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Therapeutic Effect of Telfairia Occidentalis on Protein Energy Malnutrition-Induced Liver Damage

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Abstract: Comparison was made between the efficacy of dietary protein replenishment and supplementation with Telfairia occidentalis leaves, in treatment of Protein Energy Malnutrition (PEM) induced liver damage. PEM rats were produced by feeding weaning rats a protein deficient diet (2% protein) for 28 days and then divided into four dietary treatment groups: 2% protein (group A; PEM control group); 20% protein and 10% T. occidentalis (group C); 20% protein (group D) and 10% T. occidentalis (group E). The protein deficient diet caused a significant increase (p<0.01) in hepatic malondialdehyde (MDA) level and the liver function enzymes alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST) level in the serum. It also caused a marked reduction (p<0.01) in glutathione level, significant decrease (p<0.01) in the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) and significant damage to the hepatocytes. Recovery diets of protein alone and protein supplemented with T. occidentalis had significant effects on all the parameters. The MDA level and the serum liver function enzymes were significantly reduced (p<0.01), glutathione and antioxidant enzymes levels were markedly increased (p<0.01) and a highly significant hepatocyte healing observed in the histology images. The highest recovery was however observed in group C. Results indicate the restorative ability of T. occidentalis in treatment of oxidative stress induced liver damage in PEM rats.

Key words: Protein energy malnourished, Telfairia occidentalis, oxidative liver damage

INTRODUCTION

The World Health Organization (WHO, 2006), defined malnutrition as the cellular imbalance between the supply of nutrients and energy and the body’s demand for them to ensure growth, maintenance and specific functions. Protein Energy Malnutrition (PEM) previously termed protein calorie malnutrition has assumed the position of being the leading cause of death directly or indirectly among children under 5 years of age in developing world in the past 40 years (Olu, 2001), in spite of our wide knowledge and understanding of human nutritional requirements.

The term PEM applies to a group of related disorders that include marasmus, kwashiorkor and intermediate states of marasmus-kwashiorkor. The term marasmus is derived from the Greek word marasmos, which means withering or wasting. Marasmus involves

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inadequate intake of protein and calories and is characterized by emaciation. The term Kwashiorkor is taken from the Ga language of Ghana and it refers to the disease that occurs when there is inadequate protein intake with reasonable calorie (energy) intake (Olu, 2001). A study suggests that marasmus represents an adaptive response to starvation whereas kwashiorkor represents a maladaptive response to starvation (Robert, 1999). Children may present with a mixed picture of marasmus and kwashiorkor or with milder forms of malnutrition. For this reason the term PEM was suggested to include both entities. Oxidative stress in the cells refers to an increase in the generation of Reactive Oxygen Species (ROS) and depletion in antioxidant defense system, causing an imbalance between oxidants and antioxidants (Rukumani et al., 2004). Studies have indicated that PEM results in the generation of free radicals and these have been implicated in the mechanism of lipid peroxidation (Ashour et al., 1999).

*Telfairia occidentalis* Hooker is an important staple vegetable grown in Nigeria. It is also called fluted pumpkin, oyster nut or ridge gourd and belongs to the plant family Cucurbitaceae. Investigation revealed that the leaves of *T. occidentalis* are popularly consumed in many homes in Nigeria as a result of the various medicinal potentials ascribed to it; the leaf is very rich in phytochemicals with antioxidant activity, such as phenols and ascorbic acid (Oboh, 2005).

Oyolu (1980) observed that vegetables will continue to remain the primary source of proteins, minerals and vitamins in African countries. He noted that leaves and edible shoots of fluted pumpkin together contain 85% moisture, while the dry portion of what is usually consumed contains 11% crude protein, 25% carbohydrate, 3% oil, 11% ash and as much as 700 ppm iron.

*Telfairia occidentalis* has also been found to contain the highest levels of crude fibre, ash and protein in a study involving two other plants; *Talinum triangulare* and *Amaranthus cruentus* (Fasuyi, 2006). Its protein stability profile as functions of pH indicates its high stability in both alkaline and acid media with minimum solubility (isoelectric point) at between pH 4 and 6. The potential of *T. occidentalis* as food/feed and particularly as alternative protein resource is exemplified by the proximate composition, amino acid profile, mineral composition and the gross energy content of the analyzed leaf meal samples. The crude protein values even though untested and reserved for further studies in feeding assays indicate the potentialities of *T. occidentalis* as probable alternative protein source in monogastric diets (Fasuyi, 2006). The potassium, sodium, calcium, phosphorous and magnesium contents of *T. occidentalis* is particularly high when compared with most other foods, even iron, which is commonly deficient in many diets is fairly abundant. Given the high crude protein content and the favorable amino acid profile, it is suggesting that their incorporation in food/feed formulation especially incorporation into low protein traditional foods such as maize gruel (pap), cassava and yam flour to enhance their nutritive values (Fasuyi, 2006).

