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Short Communication

Assessment of Renal and Haematological Effects of *Aspilia africana* Leaf Extracts in New Zealand Rabbits

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ABSTRACT

Aspilia africana is an indigenous medicinal plant known for it's haemostatic property in African folklore medicine. In this study, the renal and haematological effects of *Aspilia africana* leaf extracts in experimental rodents were investigated. Fifteen male rabbits of the New Zealand strain were equally assigned into three groups: Group 1, the control received normal saline, group 2 received 100 mg/kg body weight *Aspilia africana* aqueous extract while group 3 received 100 mg/kg body weight *Aspilia africana* chloroform extract for 14 consecutive days. Renal parameters (creatinine, urea, Na^+, K^+, HCO_3^-) were determined in serum samples while haematological parameters: packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC) and mean cell volume (MCV) were determined in whole blood samples. Oral administration of the aqueous extract of *Aspilia africana* induced a significant decline ($p<0.05$) of urea, sodium, potassium concentrations in serum of test animals relative to control. The chloroform extract did not significantly alter ($p>0.05$) the parameters of renal function except serum bicarbonate value which was significantly elevated. Erythropoiesis represented by red blood cell count (RBC) was significantly suppressed ($p<0.05$) in groups treated with both extracts relative to the control. Proliferation of white blood cells occurred in test groups compared to the control ($p<0.05$) after administration of both extracts. PCV and MCV were not significantly different ($p>0.05$) among the groups**.** The results indicate that the aqueous extract caused elevation in white blood cells count but induced different effects on kidney parameters depending on the extracting solvent.

***Keywords*:** *Aspilia africana*, haematological parameters, aqueous extract, chloroform extract.

Introduction

Medicinal plants as sources of bioactive agents continue to exhibit fascinating ability in terms of human health management since ancient times with more than half of modern therapeutics in circulation being of natural product origin.[1] The vital role of medicinal herbs in the treatment and prevention of diseases is not by itself an assurance of safety if not properly used by an uninformed public.[2] *Aspilia Africana,* is a tropical, semi-woody perennial herb belonging to the family, asteracea. It is used by traditional communities in Africa and Asia as a bactericidal, anti-inflammatory, astringent and wound- healing agent.[3,4] Oral consumption of the decoction of the leaves is reportedly able to relieve febrile headache, quicken delivery and cure lumbago, sciatica and stomach disorders.[5-7] Besides management of stomach disorder, others have reported that in folklore medical practice, *Aspilia africana* herbal preparations can be used for the treatment of stomach aches, dysmenorrhoea, tuberculosis, rheumatoid arthritis, gonococal infection, corneal opacity, cough and insect bites.[8] The kidney performs functions such as urine formation, regulation of acid-base balance, excretion of waste products of protein metabolism, protein conservation and hormonal function. Chronic renal failure and end-stage renal diseases constitute a huge global health challenge in developing and developed countries.[9] Treatment modalities for these ailments typically require rigorous and expensive medical procedures such as dialysis or kidney transplants.[10] Diagnosis and prognosis of renal failure in hospital patients are established by determination of serum of biochemical parameters such as urea, creatinine and electrolytes.[11,12] In experimental rodents, analysis of serum biochemical parameters gives adequate information about organ damage, particularly the liver and kidney.[13,14] Studies on the toxicological and beneficial effects of *Aspilia africana* extracts on renal and haematological parameters in experimental models have not been sufficiently documented in literature. This study was therefore designed to provide scientific information on the beneficial or toxic effects on renal and haematological indices in New Zealand rabbits that would be occasioned by oral consumption of aqueous and chloroform leaf extracts of *Aspillia africana*.

Materials and Methods

Experimental animals

Fifteen male rabbits (New Zealand breed) were housed in rabbit cages in the Animal House of Faculty of Life Sciences, University of Benin, Benin City under suitable environmental conditions (24 ± 2ºC, 12-h light and dark cycles) and were allowed to acclimatize for two weeks with free access to food and clean water before the study commenced. Ethical clearance for animal experimentation was obtained from the Animal

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Ethics Committee of the Faculty of Life Sciences, University of Benin, Benin City.

Preparation of extract

Fresh leaves of *Aspilia africana* were collected in April 2016 from a farmland in Ovwioge, Benin City, Edo State, Nigeria. The leaves were identified by a taxonomist, Dr. Odaro Timothy of the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The collected samples were washed with running tap water, dried under shade and pulverized into fine powder. The aqueous and chloroform extracts were prepared by soaking 250 g each of the powdered leaves in 500 mL of distilled H_2O and 500 mL of chloroform, respectively for 72 h. The extracts were filtered with muslin cloth. Subsequently, the filtrate of the aqueous extract was freeze dried while the filtrate of the chloroform extract was evaporated to dryness with rotary evaporator under reduced pressure. The crude extracts were reconstituted in normal saline and stored in a refrigerator at 4^{o}C until they were required for use.

