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Telfairia occidentalis Ameliorates Oxidative Brain Damage in Malnorished Rats

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Abstract: It has been observed and established that enhancing the antioxidant defense system during the early phase of rehabilitation is important to the survival of wasting protein energy malnourished patients. In this study, comparison was made between the efficacy of dietary protein replenishment and supplementation with *Telfairia occidentalis* leaves, in the treatment of oxidative brain damage in the malnourished rats. The protein energy malnourished rats were produced by feeding weanling rats a protein deficient diet (2% protein) for 28 days. The malnourished rats were then divided into three dietary treatment groups, 20% protein+10% *T. occidentalis* group (PTG), 20% Protein Group (PG) and the 10% *T. occidentalis* group (TG). Significant decrease in brain size ($p<0.01$), activity of superoxide dismutase ($p<0.01$), catalase ($p<0.01$) and increased Malondialdehyde levels ($p<0.01$), indicative of oxidative damage were observed in the malnourished rats as compared with the control group. Reduced level of oxidative damage was however observed in group TG, PG and PTG , respectively. The result indicates that *T. occidentalis* leaves supplementation with protein repletion is more effective for recovery from protein energy malnutrition induced oxidative damage in rats than protein repletion alone.

Key words: Protein energy malnourished, *Telfairia occidentalis*, oxidative brain damage

INTRODUCTION

Malnutrition affects a large number of children worldwide. Inadequate nutrition during pre- and postnatal period may alter brain development resulting in biochemical, physiological and anatomical changes which in turn could cause behavioral abnormalities (Fernanda *et al*., 2006). Eating is not an optional event in the maintenance of life, rather, it is an essential action that must be taken by animals to stay alive. Eating a balanced diet is equally essential in supporting life. An unbalanced diet is described as one deficient in at least a class of essential nutrient. Continuous ingestion of such unbalanced diet may lead to malnutrition (Keusch and Farthing, 1986).

Malnutrition is the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance and specific functions

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(World Health Organization, 2006). The problem of malnutrition is one of the most crucial tasks facing mankind today. Whereas, the problem of malnutrition has almost been eradicated in advanced countries, it is however still prevalent in the developing countries (Andrew-Lin, 2003).

Protein-Energy Malnutrition (PEM), also referred to as protein-calorie malnutrition is a potentially fatal body-depletion disorder. It develops in children and adults whose consumption of protein and energy (measured by calories) is insufficient to satisfy the body's nutritional needs. It is the leading cause of death in children in developing countries. In Nigeria, most of the data reported indicates PEM to be one of the major causes of child death, this is because most families do not provide enough protein supplementation to their weaning children while carbohydrate is usually adequate (Hamidu *et al*., 2003). The PEM applies to a group of related disorders that include Masrasmus, Kwashiorkor and intermediate states of Marasmus-Kwashiorkor.

Kwashiorkor, also called wet protein-energy malnutrition, is a form of PEM characterized primarily by protein deficiency, while Marasmus is primarily caused by energy deficiency. Children may present with a mixed picture of Marasmus and Kwashiorkor or with milder forms of malnutrition (Kayode *et al*., 2009).

Mental apathy is a feature of severe kwashiorkor and attributed to loss of potassium from the brain (Manary and Brewster, 1997). Of greater importance is the possibility that severe early malnutrition may permanently impair mental development. In pigs and rats, severe underfeeding at certain early periods in postnatal life leads to a permanent decreases in myelination of nerve cells and reduced DNA content (Yusuf *et al*., 1981).

The protein malnutrition is a worldwide problem, affecting mainly newborns and children of developing countries. This deficiency reaches the brain in the most critical period of the development. Various consequences are related to this insult, such as memory disturbance, learning and behavioral impairment (Fernanda *et al*., 2006).

Tuula (1995) reported that children who had been severely malnourished in the first year of life had at the age of seven years a small head circumference, reflecting reduced brain growth and a lower intelligence quotient than a control group, they also found that brains of children who died in the first year of life weighed less than normal and had proportional reductions of DNA and cholesterol.

