it has been shown to have several useful antimicrobial effects (Ojogbane and Nwodo, 2010).

In this study, we screened *Cyphostemma glaucophilla* aqueous leaf extract for adaptogenic potentials.

2. MATERIALS AND METHODS

2.1. Collection and Extraction of Plant Material

Cyphostemma glaucophilla leaves were collected from the bank of River Niger along Idah-Ibaji road in Kogi State of Nigeria in August 2012 and characterized by A.O Ozioko of Botany Department, University of Nigeria, Nsukka. They were washed to remove dirt, dried at room temperature and pulverized with a milling machine into a coarse powder. Some 100g quantities of the pulverized leaves were macerated in five volume (w/v) of water for 18 h with two changes of solvent. The filtrate was evaporated in a water bath to get the dried residue (yield = % starting material).

2.2. Animals

The wistar albino rats used for this study were obtained from the Faculty of Biological Sciences Animal House, University of Nigeria, Nsukka, Nigeria. The rats of either sex, aged between 7 and 9 weeks and weighing (110-150g) were housed under standard conditions

(25±2°C and 12h light/dark cycle). They were fed with standard pellets (Top feed Nigeria Ltd) and had unrestricted access to clean drinking water.

Studies on adaptogenic potential of the extract on physical, biological and chemical stressor by the method of (Phanchonpai *et al.*, 2012a).

2.3. Effect of the Extract on Physical Stressor

Albino rats of either sex (120-150g) which were divided into four groups of five animals each were respectively given (100, 250 and 500 mg kg⁻¹) body weight of the extract to groups B, C and D, normal saline (0.85% NaCl; 5mL kg⁻¹) to the control group A orally and twice daily for 10 days using stomach tubes. Animals were allowed free access to food (Top feed Nig. Ltd) and clean water. After the last dose, animals were fasted for 18 h and then immobilized individually inside specially improvised plastic containers at 4°C (in a refrigerator) for 18h. At the end of the period, the rats were sacrificed by chloroform anaesthesia, the stomach was removed and opened along the greater curvature, rinsed under a stream of water and pinned on a cork board and observed using a 10 × magnification hand lens. Erosion formed on glandular portion of the stomach was counted and each given severity rating on a 1-3 scale based on the diameter of the ulcer:

- 1 = ulcer ≤ 1 mm
- 2 = ulcer > 1 mm ≤ 2 mm
- 3 = ulcers ≤ 2 mm

The total score for each stomach was designated as the Ulcer Index (UI) for that stomach.

2.4. Effect of the Extract on Biological Stressor

Studies were conducted on albino rats of either sex (130-160g) which were divided into four groups A, B, C and D of five animals each. Groups B, C and D received (100, 250 and 500 mg kg⁻¹) body weight of the extract while group A received normal saline (0.85% NaCl; 5 mL kg⁻¹). Animals were fed commercial grows mash (Top feed Nig Ltd) and allowed access to water.

The various treatments were administered orally for 10 days on days 11 and 12, the animals were infected intra peritoneally with 0.1mL and 0.15 mL of 1×10⁹ CFU/mL glucose enriched *Klebsiella pneumonia* respectively. Bodily changes, total White Blood Cell counts (WBC) and differential counts were monitored for 21 days.

2.5. Effect of the Extract on Chemical Stressor

This was assessed by evaluating the effect of the extract on fluoroquinolone-induced histopathological changes in albino rat livers. Male albino rats (120-150g) were divided into four groups of five rats each. Group A served as the control and received normal saline (0.85%NaCl; 5 mL kg⁻¹), group B was administered (100 mg kg⁻¹ ciprofloxacin) while group D received only (100 mg kg⁻¹ of the extract). The various treatments were administered to the animals twice daily for five days with free access to food and water. On the sixth day all the animals were sacrificed and slices of the liver fixed in normal saline for 24 h. The specimen were dehydrated subsequently in different grades of alcohol (70, 80, 90, 95 and 100%) and mounted in an auto-processor, each phase requiring about 3 h. The alcohol as removed by passing the specimen through dishes containing xylene and then through containers of paraffin wax. The specimen was then transferred into a vacuum embedder which expels entrapped air in the tissue while at the same time embedding paraffin into the spaces. Thereafter, the specimen was embedded into molten wax which was allowed to solidify by placing on ice. A microtome was used to cut ribbons of the specimen of about 5 μ thick. De-waxing of the cut session was done by passing it through xylene, absolute alcohol, 80 and 70% alcohol. The sections were stained with haematoxylin and eosin and examined by light microscope.

