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Neurobehavioral and Safety Evaluation of a Polyherbal Antihypertensive Mixture in Ghana

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ABSTRACT

Neurobehavioural evaluation is an important component of testing for the neurotoxic potential of drugs. The aim of this study therefore is to ascertain the safety for use of a polyherbal anti-hypertensive product on the Ghana market (made from bark and leaf extracts of Persea Americana and Vernonia amygdalina) by assessing neurobehavioural effects and safety profile when administered to ICR mice. The effects of 55 - 550 mg/kg of the product on general health (home-cage/open-field observations), locomotory activity (motion, rearing, centering), muscular coordination and strength (grip strength and rotarod tests), vestibular function (righting reflex response), liver and kidney function, hematological profile, and urine content was assessed. One hour post-treatment observation in neurobehavioral studies revealed significant dose-dependent reduction ($P \leq 0.05$-0.001) in locomotion, rearing, centering grip strength, muscle coordination, and righting response. These observations were insignificant 24 hours post-treatment. Acute and delayed toxicity studies showed no involuntary/abnormal motor movements, stereotypy, bizarre behaviors, abnormal gait and posture, clinical signs, and mortality. There were no significant differences in measured hematological parameters except for WBCs ($P \leq 0.05$), liver function tests, and urine analysis between treated and untreated mice. While creatinine levels were normal, urea levels were significantly high ($P \leq 0.05$) at higher doses of treatment. The product is not lethal but could cause central nervous system depression, anxiolysis, and probably muscle relaxation which warrants cautioning users.

Keywords: Antihypertensive, CNS depression, ICR Mice, Spontaneous activity, Grip strength.

INTRODUCTION

Hypertension is a major public health problem in Ghana [1], and is associated with relatively low levels of awareness, drug treatment, and blood pressure control [2]. It was reported in 2007 as the leading cause of death and hospital visits (70 %) in Ghana [3]. The number of reported new cases of hypertension in outpatient public health facilities in Ghana increased more than ten-fold in 2007 [3]. In most regions in Ghana, hypertension ranks as the fifth commonest cause of outpatient morbidity. However, in the Greater Accra Region, hypertension was second to malaria as the leading cause of outpatient morbidity in 2007 [4]. Hypertension is an important cause of heart failure in Ghana and West Africa [5, 6]. These facts stimulate the quest for the search of herbal antihypertensive products with maximum efficacy and minimal adverse effects. The product under study is a polyherbal made from *Persea americana* and *Vernonia amygdalina,* registered with the Food and Drugs Board (FDB), and is effective in managing hypertension.

Persea americana Mill (Lauraceae) also known as avocado, alligator pear or butter pear has hypotensive or antihypertensive effects [7, 8]. *Vernonia amygdalina* L. (Asteraceae) also known as "bitter leaf" is a widely used medicinal plant in Africa for its antihypertensive effects [9]. Leaves from this plant serve as vegetable and culinary herb in soup [10]. In traditional Nigerian homes, extracts of the plant are used as tonic, in the control of tick and treatment of cough, feverish condition, constipation and hypertension [11-13].

When taking any form of drugs for treatment of hypertension, there is a possibility of neurobehavioural changes and toxic effects. It is for this reason that this polyherbal antihypertensive mixture commonly used in Ghana for managing hypertension is being evaluation for investigated for neurobehavioural effects and safety for use.

MATERIALS AND METHODS

2.1 Animals and Husbandry

Male Imprint Control Region (ICR) mice at 3-4 weeks of age (weighing 30-35g) were obtained from the Department of Pharmacology, KNUST, animal house and acclimated for 2 weeks prior to initiation of dosing. During this period, mice were observed (physical; in-life) daily and weighed. At initiation of treatment, animals were approximately 5 weeks old. Individual weights of mice placed on test were within ± 30% of the mean weight for each sex. All mice were examined during the acclimation period to confirm suitability for study and those considered unsuitable were eliminated.

