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Toxicological Assessment of Aqueous Extract of *Moringa Oleifera* and *Caulis Bambusae* Leaves in Rabbits

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Abstract

Twelve age-matched healthy adult male Chinchilla rabbits (2.0 ± 0.5 kg BW) were divided into three equal groups (two treatment and one control groups). The treatment groups were given 2.5 mL and 5.0 mL of aqueous extract of the leaves of *Moringa oleifera* and *Caulis bambusae* by oral intubation, while the control group received 5.0 mL of the vehicle of extraction (sterile distilled water) and examined for 30 days. The effects of the leave extracts on the heamatological parameters, selected liver enzymes, insulin level and body weights of the affected rabbits were analyzed. There were significant increases in CD_4 cells ($p<0.01$), lymphocytes ($p<0.05$) and a decrease in neutrophils ($p<0.05$). There was an enhancement in the activities of acid phosphatase, alkaline phosphatase, aspartate transaminase and alanine transaminase in rabbits exposed to 2.5 mL of the extract. There was no significant difference in the histology of major organs, weights and the physical and behavioral pattern of both test and control rabbits.

Keywords: *Moringa oleifera*; *Caulis bambusae*; Toxicological studies; Rabbits

Introduction

The plant *Moringa oleifera* Lam. commonly called drum stick plant or horse radish plant or miracle plant or mother's best friend is the most widely cultivated species of the monogeneric family *Moringaceae* (order Brassicales), which includes 13 species of trees and shrubs distributed in sub-Himalayan ranges of India, Sri Lanka, north-eastern and south–western Africa, Madagascar and Arabia [1]. *M. oleifera* is one of the most useful tropical trees. The relative ease with which it propagates through both sexual and asexual means and its low demand for soil nutrients and water after being planted makes its production and management easy. Introduction of *M. oleifera* into a farm which has a biodiverse environment can be beneficial for both the owner of the farm and the surrounding ecosystem [2].

The Moringa tree is a multi-function plant. It has been cultivated in tropical regions all over the world for high protein, vitamins, minerals and carbohydrate content of entire plant; high value of nutrition for both humans and livestock; high oil content (30-42%) of the seeds which is edible and with medicinal uses; and for its seeds coagulant properties for water and wastewater treatment [2]. This plant has been well documented for its medicinal importance for a long time. The stem bark, root bark, fruit, flowers, leaves, seeds and gum are widely used in Indian folk medicine. The pods and seeds are tastier while they are young and before they turn brown. In Malaysia, the young tender pods are cut into small pieces and added to curries for seasoning [3].

Phytochemically, *Moringa oleifera* plant family is rich in compounds containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates [4,5]. For instance, components of *Moringa* preparation that have been reported to have hypotensive, anticancer, and antibacterial activity include 4–(4'-O–acetyl-α-L rhamnopyranosyloxy) benzyl isiothiocy–anate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4–(α-L-rhamnopyranosyloxy) benzyl glucosinolate [1,6] While these compounds are relatively unique to the *Moringa* family, it is also rich in a number of vitamins and minerals, iron and essential amino acids, as well as other more commonly recognized phytochemicals such as the carotenoids, including β–carotene or provitamin A [1,7-10].

The benefit for the treatment or prevention of disease or infection that may accrue from either dietary or topical administration of *Moringa* preparations (e.g. extracts, deconcoctions, poultices, creams, oils, emollient, powders) are not quite so well known [11] *Moringa* preparations have been cited in the scientific literature as having antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti inflammatory, hypocholesterolemic and hypoglycemic activities as well as having considerable efficacy in water purification by flocculation, sedimentation and antibiosis and even reduction of *Schistosome cercaria* titer [12]; however, a second scientific judgment is required to access the efficacy of traditional cures [13].

There is strong evidence that countries in sub-Saharan Africa especially Nigeria are becoming more and more aware of the benefits of *Moringa*. Therefore, there is room to exploit the potential that *Moringa* offers in the battle against poverty and food insecurity. Even though a wide body of information on *Moringa* promises well, there is still a need for further verification and validation as part of the advocacy of its large scale exploitation.

Caulis bambusae commonly known as "bamboo shavings" leaf belongs to *Bambusoideae* family in Gramineae. They are usually found in tropical and subtropical areas of the world. China is one of main *Caulis bambusae* producing country in the world. In China, there are about 40 genera and 400 species of *Bambusoideae* and the area of *C. bambusae* groove is approximately 4,000,000 hectares [14].