The aim of the experiment is to explore the therapeutic potentials of the leaves of *T. occidentalis* in the condition of PEM.

**MATERIALS AND METHODS**

**Experimental Animals**

Forty five weanling albino rats, aged four weeks and weighing between 20-30 g were obtained from the animal house, Biochemistry Department, University of Ilorin. The animals were acclimatized for 7 days in the animal house of Biochemistry Department, University
of Ibadan before commencement of the experiment. The animals were fed *ad libitum* throughout the experiment and were allowed free access to clean drinking water. This research project was conducted from April 2006 to Feb. 2007.

**Collection of Plant Sample**

The leaves of the plant (*Telfairia occidentalis*) were bought from Bodija market in Ibadan, Oyo State, It’s botanical authentication was confirmed at the department of Botany, University of Ibadan, by Dr. E.A. Ayodele of the herbarium section. The leaves were air dried in the laboratory under room temperature, the dried leaves were milled and ready for experimental use.

**Methodology**

Forty five rats were divided into five groups of nine animals each, the groups are represented by letters A, B, C, D and E. They were fed formulated diets as shown in Table 1. Group B, representing the control group were fed normal diet (rats pellets), while all the other groups were fed protein deficient diet (2% protein) for a period of twenty eight days to induce protein energy malnutrition (kwashiorkor). On the twenty ninth day, groups A and B were sacrificed to obtain the base line values, rounding up the first phase of the experiment.

The second phase of the experiment began with the administration of recovery diet to groups C, D and E (Table 1) also for another period of twenty eight days. Group C received a diet supplemented with 20% protein and 10% pumpkin leaf powder, group D received diet supplemented with 20% protein only and group E received diet supplemented with 10% pumpkin leaf powder only. The animals were sacrificed on the 29th day.

Liver function test was carried out on the serum while, the liver tissue was used for histological studies and the post mitochondria fraction of the liver tissues was used for the antioxidant enzyme and lipid peroxidation assays.

**PREPARATION OF POST MITOCHONDRIA FRACTION**

**Reagents**

**Homogenizing Buffer**

The 6.97 g of dipotassium hydrogen orthophosphate, KHPO₄ and 1.36 g of potassium dihydrogen orthophosphate KH₂PO₄ were dissolved in little amount of distilled water and made up to 1000 mL mark in a litre standard volumetric flask. The solution was adjusted to pH 7.8.

**1.15% KCL**

The 23.0 g of KCL was dissolved in distilled water and made up to 2 L with distilled water and stored at 4°C

**Protocol**

The rats were sacrificed after blood collection by cervical dislocation, the liver tissues were quickly removed, washed in ice cold 1.15% KCL solution, blotted and weighed.
Table 2: Feed composition

<table>
<thead>
<tr>
<th>Groups</th>
<th>Soy meal</th>
<th>Corn starch</th>
<th>Vitamin/mineral mix</th>
<th>Oil (unsaturated)</th>
<th>T. occidentalis leaf</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>40</td>
<td>820</td>
<td>60</td>
<td>80</td>
<td>-</td>
<td>1000</td>
</tr>
<tr>
<td>C</td>
<td>200</td>
<td>560</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>D</td>
<td>200</td>
<td>660</td>
<td>60</td>
<td>80</td>
<td>-</td>
<td>1000</td>
</tr>
<tr>
<td>E</td>
<td>40</td>
<td>720</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td>1000</td>
</tr>
</tbody>
</table>

They were then homogenized in 4 volumes of the homogenizing buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 17000 g for 20 min in a Beckman L5-50B ultracentrifuge at 0°C. The supernatant was decanted and stored in a freezer at -4°C. All the above procedures were carried out at temperatures between 0-4°C.

Feed Preparation

The feed constituents as shown in Table 2, were mixed together thoroughly in a clean bowl to obtain a homogenous mixture, distilled water was added in bits to form a dough, the dough was then spread out on a board and cut into desired shapes and sizes. It was thereafter dried in the laboratory oven for three hours at about 70°C. The feed at this stage is ready for administration to the test rats.

The standard feed for the control rats (group B) was obtained from Ladokun feed limited, Mokola, Ibadan. The composition of the feed is; 21% protein, 67% carbohydrate, 3.5% fats, 6% fibre, 0.8% phosphorous and 0.8 % calcium.