Animals were housed in stainless steel, wire mesh cages during the acclimation and the experimental periods. The mice were kept under ambient light/dark cycle, room temperature and relative humidity. The animal had free access to pelleted mice chow (GAFCO, Tema, Ghana) and water daily.

This study was conducted in 2010 at the Department of Pharmacology, KNUST in compliance with all appropriate parts of the Animal Welfare Act Regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112–36163 effective

October 30, 1989 and 9 CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426–6505 effective March 18, 1991.

2.2 Chemicals Used

Diazepam complex (Sigma and Aldrich, St. Louis MO, USA) was used as a reference CNS depressant, anxiolytic and muscle relaxant. Caffeine complex (Sigma and Aldrich, St. Louis MO, USA) was used as a reference CNS stimulant.

2.3 The Polyherbal Product (PHA) and Dosing

A prepackage preparation made from leaves and bark of *Persea americana*, and *Vernonia amygdalina* referred to in this study as PHA is the product under study. Dosing of PHA was based on the manufacturer recommendation which was calculated to be 55 mg/kg/day. ICR mice were randomly distributed into a control group and treatment groups and received either vehicle or PHA by gavage. Dosing was a single event at a volume of 10 ml/kg body weight. Individual dose volumes were calculated based on the animal's most recent recorded body weight. The oral route of administration was used because it is the intended human exposure route.

2.4 Experimental Procedures

2.4.1 Behavioral Assessment

The spontaneous activity observed as the number of times a mouse crossed a line (motion), reared, or walked through the center of the open field (centering), of untreated ICR mice and mice treated with 55 and 550 mg/kg of PHA were observed 1 hour post-treatment in an open field previously described by Schiørring, 1979, with modification [14]. The procedure was repeated 24 hours post-treatment. The assessment was done with diazepam (0.08 mg/kg) as a reference anxiolytic and caffeine (100 mg/kg) as a reference anxiogenic drugs.

2.4.2 Neuromuscular Assessment

Neuromuscular tests for evaluating motor coordination and muscular strength was carried out using the grip strength test and the rotarod test as described by Moser, 1999, in the current protocols in toxicology [15].

2.4.2.1 Grip strength test

In this test, untreated as well as PHA-treated mice (55 and 550 mg/kg) were hung on a line and observed for how long they could hang and walk from the middle of the line to safety at the edge of the line. Animals that hang but did not move and those that fell off the line were not considered. The threshold period was three minutes. The assessment was done with diazepam (0.08 and 0.16 mg/kg) as a reference muscle relaxant

2.4.2.2 Rotarod test

The test is used to evaluate the activity of drugs interfering with motor coordination. Naive mice were placed on a 32 mm diameter horizontal wooden rod of the rotarod equipment (Model 7600; Ugo Basile, Italy) rotating at a speed of 20 rpm. Mice capable of remaining on the top for 3 min or more, in three successive trials were selected for the study. The selected animals were divided into the no treatment (control), 55 mg/kg and 550 mg/kg PHA groups (n = 5) on the test day. One hour after administration of doses each group of animals was then placed on the rod. The time taken (within three minutes) for each animal in a group to fall off the rotating rod was recorded. The total of all the recorded times per group was estimated and compared with the

control. The procedure was repeated 24 hour post treatment. The assessment was done with diazepam (0.08 and 0.16 mg/kg) as the reference muscle relaxant.

2.4.2.3 Righting Reflex Test

In this test, the untreated as well as PHA-treated mice (55 and 550 mg/kg) were held in a supine position (on its back) and then quickly released to see if they would immediately flip over to resume to a normal standing position. This procedure was done 1 hour and then 24 hours post-treatment. The assessment was done with diazepam and caffeine as the reference CNS depressant and stimulant respectively. The depressant action in the righting reflex tests was scored as no effect (-), slight depression (+), moderate depression (++), strong depression (+++), very strong depression (++++). A trained observer unaware of the experiment assigned the score for the general behavioral studies.