Reactive Oxygen Species (ROS) is well recognized for playing a dual role as both deleterious and beneficial species. Overproduction of ROS results in oxidative stress that can be an important mediator of damage to cell structures (Valko *et al*., 2007). 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; Kayode *et al*., 2009). The lipid peroxidation status in the tissues is measured by the level of Malondialdehyde (MDA) generated and its deposition in the brain has been proposed to be one of the major mechanisms of secondary damage in brain injury (Weighand *et al*., 1999).

Fluted pumpkin (*Telfairia occidentalis*) is a creeping vegetative shrub that spread low across the ground with large lobed leaves and long twisting tendrils (Horsfall and Spiff, 2005). Harvesting of fluted pumpkin takes place 120-150 days, after sowing. The seed contains 13% oil (Okoli and Nyanayo, 1988) and is used for marmalade manufacturing (Egbekun *et al*., 1998) and cookie formulations (Giami and Barber, 2004). This darkish-green leafy vegetable is popularly used in soup and in herbal preparations for the management of many diseases in Nigeria. Studies have shown that the leaf of *Telfairia occidentalis* is very rich in iron, antioxidants, phytochemicals (such as phenols) and ascorbic acid and has been found to posses antimicrobial, free radical scavenging and therapeutic activities (Oboh, 2005;

Kayode *et al*., 2009). Aderibigbe *et al*. (1999) reported that the aqueous extracts of *T. occidentalis* reduced blood glucose level and have antidiabetic effects in glucose induced hyperglycemic.

Based on the different health benefits associated with *T. occidentalis* leaves and their widespread acceptability this study was carried out to investigate the antioxidative role of *T. occidentalis* in ameliorating oxidative stress in brain caused by PEM.

MATERIALS AND METHODS

Materials

Collection and Preparation of Sample

The leaves of the plant (*Telfairia occidentalis*) were bought from Bodija market in Ibadan, Oyo State. Its 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.

The research was carried out between June 2006 and February 2007 at the research laboratory of Institute of medical research and training (IMRAT), university college hospital, Ibadan and the laboratory for nutrition and industrial biochemistry, university of Ibadan, Nigeria.

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 seven days before commencement of the experiment. The animals were fed *ad-libitum* throughout the experiment and were allowed free access to clean drinking water.

Feed Composition

Feed was compounded for the different groups as shown in Table 1.

Feed Preparation

The feed constituent 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, baked in the laboratory oven for about two hours. The feed at this stage is ready for administration to the test rats.

The standard feed for the control rats 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.

Table 1: Feed composition

Groups constituents	PEM rats (g)	PTG (g)	PG (g)	TG (g)
Soymeal	40	200	200	40
Cornstarch	820	560	660	720
Vitamin/min mix	60	60	60	60
Oil	80	80	80	80
T. occidentalis	-	100	-	100
Total	1000	1000	1000	1000

Experimental Design

Forty five rats were divided into five groups of nine animals each, the groups are represented as Control, PEM, PTG, PG and TG. 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 28 days to induce protein energy malnutrition (kwashiorkor), on the 29 day, the control and PEM groups were sacrificed to obtain the basal values, rounding up the first phase of the experiment.

The second phase of the experiment began with the administration of recovery diet to groups PTG, PG and TG, also for another period of twenty eight days, group PTG received a diet supplemented with 20% protein and 10% *T. occidentalis* preparation, group PG received diet supplemented with 20% protein only and group TG received diet supplemented with 10% *T. occidentalis* only. The animals were sacrificed on the 29th day. The post mitochondrial fraction of the brain tissue was used for antioxidant enzyme assays and lipid peroxidation studies.

Methods

Preparation of Post Mitochondria Fraction

Reagents

Homogenizing Buffer

The 6.97 g of dipotassium hydrogen orthophosphate, K_2HP0_4 and 1.36 g of potassium dihydrogen orthophosphate KH_2P0_4 were dissolved in little amount of distilled water and made up to 1000 mL mark in a liter 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.

