Is Fermented Pentactethra Macrophylla Nutritional Or Antinutritional?: Response From Haematological Studies In Protein Malnourished Guinea Pigs

H Nwanjo, I Iroagba, I Nnatuanya, N Eze

Citation

Abstract
The fermented powered seeds of Pentaclethra macrophylla were investigated for their possible anti-anaemic potentials in protein malnutrition guinea pigs by feeding the animals with a protein deficient diet containing 2% protein for 4 weeks. One group of the protein malnourished guinea pigs were therefore treated with normal diet (group C) while the other group was subsequently placed on the protein deficient diet supplemented with Pentaclethra macrophylla, 2 g/kg body weight (group D). Animals fed with normal diet and protein deficient diets throughout the experiment were used as negative control (group A) and positive control (group B) respectively. There was a significant reduction in weight change (P<0.05) of groups B, C and D when compared with Group A (negative control). This reduction in weight change is more pronounced in group B followed by D. It was observed that Hb, PCV levels and RBC, WBC counts of groups C and D were significantly increased (P<0.05) when compared with those of group B. But this positive response is more pronounced in group C. On the other hand the Hb, PCV levels and RBC, WBC counts of groups C and D were significantly decreased (P<0.05) when compared with group A. This findings therefore suggests that Pentaclethra macrophylla is nutritional but its nutritive value is slightly hindered by the presence of antinutritional factors present on the seed.

INTRODUCTION
For normal functioning, the human body requires sufficient amount of protein. Unfortunately protein foods are in short supply and therefore not within the reach of low-income households in Nigeria. This insufficient intake of protein can result to anaemia, which will also reflect on the haematological parameters. So diet plays a very important role in the prevention of anaemia.

The African Oil bean tree (Pentaclethra macrophylla Benth), is a large leguminous woody plant that belongs to the family Mimosoideae (Keay, 1989). The local names include “Congo acacia” in Congo, “Duala Kombola” in Cameroon and “Ugba”, Ukpkala” in South Eastern part of Nigeria. Fermented Pentaclethra macrophylla or African oil bean seeds have become a very important delicacy in the life of Nigerians. Its use as food among the South Eastern populace of Nigeria and as a delicacy across the different tribes in Nigeria has increasingly been shown and consequently the ready-to-eat dish is called “African Salad”. Edward (1985) reported that this snack has gained a lot of popularity among different tribes in Nigeria as a result of greater integration and changing food habits. The oil bean seeds are inedible when raw and bitter until the final stage of fermentation. The fermentation improves flavour and taste and has been described as an economic processing method used in homes to improve nutritional quality (Obiazoba and Egbuna, 1992).

Despite these varied uses of the plant, there has been insufficient information on its exact haematological potentials on the animal system. An indication of possible anti-anaemic potentials of Pentaclethra macrophylla emerged from the study of Odoemelam, (2005), who estimated the proximate and the mineral composition of the African oil bean seed and suggested that since the seed is rich in protein and minerals it should serve as a cheap source of protein thus increasing the haematological parameters. On the other hand Achinewhu (1983) has observed a depression in weight along with negative protein utilization which may also lower the haematological parameters in rats fed with various tropical legume seeds including oil bean, they attributed those adverse effects to the presence of heat stable toxicants in the seeds. And also, some antinutritional factors
have been identified and assayed in the Pentaclethra macrophylla (Onwuliri et al., 2004). This study was therefore designed primarily to assess the actual effect of an aqueous extract of Pentaclethra macrophylla seeds on some haematological parameters in protein malnourished guinea pigs.

MATERIALS AND METHODS

CHEMICAL AND PLANT MATERIALS

All chemicals used in this study were of analytical grades purchased from British Drug House (Pools, U.K). Seeds of Pentaclethra macrophylla were bought from the local markets in Owerri Metropolis. Dr. S. E. Okeke of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria confirmed the botanical identity of the seeds, and the voucher samples are kept in the Department for references.

ANIMALS AND TREATMENT

Twenty-four male guinea pigs weighing 0.5-1.0 kg were obtained from a private farm in Owerri. The animals were fed ad libitum on normal commercial chow and water. They were randomly divided into 4 groups of 6 guinea pigs each and subsequently placed on their respective study diets as described by Adetusi and Oloowokere (1986) see table 1 and 2.

