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 leaf extracts on the haematological parameters, selected liver enzymes, insulin level and body weights of the affected rabbits were analyzed. There were significant increases in CD₄ 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 monogenic 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, anticyancer, and antibacterial activity include 4-(4-O-acetyl-α-L-rhamnopyranosyl) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4–(a-L-rhamnopyranosyl) 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 ß-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, decoconctions, poultices, creams, oils, emollient, powders) are not quite so well known [11] Moringa preparations have been cited in the scientific literature as having antibiotic, antityrpanosomal, hypotensive, antispasmodic, antifulcer, anti inflammatory, hypcholesterolenic and hypoglycemiac 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 Graminaceae. 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 medicinal 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 hematology, 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 anesthetising them with ether and after laparotomy 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1 (2.5 mL)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>2.30 ± 0.50</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.10 ± 0.11</td>
</tr>
<tr>
<td>Fur appearance</td>
<td>FL</td>
</tr>
<tr>
<td>Eye</td>
<td>SP</td>
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<tr>
<td>Feces</td>
<td>N</td>
</tr>
<tr>
<td>Behavior</td>
<td>N</td>
</tr>
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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.
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 hematologic parameters of rabbit shows a significant increase in CD4 cells. CD4 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 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' antimicrobial activity. There were no significant differences between the tests and control groups for the other hematological parameters which suggest that the plants leaves could enhance hematopoietic activity and may not precipitate anaemia in a biologic system.

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 discussed in the literature.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>Control</th>
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<tbody>
<tr>
<td>CD4 (cells/mL) (×10⁵)</td>
<td>49.67 ± 39.67*</td>
<td>7.50 ± 3.15</td>
</tr>
<tr>
<td>Hb (g/100 mL)</td>
<td>9.23 ± 1.42</td>
<td>11.45 ± 0.51</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>70.25 ± 1.32</td>
<td>68.65 ± 0.72</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18.85 ± 0.74</td>
<td>20.40 ± 0.11</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>28.08 ± 0.88</td>
<td>29.70 ± 0.20</td>
</tr>
</tbody>
</table>

Values are mean ± S.E, N=4

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

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

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.
shown to have phytochemicals (thiocarbamates, nitriles, 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.

References