2.43 Safety Assessment

2.4.3.1 Acute and Delayed Toxicity Test

The mice were assigned to treatment groups 1-6, with ten (10) in a group. Group 6 was the non-drug treatment group (control). PHA was administered at doses of 55, 110, 165, 275, and 550 mg/kg (representing the stated daily dose, two, three, five and ten times the daily dose respectively). Observation for clinical and behavioral symptoms of toxicity and mortality were made by filming for the first 60 minutes after which observations were made hourly for 24 hours and then daily thereafter for 14 days. The time of onset, intensity, and duration of these symptoms, if any, was recorded.

2.4.3.2 Hematological Profile and Urine analysis

Blood samples from untreated mice (control) and 550 mg/kg PHA-treated mice were collected into EDTA treated sample tubes before treatment, 24 hours after treatment, and then 10 days post-treatment and sent to the KNUST Hospital for hematological assessment using the BC-3000plus Mindray Autohematology Analyzer (Shenzhen, China). Hematological parameters measured are as shown in Table 1. A semi-quantitative biochemical test was performed on fresh urine samples obtained from mice in metabolic cages (Ugo Basile Biological Research Equipment, Comerio,Va., Italy) prior to treatment, 24 hours, and then 10 days post-treatment using Taytec urine reagent test strips (Missisauga, Canada). Parameters measured were pH, calcium, protein, specific gravity, protein, blood, bilirubin, ketone, glucose, and ascorbic acid. The color, appearance and smell of the urine samples were also noted.

2.4.3.3 Assessment of Liver and Kidney Function

Prior to PHA treatment (control) and then 10 days post-treatment with 550 mg/kg PHA, blood samples for liver and kidney function tests were collected and centrifuged (temperature: 25°C, speed: 4000g) for 5 minutes using the Mikro 220R [Hettich Zentrifuge, USA] machine to obtain the plasma. In the kidney function tests, blood urea was determined by an enzymatic colorimetric test using the urea cromatest Kit [Linear Chemicals SL, Barcelona, Spain] and blood creatinine was determined by the creatinine kinetic colorimetric method using the creatinine cromatest Kit [Linear Chemicals SL, Barcelona, Spain]. Absorbances were measured using a microtitre plate reader (ELx808^{tm} UV Biotek Instruments Inc., Winooski, Vermont, USA) and plasma concentrations of urea and creatinine were calculated. In the liver function tests, *aspartarte aminotransferase* (AST), *alanine aminotransferase* (ALT), *alkaline phosphatase* (ALP), *gamma*

glutamyl transferase (GGT), total protein, and albumin were analyzed using methods stated in reagent kit [Fortress Diagnostics Ltd, Antrim, UK] available. Absorbances were measured.

2.5 Data Collection and Analysis

Observations were filmed using a digital camera (FujiFilm FinePix S700, Fujifilm Corporation, USA). Tracking of spontaneous activity (motion, rearing, centering), rotarod and grip strength tests was done with the aid of Techsmith Camtasio Studio 6.0 software. The statistical analysis of data was done using GraphPad Prism Version 5.0 [GraphPad Software, Inc. USA]. Statistical estimates were made with One-way Analysis of Variance (ANOVA) followed by Bonferonni's multiple comparisons test [post test] at a confidence level of 95 %. Probability values less than or equal to 5 % ($P \leq 0.05$) were considered significant.

