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Combined Extracts of *Bryophyllum pinnatum* and *Aloe Barbadensis* Induce Hepato-renal Dysfunctions and Elevated Hematological Indices in Wister Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AAA and NOI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MAM and JKF managed the analyses of the study. Author MAM managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The study was conducted to investigate the safety of combined extracts of *Bryophyllum pinnatum* (family: Crassulaceae) and *Aloe barbadensis* (family: Xanthorrhoeaceae) in rats. Forty rats were randomly selected and divided into four groups. Rats in groups I were administered with placebo as the control animals, while rats in groups II, III and IV were administered with aqueous extracts at 25mg/ml, 50mg/ml and 100mg/ml respectively for 28 days. Five milliliter (5ml) of blood was collected from either the ocular vein or aorta of each animal for evaluation of baseline and post – treatment values of AST, ALP, ALT, BUN, Total protein, and Creatinine, WBC, RBC, Platelet, MCV, MCH and MCHC. Data were expressed as Mean±Standard Error of Mean and analyzed using one-way ANOVA. Difference of means was considered statistically significant at $P<0.05$. Quantitative phytochemical assay revealed mainly alkaloids, with one gram each of *B. pinnatum* and *A. barbadensis* extracts containing 5.3mg and 9.1mg of alkaloids

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respectively. Administration of the combined extracts elevated the serum levels of Alkaline phosphatase, Aspartate amino transaminase, Total protein, Creatinine and BUN in the entire rats in test groups especially those in group IV ($P<0.05$). In ALT, there was no significant variation between the baseline and the post-treatment values especially in animals in groups III and IV ($P>0.05$). Rats in groups II, III and IV exhibited significant increase in values of WBC, RBC, Platelet, MCV and MCH ($P<0.05$) while MCHC value for rats in group IV showed insignificant differences when compared to the control rats ($P>0.05$). Administration of the combined extracts may be tolerated at ≤25mg/kg bodyweight, if prolonged administration is avoided.

Keywords: Combinatorial therapy; effect; Hepato-renal dysfunctions; haematological indices.

1. INTRODUCTION

Medicinal plants constitute a well-patronized source of genuine drugs and about 80% of people in developed countries still take natural products [1]. *Bryophyllum pinnatum* is a perennial herbs widely used by traditional healers in many regions of the world. The herb contains a wide range of active compounds otherwise known as phytochemicals such as alkaloids, triterpene, glycosides, flavonoids, steroids, lipids and organic acids. The extract from this plant is effective for use as immune-modulator, analgesics, antibiotics, anti-ulcer drugs, anti-fungals, sedatives, anti-virals and anti -inflammatory agents [2-4].

Similarly, *Aloe barbadensis* is a vital medicinal plant commonly used all over the world. It is a bitter herb with anti-fungal, antibacterial, antiviral and anti–parasitic properties. It contains biologically active compounds such as anthraquinones, saccharides, postaglandins, fatty acids, enzymes, amino acids, vitamin and minerals [5,6].

The use of natural product is fast gaining popularity due to the belief that local herbs are more readily available, affordable and have fewer side effects [7,8]. Increasing resistance of microorganisms to existing antibiotics calls for concerted effort to research for new drugs that are safe for public health consumption and that can serve as good replacements for the older antimicrobial agents [9]. The concept of combinatorial therapy of herbal products can reduce the rate at which uneconomic species (eg: pathogens and cancer cells) develop resistance to drugs.

Meanwhile, several authors [10-14] had documented antimicrobial activities of *Bryophyllum pinnatum* and *Aloe bardadensis* on some isolates of microorganism when the herbs were separately administered. However, there has been a paucity of data on the safety and toxicity of the duo, when jointly administered for treatment of ailments. The two herbs are known for their antimicrobial properties and the tendency to combine both for microbial ailment in healing homes is likely. In such a scenario, the safety of combining extract needs examination and documentation, hence, the need for this study.

This study therefore attempts to investigate the effect of combining *Bryophyllum pinnatum* and *Aloe bardadensis* extracts on some biochemical and hematological parameters in experimental rats to deduce the safety of the combined product for human consumption considering the enormous potential of both herbs in disease control.