The extract was screened for the presence of bioactive components following the method.

2.6. Statistical Analysis

Results data were analyzed by one way analysis of variance using SPSS version 18 differences of the means were considered significant at $p < 0.05$.

3. RESULTS

Extract produced a significant ($p < 0.05$) dose dependent inhibition in ulcer index. Dose of extract at 100mg kg⁻¹ protected against ulceration by 21.30%, scalar doses at (250,500 mg kg⁻¹) protected against ulceration by 34.30 and 56.09% respectively.

Extract induced a significant ($p < 0.05$) dose dependent increases in total leucocyte count with higher proportion of differential lymphocytes than neutrophil pre infection and treatment (day 0 and 8). However, the proportion of neutrophils mobilized increased from (day 10) post infection through (day 21).

4. DISCUSSION

Table 1, *Cyphostemma glaucophilla* was found to contain proteins, carbohydrates which are useful in the building up of worn out tissue and regulation of internal body temperature. Flavonoids and vitamin C are antioxidants, which could be useful in free radical scavenging in living system. Vitamin C is particularly necessary for connective tissues and promotes the healing of fracture and wounds (Phanchonpai *et al.*, 2012a). These justify the use of the extract in malnutrition and healing of fractured bone. Tannins are a stringent, bitter plant polyphenols that either bind and precipitate or shrink proteins, they are distributed all over the plant kingdom and have been considered traditionally as antinutritional but, it may be employed medicinally in anti diarrheal, hemostatic and antihemorrhoidal compounds. Its presence suggests that the extract is of medicinal value because tannins have shown potential antiviral (Klabunde, 2010; Phanchonpai *et al.*, 2012b) antibacterial and antiparasitic effect. Saponins are glycosides of steroids which are regarded as anti-nutrients. However are also believed to be useful in human diet for controlling cholesterol. Its presence suggests medicinal value of the extract.

Table 1. Phytochemical screening

Compounds	Aqueous extract
Alkaloids	-
Anthraquinone	-
Carbohydrates	++
Flavonoids	+
Cardiac glycoside	++
Proteins	++
Lipids	+
Tounins	-
Saponins	++
Steroids	+
Vitamin E	-
Vitamin C	++
Vitamin A	-

-: Absence of bioactive compound ++: Presence of bioactive compound in high concentration +: Presence of bioactive compounds in low concentration

Table 2. Extract inhibition of cold-immobilization stress induced ulcer in rats

Group	Treatment (mg/kg)	Ulcer index (mm)	Protection (%)
A	Normal saline (5 mL kg ⁻¹)	23.00 \pm 3.00	
B	100	18.10 \pm 2.10	21.30
C	250	15.11 \pm 2.12	34.30
D	500	10.10 \pm 1.15	56.09