This plant is one of the valuable natural plants all over the world. It

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is commonly called "Gold of the poor" in China for its high economic value and important long history of food and medical application and thus, has been listed by Ministry of Health PRC into the list of natural plants with dual-purposes as food and drug [15]. Different parts of *C. bambusae,* such as the leaf, rhizome system, fruit, juice, poles, stem and shoots have different therapeutic effects [16]. Effective ingredients of bamboo leaf extract include flavones, phenolic acid, lactone, polyose, amino acids and micro elements amongst others. The leaf flavonoids have been studied systematically and thoroughly and it was revealed that this natural product (flavonoids) mainly contains four kinds of C–glycosyl flavones namely; orientin, homo-orientin, vitexin and iso-vitexin. *C. bambusae* as a food additive has many kinds of biological effects such as anti-free radical, anti-oxidation, anti-senescence, anti-bacterial, anti-viral, and the prevention of cardiovascular and cerebrovascular disorders and senile degenerative diseases [16,17], improving retentive faculty, improving sleep quality, anti-cancer and skin beautification [18], lowering of serum cholesterol level and it has positive impacts on the health and longevity of human beings [19]. But studies on the active phytochemicals, structural relationship between these phytochemicals and their physiological and pharmacological activity are extremely limited.

Moringa oleifera and *Caulis bambusae* plants have been mentioned in ethnomedical practices and their phytochemistry documented. There are reports of their individual uses in the treatment of human ailments. This study therefore seeks to assess the systemic impact of the aqueous extracts of the leaves of these two plant products for possible toxic effects in mammals using heamatology, serum chemistry and histopathological changes as indices of toxicosis. It is expected that the findings from this work may add to the overall value of the medicinal and nutritional potential of these plants.

Materials and Methods

The pulverized leaves of *M. oleifera* and *C. bambusae* were obtained from the National Academy for the Advancement of Science (NAAS), Benin City, Nigeria. The powder products (NAAS/09/02) packaged in sachets and bottles are sold commercially to people for medicinal and nutritional purposes. 10 g of ground plant materials were transferred into 250 mL Pyrex flask containing 90 mL of sterile distilled water and allowed to soak for 4 h, for easy dissolution and extraction. Thereafter, the homogenate was filtered through Whatman's No. 1 filter paper to obtain the filtrate which was labeled plant aqueous extract for subsequent use.

Twelve age-matched healthy adult male Chinchilla rabbits (2.0 ± 0.5 kg BW) were used in this study. Only male rabbits were used because one was also looking at the possible effects of this extract on the testes or male reproductive organs of the rabbits in relation to fertility. They were maintained at the experimental animal house unit of the Department of Microbiology, Faculty of Life Sciences, University of Benin, Nigeria. The rabbits were divided into three equal groups (n=4) (two treatment and one control groups) and allowed to acclimatize for 7 days in their respective cages. The treatment groups (group 1 and 2) were given 2.5 mL and 5.0 mL of the plant aqueous extract by oral intubation, while the control group (group 3) received 5.0 mL of the vehicle of extraction. The animals were administered daily on this supplement for 30 days.

The body weights of rabbits were taken with a top-loading weighing balance (5 Goat Brand, China), other physical and behavioral changes of rabbits were taken during the treatment period. At the end of the treatment period, blood samples were collected from the rabbits by cardiac puncture into heparinized and non-heparinized plastic tubes for hematological and biochemical investigations. The blood in non-heparinized tubes was allowed to clot; serum separated from the clot and centrifuged into clean tubes for biochemical analysis.

Fourteen days after the last treatment, the rabbits were sacrificed by anaesthetizing them with ether and after laporactomy and evisceration, the liver, kidney, lungs, spleen and testes were removed, weighed and placed in 10% formalin for processing for histopathology.

Heamatopathology

Blood samples were analyzed for CD4 cells count using the cytoflow SL-3 flow cytometer (Partec, Gmbh, Germany) as described by Hoepelman et al. [20]. Packed Cell Volume (PCV), Haemoglobin level (Hb), White Blood Cells count (WBC), platelets and red blood cell indices (MCV, McHc, and MCH) were analyzed following the methods outlined by Dacie and Lewis [21].

Biochemistry

Sera obtained from clotted blood samples of rabbits were analyzed for Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), Aspartate Transaminase (AST) and Alanine Transaminase (ALT) using the methods outlined by Anon [22,23]. The quantitative determination of insulin in serum was carried out using DRG Insulin Enzyme Immunoassay kit (DRG Insulin ELISA EIA-2935) with reference to the method described by Starr et al. [24].

Histology

Serial sections of the formalin fixed organs were cut (5 µm thick), fixed on microscope slides, dewaxed and stained with heamatoxylin and eosin (H & E) following the methods outlined by Ibeh [25]. The sections were mounted in Canada balsam and examined under light microscopy for studying presence or absence of architectural defects.

Statistical analysis of data

Data obtained were analyzed by one-way Analysis of Variance (ANOVA) using F-test and T-test to determine the significance of differences in group results and Duncan's multiple range tests to locate points of significant differences following the methods outlined by Ogbeibu [26].