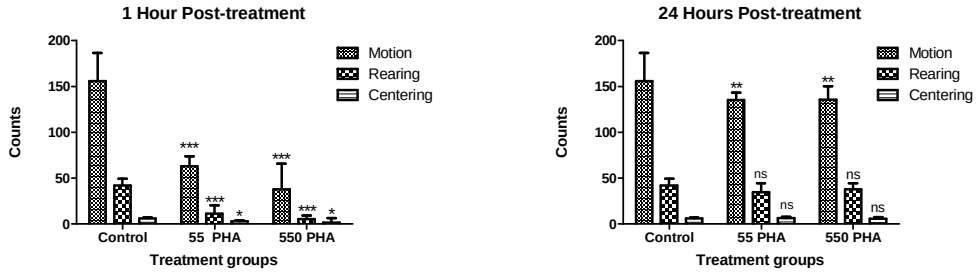

Figure 1: Effect of 55 mg/kg and 550 mg/kg of PHA on motion, rearing and centering in ICR mice in an open field observation 1 hour and 24 hours post-treatment. * implies $P \leq 0.001$; * implies $P \leq 0.001$; ns implies $P > 0.05$.**

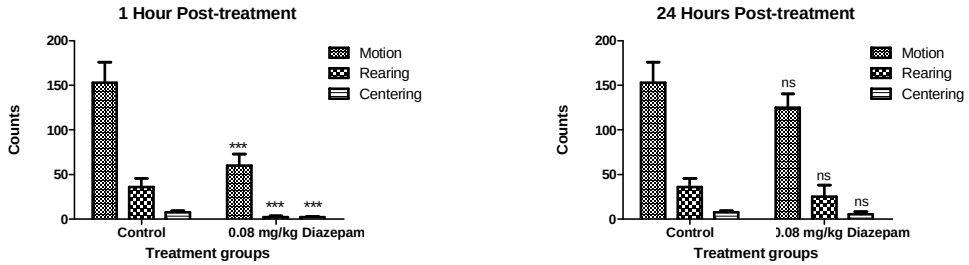

Figure 2: Effect of diazepam (0.08 mg/kg) on motion, rearing and centering in ICR mice in an open field observation 1hour and 24 hours post-treatment. * implies $P \leq 0.001$; ns implies $P > 0.05$.**

RESULTS

Results from the open field examination show that PHA caused very significant dose-dependent decreases ($P \leq 0.001$) in locomotory activity (mostly walking with little or no running) and rearing and a significant decrease ($P \leq 0.01$) in centering, one hour post-treatment but these effects were not significant 24 hours post-treatment (Figure 1). Results from the diazepam-treated mice were similar (Figure 2). The video recording revealed that the mice showed very low reactivity and arousal (somewhat slow, sluggish, slight exploratory movements). There were no significant changes in measured parameters in the caffeine-treated groups (Figure 3). With the manipulative tests, it was observed that PHA-treated mice had significant dose-

dependent reductions ($P \leq 0.01 - P \leq 0.001$) in grip strength and motor coordination (Figures 4 and 5), and righting response (++); comparable to diazepam-treated mice. These observations however were not significant after 24 hours post-treatment.

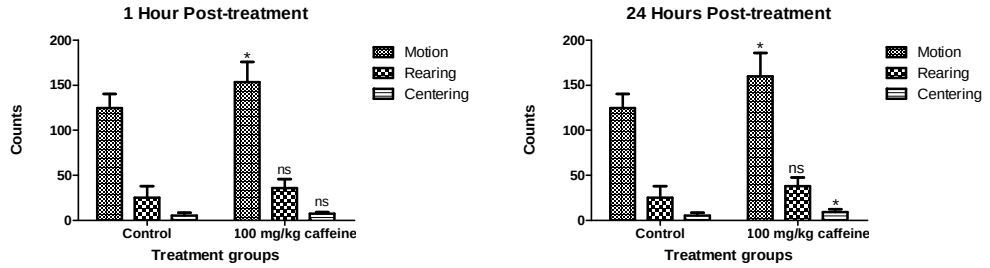

Figure 3: Effect of Caffeine (100 mg/kg) on motion, rearing and centering in ICR mice in an open field observation 1hour and 24 hours post-treatment. * implies $P \leq 0.05$; ns implies $P > 0.05$.