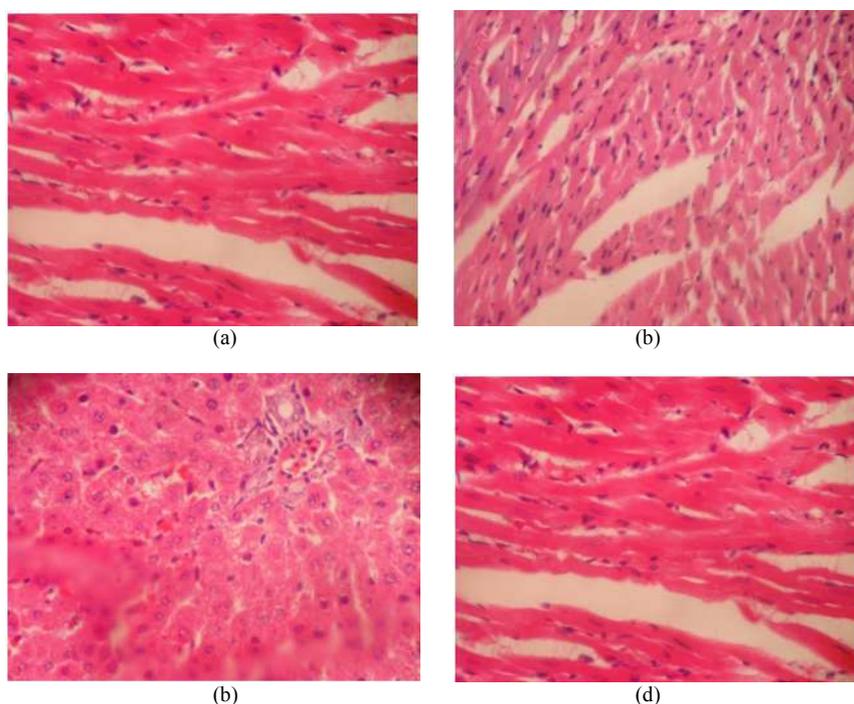


Fig. 1. Slides of liver histopathology, (a) TREATMENT: Normal saline (5 mL kg^{-1}) Control Liver architecture is normal there are no degenerative changes in the hepatocyte, there is no inflammation, (b) TREATMENT extract (150 mg kg^{-1} and Ciprofloxacin (10 mg kg^{-1}) Liver architecture is normal there are no degenerative changes in the hepatocyte, there is no inflammation. (c) TREATMENT: Ciprofloxacin (10 mg kg^{-1}) Liver mild distortion there is vacuolar degenerative, (e) TREATMENT: Extract only (150 mg kg^{-1}) Liver architecture is normal there are no degenerative changes in the hepatocyte, there is no inflammation

Observation on **Table 2** indicates the protective effect of the extract against ulceration. Gastric ulceration induced by large number of stressors is one of the most widely used paradigm to evaluate anti-stress adaptogenic activity (Menezes, 2009; Sancheti *et al.*, 2010). Flavonoids, one of the phytoconstituents of the extract had been reported by Pifferi (Panossian and Wikman, 2009) as the most important phytoconstituents associated with the anticancer activity. This could be responsible for this observation.

In the assessment of adaptogenic potential of a substance, it is desirable that the agent should be able to confer an increase in the non specific resistance against biological, physical and chemical stressors (Obimba, 2011; Park *et al.*, 2011). All the animal treated with the plant extract were very active throughout the experiment.

In **Table 3**, pre-treatment no significant change was observed in the leucocyte count of animals. After infection and treatment there was a significant ($p < 0.05$) dose dependent increase in leucocyte count with a reduction in differential lymphocyte and an increase in

differential neutrophil and monocyte count. The Polymorphonuclear Neutrophils (PNMs), which engulf and eliminates invading microorganism was the most mobilized. By the 21st day, the total leucocyte count is significantly $p < 0.05$ reduced. These observations, partly explains anti-inflammatory activity of the plant extract reported in the early study (Park *et al.*, 2011). The apparent increase in monocytosis is a direct consequence of the physiological effect of the polyphenols, which is one of the phytochemicals present in the extract (Park *et al.*, 2011; Oshikoya *et al.*, 2010). Also, (Park *et al.*, 2011) had recorded that plant polyphenols are known to stimulate monocytes. This could be a possible mechanism by which the extract treats infection and inflammation in kwashiorkor in ethno medical practice.

The histopathology results shown by the various slides in **Fig. 1**, indicates that the extract protected against the hepatic changes associated with ciprofloxacin. Hepatoprotection exhibited by some adaptogens is believed to be a major mechanism for their adaptogenic properties (Obimba, 2011; Park *et al.*, 2011).