Results

Table 1 shows the effects of the leaves extract on the body weight, temperature, behavioral and other physical parameters of rabbit. There were no significant differences in the mean body weight and temperature ($p>0.05$), fur appearance and eye sparkle, behavior and feces texture of test rabbits when compared with the control after 30 days of dietary exposure.

Parameter	Test		Control
	Group 1 (2.5 mL)	Group 2 (5.0 mL)	Group 3
Body weight (kg)	2.30 ± 0.50	2.15 ± 0.50	2.50 ± 0.05
Temperature (°C)	38.10 ± 0.11	38.20 ± 0.11	38.30 ± 0.11
Fur appearance	FL	FL	FL
Eye	SP	SP	SP
Feces	N	N	N
Behavior	N	N	N

FL: Full luster; SP: Sparkling; N: Normal

Values are mean ± S.E. where S.E. stands for standard error

Table 1: Effects of the leaves extract on physical and behavioral parameters of rabbits.

Parameter	Test		Control
	Group 1(2.5 mL)	Group 2 (5.0 mL)	Group 3
CD_4 (cells/mL)($\times 10^3$)	49.67 ± 39.67*	9.75 ± 8.66	7.50 ± 3.15
Hb (g/100 mL)	9.23 ± 1.42	9.70 ± 0.56	11.45 ± 0.51
PVC (%)	31.50 ± 5.32	32.00 ± 1.46	36.50 ± 2.06
PLT (µL)($\times 10^4$)	21.50 ± 4.24	30.00 ± 2.24	27.80 ± 5.46
WBC (µL)($\times 10^3$)	6.70 ± 0.43	4.40 ± 0.24	3.30 ± 0.75
NEUT (%)	33.50 ± 8.38	37.00 ± 9.70	50.25 ± 10.25
LYMP (%)	53.75 ± 9.41*	43.00 ± 13.59	32.50 ± 11.55
MONOCYTE (%)	8.50 ± 1.44	8.50 ± 0.65	8.75 ± 0.75
BASOPHIL (%)	0.50 ± 0.41	1.50 ± 0.29	0.75 ± 0.48
EOSINOPHIL (%)	4.75 ± 1.25	6.00 ± 0.54	4.50 ± 1.32
MCV (fl)	70.25 ± 1.32	69.78 ± 2.23	68.65 ± 0.72
MCH (p.g)	18.85 ± 0.74	19.75 ± 0.52	20.40 ± 0.11
MCHC (g/dL)	28.08 ± 0.88	28.68 ± 0.35	29.70 ± 0.20

Values are mean ± S.E, N=4

*=Location of significant difference using Duncan's multiple range tests.

PCV: Packed Cell Volume; PLT: Platelet; LYMP: Lymphocyte; WBC: White Blood Cell; NEUT: Neutrophil; Hb: Haemoglobin; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

Table 2: Effects of the leaves extract on heamatologic parameters of rabbits.

Parameter	Test		Control
	Group 1(2.5 mL)	Group 2 (5.0 mL)	Group 3
ACP (µL)	28.00 ± 2.06	26.00 ± 1.29	27.80 ± 1.11
ALP (µL)	32.30 ± 2.43	29.50 ± 1.85	31.80 ± 2.63
AST (µL)	55.30 ± 5.15	45.80 ± 5.17	53.30 ± 3.15
ALT (µL)	45.50 ± 5.17	40.00 ± 4.51	43.80 ± 3.43

Values are mean ± S.E, N=4

ACP: Acid Phosphatase; ALP: Alkaline Phosphatase; AST: Aspartate Transaminase; ALT: Alanine Transaminase

Table 3: Effects of the leaves extract on some serum enzymological parameters of rabbits.

Table 2 shows the effect of the leaves extract on heamatologic parameters of rabbits. There were significant increases in CD_4 cells ($p<0.01$) and lymphocytes ($p<0.05$) and a decrease in neutrophils. The mean CD_4 cell counts for the rabbits were 49.67 ± 39.67×10^3 cells/mL (Group 1), 9.75 ± 8.66×10^3 cells/mL (Group 2) and 7.50 ± 3.15 ×10^3 cells/mL (control). The increase in lymphocyte was in line with CD_4 cells increase and this increase were concentration dependent, that is, more in group 1 rabbits exposed to 2.5mL concentration of plant aqueous extract. The mean percentage of lymphocytes was 53.75 ± 9.41 % (Group 1), 43.00 ± 13.59 % (Group 2) and 32.50 ± 11.55 % (Control). There were no significant differences between the tests groups and control group for the other heamatological parameters.

Table 3 shows the effects of the leaves extract on some serum enzymological parameters of rabbits. There were no significant decreases or increases in the concentration of the serum enzymes analyzed between the tests and control rabbit groups.