2. MATERIALS AND METHODS

2.1 Collection of Plants and Preparation of the Extracts

Fresh *Bryophyllum pinnatum* leaves were collected from Isale Aluko garden near Baboko market in Ilorin, while the fresh *Aloe barbadensis* plant was collected from potted plant at Adewole Estate in Ilorin. All the plants and leaves were taken to Plant Biology Unit of Kwara State University for identification. Manual aqueous extraction was employed on each of the plants. The *Bryophyllum pinnatum* leaves were washed and crushed in sterilized electric blender, the paste obtained was squeezed out through a sterilized mesh of about 0.20mm in size and the residue (extract) was kept in an electronic oven, maintained at 60ºC for drying. The resulting granular powder was weighed, re-dried and weighed again until constant weight was obtained. The same procedure was repeated for the *Aloe barbadensis* leave to obtain coarse granules which were later grinded with sterilized ceramic mortar and pestle.

2.2 Fortification of Extracts

One gram of the granular extract of *Bryophyllum pinnatum* was weighed and dissolved in 10mls of sterile distilled water to obtain 100mg per ml concentration of *Bryophyllum pinnatum*. Also, one gram of powdery Aloe barbadensis was weighed and dissolved in 10mls of sterile distilled water to obtain 100mg per ml concentration of *Aloe barbadensis*, 0.6ml of the *Bryophyllum pinnatum* concentration was mixed with 0.4ml of the *Aloe barbadensis* to obtain 100mg per ml of fortified *Bryophyllum-aloe.* Half of the neat concentration was used as 50mg/ml while a quarter dilution was used as 25mg/ml per kilogram weight of animal.

2.3 Use of Experimental Animals

The procedure of experimentation obeyed the criteria of the Institutional Ethics Committee and approval was given by the committee. Also toxicity in the animals was assessed based on World Health Organization guidelines [15]. Forty apparently healthy adult Wister rats, weighing between 250g and 280g were employed in the study. They were composed of equal number of male and female, procured from the animal house of Biological Sciences Department of Achievers University, Owo–Nigeria. They were fed orally with rat pellets and clean drinking water and were maintained at room temperature (25ºC) throughout the period of study.

These animals were divided into 4 groups. Each group contains five male and five female rats. The first group was the control group while the remaining three groups were test groups. Prior to treatment of the animals, the baseline biochemical parameters of each test group were determined. This was achieved by analyzing the venous blood collected from the ocular vein of the animals. Varying concentration of the fortified extract per kilogram of animal was administered to each animal in test groups by inter-peritoneal injection. The first 10 animals in the test groups (Group 2) were administered with 100mg of the fortified extract, per kilogramme weight of the animals, the second test group (Group 3) animals were administered with 50mg of the fortified extract per kilogramme of the animal while the last ten animals in the third test group (Group 4) were administered with 25mg of the fortified extract per kilogramme weight of the animals. Each animal in the control group (Group 1) was administered 2ml of sterile distilled water as placebo. The administration of the fortified extract into the animals was done daily for 28 days after which post–treatment biochemical

investigations were conducted. The animals were monitored for any strange behaviour due to extract toxicity. Pre and post treatment weight of the animals were also noted and recorded.

2.4 Collection and Processing of Blood Samples

A total of five milliliters of either ocular or aortic blood samples was collected from each of the animals. 2ml of the sample was put in EDTA anti-coagulated container for hematological parameters while the remaining was kept in plain container for Albumin, blood urea nitrogen (BUN), Creatinine, Alkaline phosphatase, Alanine Transaminase and Aspartate Transminase tests. The blood samples in plain containers were allowed to clot and spun at 5,000 revolutions per minute for 5 minute to extract the serum needed for the analysis. The procedures described by Baker and Silveton, Ramnik, and Monica for analysis [16-18] of hematological and biochemical parameters were employed.

2.5 Statistical Analysis

Data generated were entered into the computer and analyzed using SPSS version 17 software (SPSS Inc, USA). The data were expressed as mean$\pm$Standard Error of Mean. The test of significance was performed using One- way ANOVA for baseline and post-treatment values. The level of significance for statistical difference was based on P value less than 0.05.

3. RESULTS

Analysis of some biochemical and haematological parameters were carried out to investigate the hepato-renal functions and haematological indices in Wister rats before and after treatment with the combined extract. BUN, Creatinine and Albumin levels were determined to ascertain the functions of the kidney, serum enzymes such as AST, ALT and ALP were assayed to monitor the functions of the liver while WBC, RBC, MCV, MCH, MCHC and Platelet values were evaluated to assess the effect of the extract on blood cell indices. Prior to the analyses, quantitative phytochemical assay of both extracts revealed essentially alkaloid content in addition to other components. The assay revealed that one gram each of *Bryophyllum pinnatum* and *Aloe barbadensis* extracts contained 5.3mg and 9.1mg of alkaloid respectively (Fig. 1). Effect of the combined extract on hepato-renal functions in rat is as shown in (Table 1). Administration of the extract significantly elevated the serum levels of Alkaline Phosphatase, Aspartate amino Transaminase, Total protein and Blood Urea Nitrogen (BUN) ($P<0.05$) in the entire experimental test rats especially those in group IV. In ALT however, there was no significant variation between the baseline and the post-test values ($P>0.05$) especially in group III and IV animals. Five of the test animals in group IV administered with 100mg/ml of the combined extract died within seven days of treatment while the remaining five survived till the end of the study.