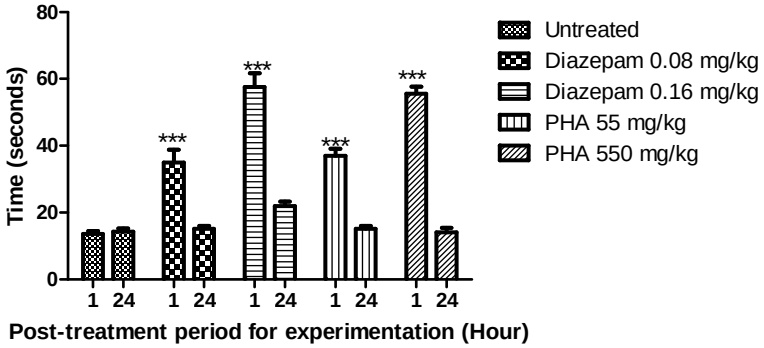

Figure 4: The effects of diazepam (0.08 and 0.16 mg/kg) and PHA (55 and 550 mg/kg) on motor coordination and muscular strength using the grip strength test for neurobehavioural assessment during an acute toxicity study. * implies $P \leq 0.001$.**

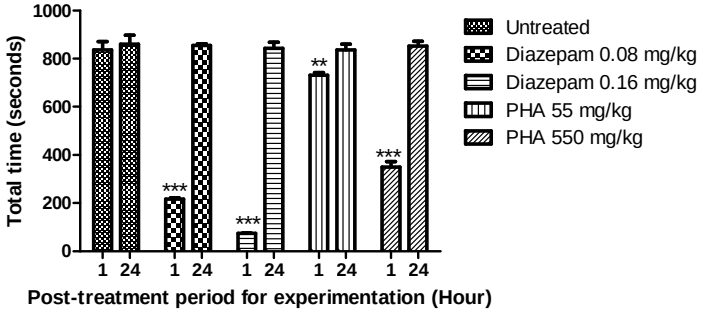

Figure 5: The effects of diazepam (0.08 and 0.16 mg/kg) and PHA (55 and 550 mg/kg) on motor coordination and muscular strength using the rotarod for neurobehavioural assessment during an acute toxicity study. * implies $P \leq 0.001$, ** implies $P \leq 0.01$**

After treatment with PHA, no deaths were recorded at any dose levels over the entire experimental period (14 days). There was no lacrimation, salivation, urination labored breathing, constipation, emaciation, skin eruptions, abnormal posture, hemorrhage, sedation, diarrhoea, polyuria, polydipsia, polyphagia, anorexia, rhinorrhoea/nasal congestion, loss of autonomic reflexes, neuromuscular inco-ordination and collapse, hyperesthesia, hypothermia, twitching, spasticity, convulsion, writhing, tremors, fasciculations and respiratory depression. Observations of gait in all groups of animal did not show, staggering, wobbly gait, hind limbs exaggerated, overcompensating, and/or splayed movements, feet (primarily hind feet) point outward from body, forelimbs dragging and/or showing abnormal positioning, nor walking on toes (the heels of the hind feet are perpendicular to the surface). The mice did not show completely flattened, pelvis flat on surface or pelvis low, dragging somewhat, but the mice did show a hunched, or raised up back.

Hematological analysis showed no significant difference ($P > 0.05$) between all parameters measured of the treated and untreated groups, except for a decrease ($P \leq 0.05$) in white blood cell count over time (Table 1). RBC values recorded were all indicative of normal values typical of laboratory mice i.e. within 5-9.5 x 10^6 cells/mm^3 of blood [16]. Hemoglobin levels recorded were within normal range (10-16.3 g/dl) and so were the MCV (48-56 fL), MCH (11.9-19.0 pg), and the MCHC (25.9 35.1 g/dl). There were no discrepancies in the RBC distribution widths between the treated and untreated animals over the entire study period. Even though platelet number was much lower compared with normal values (1084-1992 x 10^3 platelets/µl of blood), there were no significant variation among values obtained for treated and untreated animals. A similar trend was seen with MPV, PDW, and PCT (Table 1).