Table 3. Extract induced increases in total ($10^9/L$) and differential (%) leucocyte count of infected rats

Groups	Treatment	Cell count	Day 0	Day 8	Day 10	Day 14	Day 21
A	Normal	TLC	6.20±1.00	6.54±1.40	7.40±1.10	10.72±0.80	11.0±0.02
	Saline (5 mL kg ⁻¹) Control	Neutrophils	30.89±0.70	36.0±0.500	59.50±0.12	50.09±0.02	51.00±0.08
		lymphocytes	62.01±0.10	60.33±0.80	40.50±0.10	42.24±0.09	41.33±0.01
		monocytes	6.77±1.00	2.67±0.20	0.0000000	6.50±0.10	6.50±0.10
B	100 mg kg ⁻¹ extract	eosinophils	0.33±0.06	0.33±0.05	0.0000000	0.84±0.03	0.84±0.03
		TLC	6.10±0.09	7.91±1.90	7.00±1.11	9.16±0.70	8.80±0.01
		Neutrophils	31.40±0.20	38.33±0.40	55.50±0.50	49.59±0.30	45.59±0.02
		Lymphocytes	62.60±0.04	57.67±0.80	44.50±1.01	45.09±0.10	49.01±0.50
C	150 mg kg ⁻¹ extract	Monocytes	5.90±0.01	3.33±0.02	0.0000000	4.40±0.10	4.40±0.02
		Eosinophils	0.1±0.040	0.67±0.06	0.0000000	0.54±0.04	0.84±0.08
		TLC	6.76±0.10	9.39±1.70	8.90±1.40	8.26±1.20	8.00±1.00
		Neutrophils	31.67±0.05	38.0±0.100	47.00±1.01	51.67±0.05	51.67±0.08
D	200 mg kg ⁻¹ extract	Lymphocytes	61.97±0.05	54.0±1.600	55.50±0.12	42.43±0.70	42.43±0.00
		Monocytes	6.01±0.07	6.67±0.01	0.0000000	0.88±0.03	4.77±0.06
		Eosinophils	0.35±0.03	1.00±0.01	0.0000000	0.25±0.02	0.88±0.25
		TLC	6.19±0.50	10.04±0.70	9.50±0.60	6.98±1.90	6.80±0.04
E	250 mg kg ⁻¹ extract	Neutrophils	30.39±0.01	41.17±0.70	50.00±0.01	50.64±0.20	51.50±0.08
		Lymphocytes	62.20±0.10	56.5±0.100	50.00±0.05	44.36±0.20	48.50±0.03
		Monocytes	6.98±0.09	6.33±0.10	0.0000000	4.09±0.10	0.0000000
		Eosinophils	0.33±0.03	0.67±0.03	0.0000000	0.83±0.01	0.0000000
E	250 mg kg ⁻¹ extract	TLC	6.31±0.10	11.92±0.05	10.62±0.41	6.30±1.30	6.20±0.05
		Neutrophils	31.20±0.10	42.0±0.080	55.00±0.01	42.41±0.10	50.50±0.01
		Lymphocytes	62.33±0.01	51.67±0.40	45.00±0.10	51.33±0.40	49.50±0.01
		Monocytes	5.80±0.10	5.00±0.04	1.00±0.03	5.25±0.04	0.0000000
E	250 mg kg ⁻¹ extract	Eosinophils	0.67±0.03	1.33±0.05	0.0000000	0.17±0.02	0.0000000

Even though ciprofloxacin is not a standard hepatotoxin, the ability of the extract to alleviate hepatotoxicity resulting from this drug is a demonstration of its adaptogenic potential. Hepatoprotection, exhibited by adaptogens is general related to the presence of the constituents with antioxidant properties (Panossian and Wikman, 2009). The extract had earlier been reported as hepatoprotective (Ojogbane *et al.*, 2011a) and inflammatory (Oshikoya *et al.*, 2010; Ojogbane *et al.*, 2011b). These pharmacological properties, attributed principally to its biflavonoids constituents (Ojogbane and Nwodo, 2010) are believed to be related to its antioxidative activity and the ability to increase immunity in general.

5. CONCLUSION

The observations in these results lay credence to the adaptogenic properties of the extract. The antioxidant, anti-inflammatory and immunostimulatory properties of the flavonoid constituents of the extract may be responsible for the adaptogenic effect.

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