Evaluation of the Effects of Co-administration of Ethanol Leaves Extracts of *Andrographis paniculata* leaves and *Phyllanthus amarus* on Albumin, Globulin and Bilirubin in Heavy Metal Treated Albino Wistar Rats.

ABSTRACT

The effects of ethanol leaves extracts of *Andrographis paniculata* and *Phyllanthus amarus* on albumin, globulin and bilirubin in heavy metal treated albino wistar rats was evaluated. Twenty (20) male rats (130-170g) were assigned five groups of four animals. Groups 1, 2 and 3 were treated with 30 percent of the LD_{50} of cadmium each before administration of plant extracts. Group 1 was administrated with 400mg/kg of *Andrographis paniculata* and *Phyllanthus amarus* at 50:50 dosage ratio. Group 2 and 3 were administrated with 400mg/kg of *Andrographis paniculata* and *Phyllanthus amarus* respectively. Group 4 was neither treated with the heavy metal nor administrated with the leave extracts and served as control. The animals were all feed with commercial rat mash and with distilled water throughout three week of extract administration. At the end of the 21 days, the result revealed a non-significant decrease in mean albumin and globulin in the treatment groups when compared to the control group ($P > 0.05$). The result also indicated a significant decrease in direct bilirubin in the treated group when compared to the control group (<0.05), significant decrease was also recorded in the mean serum direct bilirubin in group 2 compared to the control group P (<0.05). These results show that ethanol extracts of *Andrographis paniculata* and *Phyllanthus amarus* leaves

could inhibit the oxidative stress that would have been caused by cadmium toxicity, therefore, the two plants leaves are hepatoprotective in nature and could be used as an hepatoprotective agents.

Keywords: *Andrographis paniculata, Phyllanthus amarus,* Albumin, Globulin, Bilirubin, Albino Wistar rats.

INTRODUCTION

Medicinal plants are integral part of human life to combat the sufferings from the dawn of civilization (Chaudhary *et al.,* 2010). It is estimated that more than 80,000 of total plants species have been identified and used as medicinal plants around the world. The indigenous medicinal plants and plant-derived drugs are the potential source of alternative medicine and are extensively used to treat various health ailments (Karishankar *et al.,* 2011). Use of medicinal plants is a core component of primary health care level due to availability, acceptability, compatibility and affordability. Example of such medicinal plants are *Andrographis paniculata* and *Phyllanthus amarus.*

Andrographis paniculata is an important medicinal plant and widely used around the world. It belongs to the family, Acanthaceae. *Andrographis paniculata* is used as traditional herbal medicine in Bangladesh, China, Hong Kong, India, Pakistan (Kabir *et al.*, 2014). Ethnobotanically, *Andrographis paniculata* is used for the treatment of snake bite, bug bite, diabetes, dysentery, fever and malaria (Burkill *et al.,* 2006). In recent times, commercial preparations of this plants extracts are also used in certain countries. However, the preparations yet need to be standardized for their better efficacy. *Andrographis paniculata* has been reported to have a broad range of pharmacological effects including anticancer, antidiarrheal, antihepatitis, anti-HIV, antimicrobial, antimalarial, antioxidant, cardiovascular, cytotoxic, hepatoprotective, immunostimulatory and anti-sexual dysfunctional activities (Akbarsha and Murugaian, 2000).

The genus Phyllanthus is one of the most important groups of plants traded as a raw herbal drug in India (Ved and Goraya, 2008). The genus Phyllanthus of family *Euphorbiaceae*, consists of approximately 1,000 species, spread over tropical and sub-tropical continents like America, Africa, Australia and Asia (Unander *et al.,* 2005). In India, *Phyllanthus amarus* is widely distributed as a weed in cultivated and waste lands. All three major habits (i.e, trees, shrubs and herb) are seen amongst the *Phyllanthus* species. It plays important role in the development of green medicines which are safer to used and more dependable than costly synthetic drugs with no adverse effects. *Phyllanthus amarus* uses are gaining momentum because of its novel antiviral activity against hepatitis B virus and for several other biological activities such as kidney and gallbladder, strong cold, flu, tuberculosis, liver diseases etc (Unander *et al.,* 2005).

MATERIALS AND METHODS

Collection of the Plant Samples

The fresh leaves of *Andrographis paniculata* and *Phyllanthus amarus* were collected in May, 2019 from the botanical garden in Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene Local Government Area. Both plants were authenticated by a Taxonomist in the Department of Botany, University of Uyo, Akwa Ibom State, Nigeria and were later taken to Biochemistry Laboratory in Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene for preparation and use in the study.