Table 1: Hematological assessment values obtained before and after treatment of ICR mice with 550 mg/kg of PHA.

	Control	24 hours post-treatment	10 days post-treatment
WBC (x10^9/L)	8.48 ± 2.45	7.27 ± 4.67	2.87 ± 1.46*
HGB (g/dl)	13.98 ± 1.47	14.92 ± 1.13	13.87 ± 1.05
RBC (x10^{12}/L)	7.52 ± 0.63	7.67 ± 0.75	7.28 ± 0.69
HCT (%)	39.48 ± 4.14	41.33 ± 2.69	37.57 ± 3.15
MCV (fL)	52.53 ± 2.38	51.35 ± 0.60	51.82 ± 3.40
MCH (pg)	18.52 ± 0.78	18.47 ± 0.29	19.03 ± 1.07
MCHC (g/dl)	35.35 ± 0.24	35.35 ± 0.24	36.88 ± 0.76
RDW-CV (%)	14.73 ± 0.64	16.17 ± 0.34	16.35 ± 0.93
RDW-SD (fL)	28.25 ± 1.85	29.22 ± 0.62	29.68 ± 2.55
PLT (x10^9/L)	700.00 ± 134.51	706.33 ± 120.53	791.83 ± 197.64
MPV (fL)	6.28 ± 0.57	6.48 ± 0.35	6.18 ± 0.67
PDW	16.52 ± 0.71	17.07 ± 0.58	16.47 ± 1.21
PCT (%)	0.43 ± 0.06	0.46 ± 0.07	0.49 ± 0.12

*Values recorded are means and standard deviations (N=6). Values obtained for the various parameters before treatment (control, 24 h and 10 days post treatment are not significantly different from each other but for WBC. * implies $P \leq 0.05$. Level of significance was analyzed using One-Way Analysis of Variance (ANOVA) followed by Dunnet's post-hoc test.*

Urinalysis of treated and untreated mice, showed no significant changes except for the increase in pH which become slightly basic (from 5.4 ± 0.55 to 7.8 ± 1.60) and the change of urine color from straw to amber after 24 hours of drug treatment but returns to normal (Table 2). Urine protein was also high compared to normal values of urine analytes for laboratory mice (20–40 mg/24 h) [17]. That notwithstanding, variation among treatment groups were not significant. Urine colour, smell, appearance and volumes were similar to the control.

Table 2: Semi-quantitative urine analysis values obtained with Taytac urine reagent strips before and after treatment with 550 mg/kg of PHA.

Parameters	Before Drug Treatment	24 h after Treatment	Day 10 after Treatment
pH	5.4 ± 0.55	7.8 ± 1.60	5.6 ± 0.54
Nitrite (mg/dL)	(-)	(-)	(-)
Urobilinogen (mg/dL)	0.2 ± 0.00	0.2 ± 0.00	0.2 ± 0.00
Protein (mg/dL)	72 ± 38.3	86 ± 31.3	58 ± 38.3
Specific gravity	1.000 ± 0.000	1.004 ± 0.002	1.000 ± 0.000
Blood(Ery/uL)	(-)	(-)	(-)
Bilirubin (mg/dL)	(-)	(-)	(-)
Ketones (mg/dL)	(-)	(-)	(-)
Glucose (mg/dL)	(-)	(-)	(-)
Ascorbic (mg/dL)	32 ± 11.0	36 ± 8.0	32 ± 11.0
Color	Straw	Amber	Straw
Appearance	Clear	Clear	Clear
Smell	Pungent	Pungent	Pungent
Volume	1.14 ± 0.28	1.24 ± 0.27	1.10 ± 0.20

Values are Means ± Standard Deviations. N=5

In the liver function tests there were no significant differences ($P > 0.05$) in AST, ALT, and GGT levels. ALP and total protein levels increased significantly ($P \leq 0.01$-0.001) 24 h after treatment but by day 10, levels were not significantly different from that of the control (Table 3). In the kidney function tests, changed in plasma creatinine concentration was not significantly, while plasma urea increased significantly ($P \leq 0.05$) (Table 4).