(Table 2) depicts the effect of the combined extract on the heamatological parameters of experimental animal. Rats in groups II, III and IV exhibited significant increase in values of WBC, RBC, Platelet, MCV and MCH ($P<0.05$) while MCHC value for rats in group IV did not show any significant difference ($P>0.05$) when compared to the control rats.

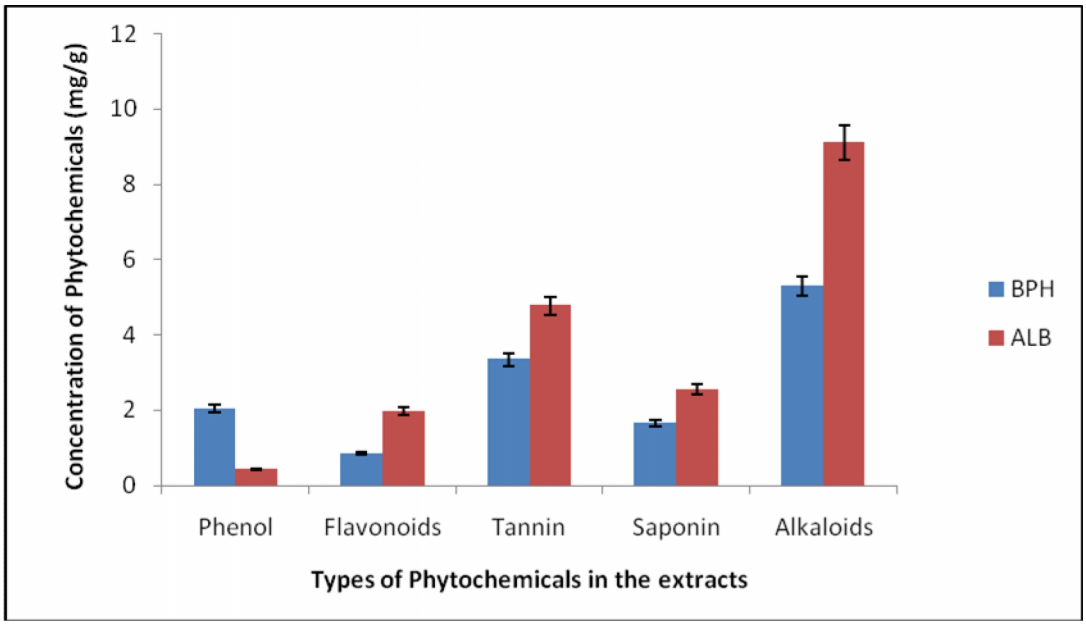

Fig. 1. Quantitative phytochemical assay of *Bryophyllum pinnatum* (BPH) and *Aloe barbadensis* (ALB) extracts

Table 1. Effects of the extract on biochemical parameters in rat (mean±standard error of mean)

Group/ parameter	Group I (Control)	Group II (25 mg/ml)	Group III (50 mg/ml)	Group IV (100 mg/ml)
Albumin (g/dl)	3.662±0.086	3.720±0.034[a]	3.818±0.024	3.900±0.055
BUN (mg/dl)	15.460±0.189	13.630±0.161[c]	13.990±0.714	18.868±0.048
Creatinine (mg/dl)	0.402±0.092	0.412±0.004	0.386±0.068[a]	0.950±0.167
ALT(IU/L)	15.720±0.073	16.100±0.055	16.060±0.068[ab]	16.168±0.233
AST(IU/L)	19.860±0.125	22.180±0.058	20.900±0.118[b]	20.368±0.355
ALP(IU/L)	61.074±0.100	64.260±0.051	62.700±0.416[c]	96.332±0.539

Key; AST=Aspartate Amino transferase; ALP=Alkaline Phosphatase, ALT=Alanine amino transferase; BUN=blood urea nitrogen