Experimental Design

The animals were randomly assigned into three groups of five animals each. Group 1 animals, the control were administered normal saline solution. The animals in group 2 were administered 100 mg kg^{-1} body weight of *Aspilia africana* aqueous extract while group 3 received the same dose of the chloroform extract. Oral administration of saline or extract occurred once daily for 14 consecutive days.

Analysis of Haematological and Biochemical Parameters

At the end of the treatment period, the rabbits were anaesthetized after an overnight fast by chloroform inhalation in a closed chamber. Blood samples (5 mL) were collected by cardiac puncture and separated into two lots for biochemical assays and haematological measurements. For the haematological measurements, 2.5 mL of whole blood was taken from each sample into labeled sterile EDTA universal bottle. Haematological parameters (PCV, RBC, WBC, MCV) were then determined using a Medonic M32M Haematology Blood Analyzer. For evaluation of serum biochemical parameters, 2.5 ml of the 5 ml blood were put in anti-coagulant free bottles. The blood was allowed to clot at room temperature and serum separated by centrifuging each sample within three hours of collection at 2000 x g for 10 min. The concentrations of creatinine and urea were determined in serum based on extant methods.[15,16] Serum electrolytes were also assessed as described by Kinsley and Schaffert [17].

Statistical analysis

*Data were presente*d as mean ± standard error of mean (Mean ± S.E.M). Statistical analyses were performed with SPSS 11.5 software. Group comparisons were made using one way analysis of variance (ANOVA) and Duncan Multiple Comparison Test. A p- value of < 0.05 was considered significant.

Results and Discussion

In developing countries, the use of herbal preparations which is an age-long practice remains widespread.[18] End-users of these formulations consumes it indiscriminately unaware of attendant health risks.[19] To protect the uninformed public from the adverse effects of arbitrary application of herbal medicine, health policies grounded on scientific research on the systemic effects of plant extracts is imperative. *Aspilia africana* has been traditionally used to treat wounds.[20] However, its effects on kidney functions are undocumented as far as we know. Thus, we investigated the effects of the aqueous and chloroform extracts on urea, creatinine, electrolyte profile and haematological parameters in New Zealand rabbits. Urea is a major constituent of urine which is excreted in urine by the kidneys. Creatinine, a waste product of muscle energy metabolism and urea are used clinically to assess the filtration capacity of the kidneys in organisms.[21] However, creatinine is a better index of renal function because it is relatively constant and closely varies with glomerular filteration rate unlike urea which fluctuates according to protein intake.[22] In this study, urea level in the aqueous extract-treated group (31.00 ± 1.30 mg/dL) was significantly lower ($p<0.05$) than control value (41.80 ± 2.68 mg/dl) and chloroform-treated group (39.00 ± 2.07 mg/dL) which was similar to the control (Table 1). Thus, oral administration of the aqueous extract caused a marked decline in plasma urea concentration in the group that received the aqueous extract when compared to the placebo group. In contrast, the chloroform extract had an insignificant effect on the plasma urea of the control. A previous study by Jiwuba *et al* [23] showed that decreased urea levels were associated with intake of high quality proteins in experimental rodent. It would therefore appear that the aqueous extract of *Aspilia africana* was able to induce the drop in urea level because of its nutritional value as a source of balanced amino acids.[24] Hence, *Aspilia africana* can potentially attenuate uraemia in patients with acute renal failure. Several studies report that mild or moderate elevation of serum creatinine is a good predictor of severity or otherwise of poor kidney function in patients irrespective of cause.[25-27] As presented in Table 1, the control group had similar ($p>0.05$) creatinine value (0.720 ± 0.07 mg/dL) when compared to the aqueous extract treated group (0.780 ± 0.05 mg/dL) and chloroform treated group (0.560 ± 0.16 mg/dL). Hence, it could be hypothesized that oral consumption of the plant could normalize creatinine values in patients diagnosed with kidney failure. The sodium concentration in the group exposed to chloroform extract (157.20 ± 4.49 mmol/L) was not significantly different ($p>0.05$) from the values obtained from control (155.60 ± 2.02 mmol/L) (Table 1). However, they were significantly higher than the values obtained for the aqueous extract treated group (140.00 ± 11.18 mmol/L). In the same vein, the mean potassium concentration in the aqueous extract treated group (6.40 ± 0.32 mmol/L) was significantly lower ($p<0.05$) than both the control (8.62 ± 0.84 mmol/L) and chloroform groups (9.76 ± 1.33 mmol/L) (Table 1). The administration of *Aspilia africana* aqueous extract thus caused a reduction in sodium and potassium levels of test animals compared to the control. The aqueous extract of the plant may be beneficial to hypertensive individuals but harmful to patients with chronic renal failure. The bicarbonate/carbon dioxide is the major buffering system in the blood with capacity to stabilize pH during conditions of acidosis.[28] The mean serum bicarbonate concentration of the aqueous extract treated group (21.40 ± 0.51 mmol/L) was not significantly different ($p>0.05$) from control values (21.60 ± 1.47 mmol/L). The chloroform extract had the highest concentration of serum bicarbonate among the groups (24.80 ± 1.07 mmol/L) (Table 1). The significant elevation of bicarbonate observed in the chloroform extract treated group could be associated with concurrent potassium depletion which as reported by other investigators [29] stimulates increased renal reabsorption of bicarbonate. According to an earlier report,[30] decreased potassium and sodium levels are associated with elevated bicarbonate in conditions of electrolyte derangement. Because sodium and potassium are required for muscular contraction, the depletion of these micro-