Table 3: Liver function test results obtained before and after treatment with 550 mg/kg of PHA

	Control	24 h post-treatment	10 days post-treatment
AST/GOT (U/I)	8.85 ± 6.80	12.08 ± 9.47	12.51 ± 11.87
ALT/GPT (U/I)	8.23 ± 4.83	3.88 ± 3.11	5.42 ± 4.98
GGT (µmol/l)	4.17 ± 2.24	7.33 ± 9.36	3.40 ± 2.32
ALP (U/I)	16.74 ± 9.09	44.53 ± 4.09 ***	13.62 ± 8.21
Total protein (g/l)	58.93 ± 14.87	97.65 ± 28.07 **	45.70 ± 7.92
Albumin (g/l)	26.94 ± 21.79	23.59 ± 5.35	15.15 ± 2.41

*Values are Means ± Standard Deviations. N=6. ** implies $P \leq 0.01$; *** implies $P \leq 0.001$. Level of significance was analyzed using One-Way Analysis of Variance (ANOVA) followed by Dunnet's post-hoc test. ALT/GPT = Alanine Transaminase/Glutamic Pyruvate Transaminase, AST/GOT = Aspartate transaminase/Glutamic Oxaloacetic transaminase, ALP = Alkaline Phosphatase, GGT = Gamma GlutamylTransferase.*

Table 4: Plasma urea and creatinine concentrations obtained for kidney function tests before and after treatment with 550 mg/kg of PHA.

	Control	24 h post-treatment	10 days post-treatment
Urea (mmol/l)	26.68 ± 19.46	41.72 ± 16.88	56.92 ± 12.15 *
Creatinine (µmol/l)	0.49 ± 0.45	0.60 ± 0.57	0.45 ± 0.42

*Values are Means ± Standard Deviations. N=6; Values for plasma creatinine obtained for PHA-treated mice were not significantly different from that of the control. * implies $P \leq 0.05$. Level of significance was analyzed using One-Way Analysis of Variance (ANOVA) followed by Dunnet's post-hoc test.*

DISCUSSION

Decreasing spontaneous activity motor coordination and muscular strength could imply that PHA could have CNS depressant, anxiolytic and/or muscle relaxant effect (similar effects were

seen in diazepam-treated groups) and thus may possibly be acting by; activate inhibitory $GABA_A$, inhibiting excitatory AMPA receptor**s,** inhibiting the NMDA glutamate receptor (inhibits an excitatory effect), and/ or potentiating the action of serotonin (5-HT) at excitatory 5-HT_3 receptors [18]. Serotonin (5-HT) potentiates the inhibitory effect of dopamine on dopaminergic ventral tegmental area (VTA) neurons [19]. Since these receptors are often localized on inhibitory interneurons, enhanced activation results in CNS inhibitory effects. It could dissolves into lipid membranes affecting the function of membrane proteins, such as receptors and ion channels.

The $GABA_A$ receptor is an inhibitory channel which when activated decreases neuronal activity and enhances the effects of GABA [20]. When GABA binds to its site on the $GABA_A$ receptor more chloride ions enter and cause hyperpolarization of the post synaptic neuron resulting in an enhanced central nervous system depressant effect [21, 22].

α-*amino-3-hydroxy-5-methyl-4-isoxazole propionic acid* (AMPA) receptors are the main mediators of fast excitatory neurotransmission in the CNS [23]. Inhibiting these excitatory AMPA receptors therefore results in CNS depression. The NMDA receptor is an ionotropic receptor that allows for the transfer of electrical signals between neurons in the brain and in the spinal column. For electrical signals to pass, the NMDA receptor must be activated. To remain activated, an NMDA receptor must bind to glutamate and to glycine. Chemicals that deactivate the NMDA receptor would inhibit excitatory effects resulting in CNS depression.