Preparation of the Plant Samples

Andrographis paniculata and *Phyllanthus amarus* plant leaves were plucked from the stems and were separately rinsed with distilled water to remove debris, shred and were dried for 48 hours under shade. The dried samples were ground separately to powdery form using an electric blender

and were stored in airtight containers and labeled correctly. About 250kg of powdered *Andrographis paniculata* was macerated in 1900ml of 70% ethanol and 220kg of powered *Phyllanthus amarus* was macerated in 2500ml 70% of ethanol for 72hours respectively at room temperature (25^0C). The mixtures were then filtered separately using Whatmans No.1 filter paper over a funnel. The filtrates were separately concentrated in water bath at 40-50^OC for three consecutive days to get the slurry form of the extracts. They were preserved in a refrigerator at 4^OC for further use.

Experimental Design, grouping and Treatment of the Animals

Twenty (20) male albino wistar rats weighing (130-170g) were obtained from the disease free stock of the animal house, Biochemistry Unit, Department of Science Technology, Akwa Ibom State Polytechnic, Ikot Osurua, and were randomly assigned four (4) groups of five rats each. They were housed in wooden cages under standard

conditions for acclimatization for one week in the animal house before the commencement of the experiment. Each group was weighed to obtain the mean body weight Group 1, 2 and 3 were administered with 30 percent of the lethal dose of cadmium each before the commencement of extract treatment. Group 1 received a combined extract of *Andrographis paniculata* (200mg) and *Phyllanthus amarus* (200mg). Group 2 received 400mg *Phyllanthus amarus* while Group 3 received 400mg of *Andrographis paniculata.* Group 4 did not receive either heavy metal nor the extract and served as control. All animals in the groups were fed with rat mash and distilled water throughout the period of treatment. The extracts were administered daily through oral route. Good hygiene was maintained by constant cleaning and removal of faeces and spelled feeds from the cages daily.

Collection of Serum Sample

After 21 days of extracts administration and feeding, the animals were subjected to overnight fast, then they were anaesthetized with chloroform vapour and were sacrificed by dissecting medioventrically and the blood sample were collected via cardiac puncture by means of syringe and needle into well labeled anticoagulant (EDTA) bottles and gently shaken and allowed to stand for 1 hour after which they were centrifuged at 4, 000rpm for 10minutes to separate serum from the blood cells. Serum levels of albumin, globulin and bilirubin were determined in the serum obtained.

Determination of Serum Albumin

This was done using the method of Grant *et al.* (1987). Standard commercial Kit supplied by Randox Laboratories UK, was used.

Procedure: About 10 µl of distilled water, 10 µl of standard and 10 µl of serum (sample) was pipetted into three different test-tubes labeled blank, standard and sample

respectively. Three hundred microliters (300 µl) of Bromocresol Green (BDG) reagent was measured into each of the test tubes, mixed and incubated at room temperature (15-24^{O}C) for 5 minutes after which the absorbance of the sample and standard were measured against that of the reagent blank at 578nm with a spectrophotometer. The procedure was repeated for each of the respective samples in each group.

Calculation:

Concentration of Serum Albumin =

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

Determination of Serum Total Bilirubin

Total bilirubin level was determined based on the method of Jendrassik and Grof (1938). Standard assay Kit was supplied by Randox Laboratories UK, was used.

Procedure: About 200ml of reagent 1 (Supharidic acid) was pipetted into two different test-tubes labeled blank and

sample respectively. Fifty microliters (50 µl) of reagent 2 (Sodium nitrate) was added to the test tube labeled sample. One thousand microliters (1000 µl) of reagent 3 (caffeine) was then added to each test tubes respectively followed by 200 µl of sample being added to each test tube. The contents of each test tube was properly mixed and incubated at room temperature ($\pm 25^{O}C$), after which 1000 µl of reagent 4 (tartrate) was added to each test tube, mixed and further incubated for 30 minutes at $25^{0}C$. The absorbance of the sample was measured against absorbance of the blank at 578nm with a spectrophotometer. The procedure was repeated for each of the samples in each group.

Calculation:

Concentration of Serum Total bilirubin = $10.8 \times A_{TB}$

Where A_{TB} = Absorbance of Total Bilirubin

Determination of Serum Direct Bilirubin

This was determined using method of Jendrassik and Grof (1938). Standard assay Kit was supplied by Randox Laboratories UK, was used.