The effects of the leaves extract on rabbit blood insulin are shown in Figure 1. There were increases in the insulin levels of test rabbits monitored with Group 2 rabbits exposed to 5.0 mL of plant aqueous extract having the highest insulin concentration. Exposure to aqueous extract of *M. oleifera* and *C. bambusae* leaves and their control showed no significant difference in the organ structure ($p>0.05$) in all the groups of rabbit.

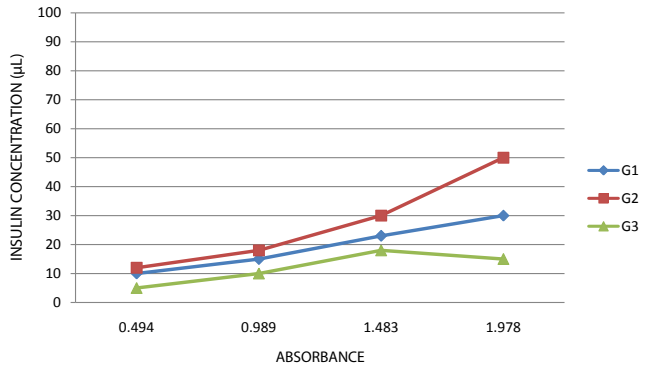
Discussion

The impact of extract of *M. oleifera* and *C. bambusae* on rabbits was determined in this study using short-term investigation protocol. The results in Table 1 suggest that exposure to these plants aqueous extract did not change significantly the body weights and other physical and behavioral characteristics of affected rabbits which implies no adverse effect on metabolic activities of these animals.

The effect of the leaves extract on heamatologic parameters of rabbit shows a significant increase in CD_4 cells. CD_4 cells are T-Helper cells which stimulate cell mediated immunity and help B-cells make antibodies which fight against antigens, thus, this suggest that the plant could be a good positive immunomodulator. There was a significant shift to lymphocytes in the population of white blood cells, which suggests presence of lymphocytosis in the treated rabbits. This result may be due to the immune response of the rabbit to the extract, which led to the mobilization of immune competent cells. The implication of this finding is that the leaves extract were immunogenic, with plant aqueous extract at a dosage of 2.5 mL providing a more effective stimulus than the 5.0 mL dosage. This opinion is not at variance with the report of Fudenberg et al. [27] concerning the functions of immune-competent cells. Also, increase in lymphocyte might be indicative that the plants leaves enhanced the animal's ability to wade off infection and this may account for the plants' antimicrobial activity. There were no significant differences between the tests and control groups for the other heamatological parameters which suggest that the plants leaves could enhance hematopoietic activity and may not precipitate anaemia in a biologic system.

The effect of *M. oleifera* and *C. bambusae* leaves extracts on selected enzymes showed an enhancement in the activities of alkaline phosphatase, acid phosphatase, aspartate transaminase and alanine transaminase. This finding suggests that the plants leaves may have the capacity to enhance the proper functioning of the liver, prostate gland, and hepatobiliary activity. These views are not at variance with the report of Ibeh [28] with respect to the functions of enzymes.

The results in Figure 1 suggest that exposure to *M. oleifera* and *C. bambusae* leaves extract caused significant increases in the insulin levels of the test animals monitored. Insulin is a hormone produced in the body which helps normalize blood sugar and supports the pancreas that produces it. Thus, this increase in insulin level confirms the anti-diabetic action of *M. oleifera* and *C. bambusae* leaves which have been



G1=Group 1 rabbits exposed to 2.5 mL aqueous leaves extract
G2=Group 2 rabbits exposed to 5.0 mL aqueous leaves extract
G3=Group 3 rabbits (control)

Figure 1: Effects of the leaves extract on rabbit blood insulin.

shown to have phytochemicals (thiocarbamates, nitrites, and beta-sitosterol) which stimulates insulin release in animals [29].

Organ pathology showed that no significant lesions were observed in both treatment and control groups ($p>0.05$). The implications of these results are that the aqueous extracts of these plants leaves at the dosage levels employed in this investigation did not exhibit marked toxicity in the animals and therefore could be regarded as safe doses (approximately 250 mg–500 mg / 2 kg body weight). This may also point to the fact that the plants leaves are relatively safe for use nutritionally and medicinally.

Conclusion

This research has shown that the dietary exposure of mammals to *M. oleifera* and *C. bambusae* leaves increases CD4 cells, increases blood insulin concentration and enhances the activities of enzymes analyzed. Therefore, the plants could be a positive immunomodulator, enhance glucose metabolism and help in the proper functioning of the liver, prostate glands and hepatobiliary activities, respectively. However, more studies are needed to properly evaluate the toxicity of these plants using long term study protocol.

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