A possible PHA depressant activity on the limbic system, thalamus, and hypothalamus, could account for its anxiolytic effects. Though CNS depressant effect has been observed as an adverse effect with the use of PHA, it could possibly be the mechanism by which the polyherbal exerts its antihypertensive effect.

Observations made at 1-2 hour intervals for 24 hours and then daily for 14 days makes the study convenient for an acute and delayed toxicity study because most of the observable symptoms of acute toxicity occur within the first 1-2 hours of drug administration. No death recorded implies that the product was not lethal even when up to ten times the daily dose was taken as a single dose. Sedation, lethargy and reduced activity observed with the administration of PHA may be a result of CNS depression. CNS depression often results from the use of depressant drugs such as alcohol, opioids, barbiturates, benzodiazepines, and general anesthetics. When these are used, effects may include anxiolysis, analgesia, sedation, somnolence, dissociation, muscle relaxation, lowered blood pressure/heart rate, respiratory depression, anesthesia, and anticonvulsant effects.

RBC values being normal indicate that PHA does not cause hemolysis of RBCs and does not have hemorrhagic tendencies. The normal levels of HGB, MCV and RDW confirm this. All thing being equal, hemoglobin concentration should be proportional to the RBC count as hemoglobin is found in red blood cells. Some herbal product can cause massive hemolysis soon after they have been administered resulting in a low RBC counts and an increased plasma and urine bilirubin and urobilinogen levels. The negative urine bilirubin and low urobilinogen confirms the “no hemolysis”. Abnormal and accelerated destruction of red cells and an increased breakdown of hemoglobin would result in increased bilirubin level (mainly indirect-reacting) with jaundice and increased fecal and urinary urobilinogen [24].

It cannot be said that he liver was injured by drug administration as indicated by the insignificant differences in AST and ALT levels measured between the control and treated groups. Liver transaminases are not indicators of liver dysfunction but are biomarkers of liver injury in a patient with some degree of intact liver function [25-26]. However calculating the AST/ALT ratios 24 h and 10 days post-treatment (3.11 and 2.3 respectively) suggests that the drug possibly causes acute hepatitis at very high doses. The AST/ALT ratio is sometimes useful in differentiating between causes of liver damage [27-29].

The liver's synthetic abilities necessary for normal vital functions was not impaired as albumin levels were not significantly different between control and treated animals. Although ALP levels were elevated significantly 24 h post treatment, it cannot be said vividly as an indicator of liver injury because ALP is associated with cellular membrane and elevated levels may be caused by injury to the liver, bone, kidney, intestine, placenta, or leucocytes. In the liver, ALP is located in the bile canaliculi. Biliary obstruction induces increased synthesis of ALP and spillage into the circulation [26].

The high blood urea nitrogen levels observed in the kidney function tests can indicate kidney dysfunction, but because blood urea nitrogen is also affected by protein intake and liver function, it is therefore not a specific indicator of kidney function [30]. Blood creatinine, which is a more sensitive indication of kidney dysfunction [30], was however not elevated. This could imply that PHA has no detrimental effect on the kidney. This was confirmed in the urine analysis results which showed no observable differences in total protein and all other measured between PHA - treated and untreated mice. Mice as well as other rodents secrete substantial amounts of protein in their urine, which is not generally observed in humans or other mammals [31].

CONCLUSION

The commonly used polyherbal anti-hypertensive preparation has CNS depressant, anxiolytic, and probably muscle relaxant activity which affects neurological behaviors. It is however not lethal up to a dose level of 550 mg/kg. Within limits of acute and delayed toxicity, it can be said that the product is safe to use.

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