Collection of Blood

Blood was collected from the rats through the eyes, a part was collected in heparinized bottles (using EDTA) and the other part was collected in non heparinized bottles (whole blood) and this was allowed to clot on exposure to air and then spun in centrifuge to collect the serum.

© Determination of AST: According to the method of Reitman and Frankel (1957)
© Determination of ALP: Estimation according to the method of Belfield and Goldberg (1971)
© Determination of Tissue Peroxidation: The extent of lipid peroxidation was assessed by measuring the level of malondialdehyde (Varshney and Kale, 1990)

Enzyme Measurement

- Catalase: Catalase activity was determined by the method of Sinha (1971)
- Superoxide Dismutase: The activity of SOD was determined by the method of Misra and Fridovich (1972)
- Determination of Reduced Glutathione: The level of reduced glutathione was determined by the method of Jollow et al. (1973)

Statistical Analysis

Data were expressed as Mean±SEM. Significant differences were tested with the student-t test using significant level of p<0.05.
RESULTS

Figure 1 shows the weekly weights of rats placed on the test and control diets. It was observed that the rats placed on the control diet showed marked weekly gain in weight (p<0.05), they were also very active, scattering their food all over their cages, defecating and urinating more than the test animals. The rats placed on the test diet showed significant increase in weight also during the first two weeks (p<0.05), but was followed by a significant decrease in the 3rd and 4th weeks. There was a significant difference (p<0.01) between the final weight of the rats in the two groups, the control group being significantly higher than the test group. The test feed was able to induce the condition of protein energy malnutrition as depicted by the body emaciation seen in terms of weight loss. Also, the fur of the rats placed on PEM diet turned yellowish, thin and dry, after about two weeks, the hair began flaking off until the bodies of the rats were devoid of fur.

Figure 2 shows the weekly weights of rats in the phase two experiment, the animals in group C with protein and T. occidentalis supplemented in their diet had a marked increase in weight (p<0.05) all through the four weeks, group D, fed with protein supplemented diet also had increment in weight (p<0.05) all through the four weeks while group E with diet supplemented with T. occidentalis only had an insignificant increase (p>0.05) in the first two weeks, a significant increase (p<0.05) in the third week and this was followed by a significant decrease (p<0.05) in the fourth week. Group C had the highest weight gain, followed by group D and E, respectively, the result also shows that group C has the highest rate of recovery in terms of weight gain, while group E has the lowest recovery rate, group D is intermediate between the two.

The result of the lipid peroxidation in the liver is shown in Fig. 3. The MDA level was significantly (p<0.01) higher in the protein malnourished group A when compared with the control group B. All the recovery diets caused significant (p<0.01) decreases in MDA levels. The highest decrease was however observed in group C rats fed with protein and T. occidentalis diet, followed by group E fed diet supplemented with T. occidentalis alone. The least depression of MDA was observed in groups on protein alone supplemented diet.

Fig. 1: Weekly weights of rats. Group A: Test group fed protein malnourished diet, Group B: Control group fed normal rats pellets
Fig. 2: Weekly weights of rats. Group C: Group fed recovery diet protein + *T. occidentalis*, Group D: Group fed recovery diet protein, Group E: Group fed recovery diet *T. occidentalis*.

Fig. 3: Values are Mean±SD of nine rats. Data were analyzed by students t-test to examine difference between average means. *p*<0.01; Group A: Test: Group fed protein malnourished diet, Group B: Control; Group fed normal rats pellets; Group C: Group fed recovery diet protein + *T. occidentalis*, Group D: Group fed recovery diet protein; Group E: Group fed recovery diet *T. occidentalis*, Comparisons: a: Group A vs all; b: Group D vs. group C and group C vs. group E.

Results therefore show that the diet containing protein and *T. occidentalis* offered a superior recovery from lipid peroxidation than protein or *T. occidentalis* alone.

The result of the reduced glutathione (GSH) level in the liver is as shown in Fig. 4. The GSH level was significantly reduced (*p*<0.01) in the protein malnourished group A when compared with the control group B. All the recovery diets caused significant (*p*<0.01) increase in the GSH levels. The highest increase was however observed in group C rats fed...
Fig. 4: Values are Mean±SD of nine rats. Data were analyzed by students t-test to examine difference between average means. ††: p<0.01; ‡‡: p<0.05. Group A: Test; Group fed protein malnourished diet, Group B: Control; Group fed normal rats pellets, Group C: Group fed recovery diet protein + T. occidentalis, Group D: Group fed recovery diet protein; Group E: Group fed recovery diet T. occidentalis, Comparisons: a: Group A vs. all; b: Group D vs. group C and group C vs. group E.