Table 2. Effects of the extract on haematological values of rats (mean±standard error of mean)

Group/ parameter	Group I (Control)	GROUP II (25 mg/ml)	Group III (50 mg/ml)	Group IV (100 mg/ml)
WBC($x10^9$/L)	3.000±0.045	4.650±0.134	8.510±0.090	14.132±0.483
RBC($x10^{12}$/L)	2.080±0.037	2.626±0.112	3.210±0.082	3.900±0.000
PLATELET ($x10^9$/L)	4.480±0.374	5.820±0.370	6.080±0.080	6.536±0.018
MCV(FL)	46.560±0.040	46.820±0.139	88.406±0.058	91.236±0.018
MCH(pg)	21.400±0.190	28.260±0.068	29.340±0.051	34.304±0.041
MCHC(g/dl)	33.000±0.032	34.060±0.460	34.860±0.125	34.336±0.370

Key; RBC=Red Blood Cell ($x10^{12}$/L); WBC=White Blood Cell ($x10^9$/L); MCV=Mean Corpuscular Volume (fl); MCH=Mean Corpuscular Haemoglobin (pg); MCHC=Mean Corpuscular Haemoglobin Concentration (g/dl)

4. DISCUSSION

The findings from this study gave a clue on how biochemical and haematological variations in animal model can be used to predict the danger in human if treated with the same extract. In the present investigation, administration of the extract significantly elevated the serum levels of Alkaline Phosphatase, Aspartate amino Transaminase, Total protein, Creatinine and Blood Urea Nitrogen(($P<0.05$) in the entire experimental test rats most especially those treated with 100mg/kg body weight. It is worthy of note that five of the ten animals that were given the highest concentration of 100mg/kg body weight died within seven days. The results from the remaining five animals revealed a significant increase in values of the evaluated parameters when compared with baseline values of the control animals. In order to ascertain the component of the extracts that could be responsible for the elevation of the parameters, quantitative phytochemical screening was carried out on both extracts and the findings revealed that both extracts contained essentially alkaloids in larger quantity with some other components such as phenol, flavonoids, tannin in lower amount. The assay further showed that one gram each of *Bryophyllum pinnatum* and *Aloe barbadensis* extracts contains 5.3mg and 9.1mg of alkaloid respectively. The investigation therefore linked the bioactivities of the combined extract with the alkaloid composition. Structurally, the hydroxyl (OH) and amine (NH) functional groups of alkaloid could interfere with synthesis of nucleic acid and protein in liver leading to liver dysfunctions and elevation in serum Alkaline Phophatase and Aspartate amino Transaminase. Although ALT, a known vital liver enzyme showed no significant increase in values, significant increase in other enzymes like Alkaline Phosphatase, Aspartate amino Transaminase and Total protein could be a good pointer to hepatic malfunctioning [19].

The herb could also cause damages to the kidney and affect the ultra filtration activities of nephron leading to abnormal elevation in the serum Total Protein, Creatinine and BUN. The significant variation in the mean value of blood urea nitrogen and serum Alkaline Phophatase recorded in this study is in consonance with the findings of Ghasi et al. in which 150mg/ml of *Bryophyllum pinnatum* extract singly produced significant increase in the mean values of urea and Alkaline Phosphatase [20]. The weight gain in the treated animals is not surprising, as this could be due to enlargement of internal organs. Association between weight gain and damaged internal organs had earlier been documented [21].

This study further showed that inter-peritoneal administration of the combined extract to the animal produced a significant increase in hematological parameters. All the test rats exhibited significant increase in values of WBC, RBC, Platelet, MCV and MCH ($P<0.05$) while MCHC value for rats treated with 100mg/ml did not show any significant difference ($P>0.05$) when compared to the control rats. The reason for this scenario could probably be due to effect of acute dehydration of blood and haemoglobin concentration per red cell. The dehydration could be attributed to the presence of hydroxyl group in the alkaliodal extract. This result is in line with the findings of Gupta et al. conducted in India on effect of alkaloidal extract on hematological values [22].

5. CONCLUSION

Going by the findings from this study, consumption of the combined extracts by rats induced hepato-renal dysfunctions at all concentrations of the combined extracts, but most pronounced at the dose of 100mg/kg body weight. The extract also caused elevation of blood cell indices that is proportional to the concentration of the combined extract. However,

since 25mg/kg body weight recorded the least toxic effect, treatment with the combined extract may be considered to be safe in humans, especially if prolonged dosage is avoided. It seems however, that such a decision should await histological confirmation.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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