Table 1: Serum urea, creatinine and electrolyte profile.

Group	Urea (mg/dL)	Creatinine (mg/dL)	Na^+(mmol/dL)	K^+(mmol/L)	HCO_3^- (mmol/L)
1	41.80 ± 2.68^{b}	0.720 ± 0.07^{ab}	157.20 ± 4.49^{b}	8.62 ± 0.84^{b}	21.60 ± 1.47^{a}
2	31.00 ± 1.30^{a}	0.780 ± 0.05^{b}	140.00 ± 11.18^{a}	6.40 ± 0.32^{a}	21.40 ± 0.51^{a}
3	39.00 ± 2.07^{b}	0.560 ± 0.16^{a}	155.60 ± 2.02^{b}	9.76 ± 1.33^{ab}	24.80 ± 1.07^{b}

Values within the same column bearing different superscripts are statistically significant different from each other ($p<0.05$).

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Table 2: Haematological profile.

Group	WBC count (x 10^{12} Cell/L)	RBC count (x 10^{12} Cell/L)	PCV(%)	MCV (fL/Cell)
1	5.90 ± 1.34[a]	7.70 ± 0.23[b]	39.62 ± 1.60[a]	66.88 ± 0.55[a]
2	14.34 ± 2.88[b]	5.11 ± 0.32[a]	37.48 ± 0.93[a]	62.92 ± 1.68[a]
3	11.54 ± 0.48[b]	5.55 ± 0.37[a]	40.70 ± 0.83[a]	64.42 ± 1.26[a]

Values within the same column bearing different superscripts are statistically significant different from each other (p<0.05). WBC = White Blood Cell Count (x 10^{12} Cell/l) RBC = Red Blood Cell Count (x 10^{12} Cell/l) PCV= Packed Cell Volume % MCV= Mean Cell Volume (fL/Cell).

nutrients may increase the risk of developing arrhythmias and muscular weakness.[31] In the haematological studies (Table 2), mean red were significantly higher (p<0.05) than the counts recorded for the groups orally treated with aqueous extract (5.11 ± 0.32 x 10^{12} Cell/L) and chloroform extract (5.55 ± 0.37 x 10^{12} Cell/L) both of which had similar RBC values indicating a mild drop in red blood cell count of test groups. An earlier study on the phytochemistry of *Aspilia africana* [32] had demonstrated that *Aspilia africana* contains significant quantities of saponins which had been shown to induce anaemia in rodents.[33] In contrast, it has previously been reported that at higher doses (250 - 500 mg/kg body weight), the aqueous extract of *Aspilia africana* stimulated erythropoiesis.[34] Mean corpuscular volume was not significantly different (p>0.05) among the groups, an indication that the plant did not induce significant alterations in blood cell volume. In addition, packed cell volume of blood was not significantly different (p>0.05) (Table 2). As shown in Table 2, white blood cell count (WBC) in the plasma of the aqueous extract treated group (14.34 ± 2.88 x 10^{12} Cell/L) and chloroform extract (11.54 ± 0.48 x 10^{12} Cell/L) treated groups increased significantly (p<0.05) compared to the control (5.90 ± 1.34 x 10^{12} Cell/L). This revealed that administration of *A. africana* extracts stimulated leucocyte expansion in test animals with the aqueous extract showing more potency in this regard. This is consistent with the finding of an earlier study in which *Aspilia africana* aqueous extract stimulated white blood cell proliferation.[34] The rise in white blood cell count in the present investigation may not necessarily be attributed to the presence of infection but may have been triggered by the method of blood collection or by stress factors induced by oral intake of the extracts.[35, 36] The effects of the aqueous extract on renal parameters when compared to the chloroform extract suggest that the components in *Aspilia africana* that influenced kidney function are likely polar in nature.

Conclusion

Based on the results of the haematological and renal evaluations presented here, it does appear that the aqueous extract of *Aspilia africana* demonstrated more activity than the chloroform extract. The water extract demonstrated pleiotropic effects on haematological parameters. The ability of the aqueous extract to induce adverse effects on electrolyte levels reveals that its consumption could be a potential risk factor for cardiovascular diseases or renal impairment.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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