Fig. 5: Values are Mean±SD of nine rats. Data were analyzed by students t-test to examine difference between average means, ††: p<0.05; ‡‡: p<0.01; Group A: Test; Group fed protein malnourished diet, Group B: Control; Group fed normal rats pellets; Group C: Group fed recovery diet protein + T. occidentalis, Group D: Group fed recovery diet protein; Group E: Group fed recovery diet T. occidentalis, Comparisons: a: Group A vs. all; b: Group D vs. group C and group C vs. group E.

with protein and T. occidentalis diet, followed by group E fed diet supplemented with T. occidentalis alone. The least increment of GSH was observed in groups on protein alone supplemented diet. Results show that the diet containing protein and T. occidentalis offered a higher level of recovery from glutathione depletion than protein or T. occidentalis alone.
The Superoxide Dismutase (SOD) and Catalase (CAT) activities in the liver are presented in Fig. 5. Compared to the control group, the SOD and CAT activities were significantly (p<0.01) reduced in the protein deficient Group A animals compared with the normal control group B. The recovery groups however showed significant increases in the enzyme activity with Group C having the highest increase (p<0.01) in both SOD and CAT compared with the test group A. While Group D had the lowest increase (p<0.01) in activity for the two enzymes, Group E was intermediate between groups C and D. This result further confirms the efficacy of the protein and *T. occidentalis* diet relative to the other diets in, reversing damage induced by the protein deficient diet.

The results of the ALT, ALP and AST activity in the serum are presented in Fig. 6. Feeding with protein deficient diet resulted in significant elevation (p<0.01) in the activities of serum ALT, ALP and AST. All the recovery diets caused significant reductions (p<0.01) in the activities of the enzymes with the protein and *T. occidentalis* diet producing the highest significant depression (p<0.01) than groups D and E on protein alone and *T. occidentalis* alone, respectively.

**DISCUSSION**

Present study suggests *T. occidentalis* as probable alternative protein and mineral source in animal diets. This finding is supported by a previous *in vitro* study done by Fasuyi (2006) as shown in Table 3.

The weight loss observed in the malnourished animals was consistent with similar study by Rana *et al.* (1996), they concluded that PEM is characterized by muscle wasting, loss of subcutaneous tissue and reduction in appetite and body weight after feeding rats a low protein (5%) or adequate protein (20%) diet for four weeks, while the loss of hair might be due to lack of synthesis of keratin (Ajayi and Ajimoko, 2005). Establishment of the
Table 3: Proximate composition and mineral content of *T. occidentalis* leaves (g kg⁻¹ DM)

<table>
<thead>
<tr>
<th>Composition</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>91.0±2.0</td>
</tr>
<tr>
<td>Crude protein</td>
<td>35.1±1.7</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>12.7±4.2</td>
</tr>
<tr>
<td>Ether extract</td>
<td>9.6±4.1</td>
</tr>
<tr>
<td>ASH</td>
<td>10.9±6.2</td>
</tr>
<tr>
<td>N-free extract</td>
<td>31.7±0.8</td>
</tr>
<tr>
<td>Ca</td>
<td>1.8</td>
</tr>
<tr>
<td>Mg</td>
<td>1.2</td>
</tr>
<tr>
<td>Zn</td>
<td>0.7</td>
</tr>
<tr>
<td>Ni</td>
<td>0.8</td>
</tr>
<tr>
<td>Na</td>
<td>8.2</td>
</tr>
<tr>
<td>K</td>
<td>3.7</td>
</tr>
<tr>
<td>P</td>
<td>1.028</td>
</tr>
<tr>
<td>Fe</td>
<td>0.992</td>
</tr>
</tbody>
</table>

(Fasuyi, 2006)

![Image of hepatocyte]

**Fig. 7:** Fused septation of the hepatocyte. x40 magnification, H and E stain

![Image of hepatocytes]

**Fig. 8:** Hepatocytes of group B rats. Observation: Normal hepatocytes, no visible lesion. x40 magnification, H and E stain

Differences in weight between the PEM group and the control group indicated that the body draws on its own stores, resulting in emaciation and severe wasting depicting onset of protein energy malnutrition (Noah et al., 2003), but this was reversed in the animals on recovery diet, most especially in the plant and protein group depicting possibility of complete recovery via adequate dietary replenishment.