An animals in group A were placed on the normal diet with 21% protein throughout the period of the experiment i.e. eight weeks (negative control). Animals in group B were placed on the protein deficient diet with 2% protein throughout the period of the experiment and served as the positive control. Group C animal were placed on the protein deficient diet containing 2% protein for 4 weeks and subsequently with normal diet (as for group A) for another 4 weeks. Guinea pigs in Group D were placed on the protein deficient diet containing 2% protein for 4 weeks and subsequently with the protein deficient diet supplement with the Pentaclethra macrophylla (2 g/kg body weight) for the remaining 4 weeks.

Figure 1

Table 1: Preparation of diet for the first 4 weeks

<table>
<thead>
<tr>
<th>Dietary Component</th>
<th>Source</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>&quot;Gari&quot;</td>
<td>63</td>
<td>82</td>
<td>82</td>
<td>82</td>
</tr>
<tr>
<td>Protein</td>
<td>Soya beans</td>
<td>21</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fat</td>
<td>Palm Oil Seed</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Mineral</td>
<td>Salt</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 2

Table 2: Preparation of study Diet for the remaining 4 weeks.

<table>
<thead>
<tr>
<th>Dietary Component</th>
<th>Source</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
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<td>82</td>
<td>82</td>
</tr>
<tr>
<td>Protein</td>
<td>Soya beans</td>
<td>21</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fat</td>
<td>Palm Oil</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Mineral</td>
<td>Salt</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>African Oil bean</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4g/kg</td>
</tr>
</tbody>
</table>

Source: (Adetusi and Oloowokere 1986)

ANALYTICAL PROCEDURE

At the end of the treatment i.e. 8 weeks, the animals were fasted for 24hrs, re-weighed and then sacrificed by cervical dislocation. With a sterile syringe and needle blood was collected from each animal by cardiac puncture. A Blood sample for each animal was collected in EDTA anticoagulated bottle for haematological analysis.

HAEMATOLOGICAL ANALYSIS

Haemoglobin (Hb) level was estimated by the method of Drabkin and Austin (1932). Packed cell volume (PCV) level was determined by the Micro Heamatocrict method. RBC and WBC count was determined by the method of Hoffbrand and Pettit, (2000).

RESULT

Table 3 shows the body weight change in the Guinea Pig placed on the study diet. The body weight change in group B was significantly reduced (P<0.05) when compared with group A and then increased in C and D when compared with group B but group D was significantly reduced than groups C.
Is Fermented Pentaclethra Macrophylla Nutritional Or Antinutritional?: Response From Haematological Studies In Protein Malnourished Guinea Pigs

**Figure 3**
Table 3: Mean weight change of both experimental and control animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial weight (kg)</th>
<th>Final weight (kg)</th>
<th>Weight change (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.86 ± 0.34</td>
<td>1.07 ± 0.12</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>B</td>
<td>0.87 ± 0.26</td>
<td>0.59 ± 0.21</td>
<td>-0.28 ± 0.10</td>
</tr>
<tr>
<td>C</td>
<td>0.84 ± 0.34</td>
<td>0.94 ± 0.17</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td>D</td>
<td>0.85 ± 0.30</td>
<td>0.89 ± 0.11</td>
<td>0.04 ± 0.05</td>
</tr>
</tbody>
</table>

*Significant reduced when compared with (group A) at P< 0.05 ** Significant reduced when compared with (group A, and C) at P< 0.05 *** Significant reduced when compared with (group A, C and D and) at P< 0.05 A Negative control, animals fed with normal Commercial chow B Positive control, animals fed with protein deficient diet. C Animals fed with protein deficient diet for 4 weeks and then with normal diet for 4 weeks. D Animals fed with protein deficient diet for 4 weeks and subsequently with protein deficient diet supplemented with fermented Pentaclethra macrophylla 2 g/kg body weight for 4 weeks.