Procedure: Two test tubes were lebeled blank and sample, then 200 µl each of the reagent 1 (Sulphauilic acid) was measured into each of the test tube). About 50 µl of reagent 2 (Sodium nitrate) was measured into the test tube labeled sample. About 200 µl of 0.9% Sodium Chloride (NaCl) solution was measured into each test tube, followed with 200 µl of sample to each of the tubes. The contents in each test tube was mixed and incubated for 10 minutes at room temperature, after which the absorbance of the sample was read against that of the blank at at 578nm with a spectrophotometer. The procedure was repeated for each of the samples in each group.

Calculation:

Concentration of Serum Direct Bilirubin = 14.1 x A_{DB} (mg/dl)

Where A_{DB} = Absorbance of direct bilirubin

Determination of Serum Globulin

Serum globulin was determined as the difference between total protein and albumin

Globulin (mg/dl) = Total protein (g/dl)- Albumin (g/dl)

Statistical Analysis

The data obtained from the test were subjected to one way analysis of variance (ANOVA). Significant difference were obtained at $P< 0.05$ by Boniferroni multiple range test. The results were expressed as mean ± standard error of mean (SEM). This was estimated using statistical package for Social Science (SPSS) verson 23.

Table 1: Mean Serum Albumin, Globulin, Total Bilirubin and Direct Bilirubin in a Heavy Metal Treated Albino Wistar Rats Treated with Ethanol Extracts of *A. paniculata* and *P. amarus* Leaves.

Parameters	Group 1	Group 2	Group 3	Group 4
Albumin (mg/dl)	3.11± 0.71	2.84± 0.27	2.77± 0.21	3.68± 0.43
Globulin (mg/dl)	4.44± 1.36	2.52± 0.01	0.69± 0.01	0.66± 0.02
Total Bilirubin (mg/dl)	0.63± 0.06	0.52± 0.01	0.69± 0.01	0.66± 0.02
Direct Bilirubin (mg/dl)	0.76± 0.62	0.44± 0.04	0.59± 0.05	0.77± 0.06

Results are presented as mean ± SEM of triplicate determinations

DISCUSSION

Medicinal plant have a long standing history in many indigenous communities and continue to provide useful tools for treating various aliment. The effects of co-administration of ethanol leaves extracts of *Andrographis paniculata* and *Phyllanthus amarus* on albumin, globulin, and bilirubin in heavy metal (Cadmium) treated albino wistar rats was evaluated. There was a significant decrease in direct bilirubin level in group 2 compared to the control group. This may indicate that *Phyllanthus amarus* have the potency to protect the animals against oxidative stress caused by heavy metal toxicity. It appears that *Phyllanthus amarus* and *Andrographis paniculata* have antagonistic joint action since the group co-administered with the two leaf extracts showed very minimal or negligible change in the albumin, globulin total bilirubin and direct bilirubin levels in all the treatment groups compared to the control group.

There was a significant decrease in the level of serum direct bilirubin in Group 2 when compared to Group

1. This helps to affirm the assertion that *Phyllanthus amarus* is more effective in lowering the direct bilirubin level in the serum when used alone, rather than when used in combination with *Andrographis paniculata*. This result is not in agreement with the report of Narsir *et al.* (2013), that significant increase was observed in direct bilirubin in their investigation on the effect of aqueous leaf extract of *Andrographis paniculata* against carbon tetrachloride-induced hepatoxicity in rats. There was no significant difference in the mean serum globulin and albumin levels in all the treatment groups when compared to their control groups. This observation was in accordance with the report of Bukoye and Musbau (2011), who investigated the effect of chronic administration of aqueous leaf extract of *Andrographis paniculata* on protein profiles. They reported a significant decrease in globulin level. This implies that the leaf extract of *Andrographis paniculata* and *Phyllanthus amarus* have the potency to reduce the serum protein. This finding support the assertion that the plant extracts

have the potency to ameliorate the effect of oxidative stress due to toxicity of heavy metals. From the results of this work, it can be suggested that *Phyllanthus amarus* is more effective in the treatment of oxidative stress due to exposure of the animals to heavy metal, but the combined leaf extracts exhibited less effectiveness, which could be due to their antagonistic action.

CONCLUSION

Based on the results obtained in this work, it could be concluded that combined extract of the two plants studied demonstrated antagonistic effect against each other, whereas individual plant extracts exhibited hepatoprotective effect on the experimental animals and could be advantageous in treating hepatic disorders.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and

country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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