The histology results as shown in Fig. 7-11, indicated liver damage in the protein deficient animals exemplified by diffused septation and necrosis of the hepatocytes in which the damaged cells are converted into fatty residues. This probably contributes to the fatty liver characteristic of the liver in protein energy malnutrition (Bobyn et al., 2002). The
Fig. 9: Hepatocytes of group C rats. Observation: High level of nuclei regeneration, highly reduced necrosis and septation of the hepatocytes. x40 magnification, H and E stain

Fig. 10: Hepatocytes of group D rats. Observation: Mild level of hepatocyte regeneration, mild reduction of septation (a). x40 magnification, H and E stain

Fig. 11: Hepatocytes of group E rats. Observation: Moderate hepatocyte regeneration, highly reduced septation (A). x40 magnification, H and E stain. Group A: Test group fed protein malnourished diet, Group B: Control group fed normal rats pellets, Group C: Group fed recovery diet protein + *T. occidentalis*, Group D: Group fed recovery diet protein, Group E: Group fed recovery diet *T. occidentalis*. H and E stain: Hematoxylin and Eosin stain, x40 magnification: Magnification of microscope.

recovery groups however, showed tremendous regeneration and healing of the hepatocytes, most especially the protein and *T. occidentalis* recovery group. The *T. occidentalis* supplement group also had high level of regeneration, greater than those of the protein supplemented group indicating that the *T. occidentalis* leaves supplementation had the most healing effect on the previously observed liver damage rather than protein repletion alone. This effect may be due to the antioxidative property of the plant (Oboh, 2005).
The significant increase in the concentration of Malondialdehyde observed in the PEM animals is an indication of increased lipid peroxidation which is an index of oxidative damage to membrane lipids during oxidative stress (Jimoh et al., 2005). Studies have indicated that PEM amongst other factors results in the generation of free radicals and these have been implicated in the mechanism of lipid peroxidation (Ashour et al., 1999). The co-administration of the protein and *T. occidentalis* successfully depressed the PEM induced lipid peroxidation thus suggesting an efficient restorative role of the diet. This role of the *T. occidentalis* leaves may be attributed to its antioxidant properties probably derived from inherent flavonoids which are known to be potent oxygen free radical scavengers and metal chelators (Oboh, 2005).

The GSH level in the liver was significantly reduced in the PEM rats. This result was similar to that obtained in gerbil model by Bobyn et al. (2002). Cellular GSH is highly sensitive to oxidative stress, thus exposure to the reactive oxygen species (ROS) and free radicals generated by the condition of protein energy malnutrition may result in rapid depletion of GSH levels in the liver. Moreover, since GSH is a protein that is mostly synthesized endogenously, its production will likely reduce in malnutrition due to the reduced energy to drive the processes of transcription and translation. The recovery diet administered to the PEM rats significantly enhanced the hepatic GSH levels with the highest enhancement recorded in the group fed protein with *T. occidentalis*, the positive modulatory effect may stem in part from the upregulation of the rate determining enzyme, γ-glutamyl cysteine synthetase in the biosynthesis of GSH. Tissue GSH synthesis is also known to be dependent on dietary amino acid supplies, this might have been supplied either by the inherent amino acids in *T. occidentalis* leaves or in the replenished dietary protein.

The activity of SOD and CAT were significantly increased in all the recovery diet groups. The increase in activity of SOD and CAT following the administration of the recovery diets may be due to the protein replenishment which is essential for the synthesis of the enzymes. This protein replenishment is highest in the protein and *T. occidentalis* group, therefore, making this dietary group a better choice for recovery.

In the present study, there was significant increase in the levels of ALT, ALP and AST in the serum of the PEM animals compared with the control animal, suggesting the possibility of liver damage in the PEM rats (Osifo and Bolodeoku, 1982). It may also be that since membranes are made up of proteins, the diet could have weakened the structural integrity of organs in rats, fed low protein diet leading to leakage of enzymes to extracellular fluids (Ajayi and Ajimoko, 2005). In the Recovery groups, there was a significant level of recovery for the three enzymes, with the highest level in the protein and *T. occidentalis* group, followed by the protein group and the *T. occidentalis* group. The protein replenishment may have mediated changes in the membrane proteins to prevent further leakages of the enzyme while the *T. occidentalis* supplementation may have contributed to the repair of damaged cells. The *T. occidentalis* preparation is rich in antioxidant phytochemicals such as vitamin C, Chlorophyll and phenols (Oboh and Akindahunsi, 2004). These may facilitate hepatocytes healing and can protect the liver cells from free radical damage (Appel et al., 1997). Present findings justifies our aim for the experiment, revealing the therapeutic ability of *T. occidentalis* leaves in PEM condition.

The antioxidative property of *T. occidentalis* worked on in this study suggests that the plant is a good dietary supplement in the treatment of PEM. A faster rate of recovery is therefore expected when *T. occidentalis* is used in the treatment of PEM than protein replenishment alone.
REFERENCES