Table 4 summarizes the Hb, PCV and WBC levels in normal and protein deficient guinea pigs. The Hb levels were significantly decreased in the group B (P<0.05) when compared with group A but significantly increased to near normal in groups C, D. This increase is more pronounced in group C. A similar trend was observed for the PCV level, RBC and WBC counts.

**Figure 4**
Table 4: Mean values of Hb, PCV, RBC and WBC of both the control and experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Hb (g/L)</th>
<th>PCV (%)</th>
<th>RBC (10⁶/uL)</th>
<th>WBC (10⁶/uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>126.1 ± 13.50</td>
<td>0.89 ± 0.03</td>
<td>4.59 ± 0.20</td>
<td>3.86 ± 0.19</td>
</tr>
<tr>
<td>B</td>
<td>83.3 ± 13.55</td>
<td>0.42 ± 0.20</td>
<td>4.18 ± 0.40</td>
<td>3.00 ± 0.34</td>
</tr>
<tr>
<td>C</td>
<td>94.4 ± 7.90</td>
<td>0.34 ± 0.20</td>
<td>3.68 ± 0.19</td>
<td>2.08 ± 0.33</td>
</tr>
</tbody>
</table>

*Significant different from positive controls (group B) at P< 0.05

A Negative control, animals fed with normal Commercial chow B Positive control, animals fed with protein deficient diet. C Animals fed with protein deficient diet for 4 weeks and subsequently with standard diet for 4 weeks. D Animals fed with protein deficient diet for 4 weeks and subsequently with protein deficient diet supplemented with fermented Pentaclethra macrophylla 2 g/kg body weight for 4 weeks.

**DISCUSSION**

The onset of kwashiokorigenic anaemia as observed in the present study was characterized by (a) Reduced body weight (b) Failure to thrive (c) Anorexia (d) Flecking off of hair (e) Reduced Hb, RBC and WBC. There was significant reduction (P<0.05) in the weight change of group B when compared to Group A, this could be attributed to the malnourished state of the animal which conform to earlier studies (Adelusi and Olowookere, 1985). However the observed significant decrease (p<0.05) in the body weight change of group D animals when compared to Group C, notwithstanding the presence of protein in the Pentaclethra macrophylla may be due to the presence of some anti-nutritional factors in the seed. It has been reported that rat fed with Pentaclethra macrophylla experienced a weight reduction with marked reduction occurring in unfermented Pentaclethra macrophylla (Achinewhu, 1982). The phytochemical screening of the seed as done by Onwuliri et al, 2004, revealed that the seed posses some anti-nutritional factors such as panceine, cyanide, oxalate, saponin. These anti-nutritional factors can invariably bind to some of the protein and make it unavailable (Ladeji et al, 1995).

There were reduced Hb, PCV, and WBC of group B animals due to the poor nutritional state of the animals. This is in confirmation with earlier work of Umoh and Maduagu (1986), which also showed that these haematological parameters were decreased in rats place on a protein energy malnourished (PEM) diets, which indicated the onset of the kwashiokorigenic syndrome. On the other hand the observed increase in these haematological parameters in group D when compared to group B may be attributed to the presence of some additional nutrients in the seed, which are necessary for erythropoiesis and haeme synthesis, as earlier reports had shown that the seed contains high levels of iron, calcium, potassium, thiamine and riboflavin (Odoemelam, 2005: Achinewhu and Ryley, 1986). All of which may contribute to increases in haematological parameters. Furthermore, reduced oxygenation of tissues (hypoxia), which is usually associated with anaemic conditions, can easily trigger off the production of the circulating hormone erythropoietin. Erythropoietin production stimulate increased red blood cell production and the presence of high iron level and other required nutrients in the seed may further increase the red blood cell production perhaps or more times normal (Guyton, 1981). However in Group C, the haematological parameters were more increased than in group D, this strongly suggests that the oil bean contains anti-nutritional factors which did not allow for maximum protein utilization by the animals.

It may therefore be concluded from the evidence from this
study that Pentaclethra macrophylla possesses anti-anaemic property which is slightly hindered by anti-nutritional factors present in the seed. Further studies are needed on the appropriate method needed to inactivate these anti-nutritional factors, which will in turn increase the nutritive value of this seed.

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