The rats were sacrificed by cervical dislocation, the brain tissues were quickly removed, washed in ice cold 1.15% KCL solution, blotted and weighed. 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.

DETERMINATION OF TISSUE PEROXIDATION

The extent of lipid peroxidation in brain was assessed by measuring the level of Thiobarbituric Acid Reacting Substances (TBARS) taken as lipid peroxide index according to the method of Varshney and Kale (1990).

Enzyme Measurement

Catalase

Catalase (CAT) activity of the post mitochondria fraction of the brain was according to the procedure of Sinha (1971) by following the absorbance of hydrogen peroxide at 240 nm, pH 7.0 and 250°C.

Superoxide Dismutase

The procedure of Misra and Fridovich (1972) as described by Magwere *et al*. (1997) was used for the determination of Superoxide Dismutase (SOD) activity in the brain by measuring the inhibition of autooxidation of epinephrine at pH 10.2 at 30°C.

Statistical Analysis

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

RESULTS

Table 2 shows the percentage increase in weight, that is the rate at which the rats increased in weight throughout the experimental period, the control group fed normal rats diet had the highest percentage increase in weight, closely followed by Group PTG on the protein and *T. occidentalis* supplemented diet, Group PG was next and then the test group. Group TG had the least percentage increase in weight.

The result of the weights of brain tissues for the different groups are presented in Table 3. There was a significant decrease in brain weight ($p<0.05$) of the PEM animals fed with protein deficient diet when compared with the control animals. The animals in the recovery Group PTG had a marked increase in brain weight compared with the PEM group ($p<0.01$) and group PG ($p<0.01$). Group PG had a non significant increase ($p>0.05$) in brain weight compared with PEM Group and Group TG had a non significant decrease when compared with PEM Group ($p>0.05$) and Group PG ($p>0.05$). Conclusively, the results showed that the recovery diet containing protein and *T. occidentalis* caused the most significant improvement on the weight of brain. The effect of *T. occidentalis* alone was not significantly different from that of protein alone.

Table 4 shows the result of the lipid peroxidation in the brain. The MDA level was significantly higher in the PEM group when compared with the control group. All the recovery diets caused significant ($p<0.01$) decreases in MDA levels. The highest decrease was however observed in group PTG rats fed with protein and *T. occidentalis* diet, followed by group TG fed diet supplemented with *T. occidentalis* alone. The least depression of MDA was observed in groups on protein alone supplemented diet PG. Results therefore show that the diet containing protein and *T. occidentalis* offered a superior recovery from lipid peroxidation than protein or *T. occidentalis* alone.

Table 2: Percentage increase in weight

Groups	Percentage
PEM	15.90
Control	124.00
PTG	71.40
PG	28.70
TG	4.08

PEM: Group fed protein malnourished diet, Control: Group fed normal rats pellets PTG: Group fed recovery diet protein+*T. occidentalis*, PG: Group fed recovery diet protein, TG: Group fed recovery diet *T. occidentalis*

Table 3: Effect of different dietary treatments on weight of brain

Groups	Brain weight (g)	Significance	Brain weight/body weight ($\times10^{-2}$)	Significance
PEM	1.286±0.17		2.49±0.32	
Control	2.034±0.79	†a	3.37±0.32	‡a
PTG	1.812±0.12	‡ab	2.97±0.10	‡ab
PG	1.294±0.55	#a	2.93±0.30	†a
TG	1.200±0.48	#ab	2.70±0.37	‡a, †b

Values are Mean±S.D of nine rats. Data were analyzed by students t- test to examine difference between average means. †: $p<0.05$, ‡: $p<0.01$, #: $p>0.05$. PEM: Group fed protein malnourished diet, Control: Group fed normal rats pellets, PTG: Group fed recovery diet protein+ *T. occidentalis*, PG: Group fed recovery diet protein; TG: Group fed recovery diet *T. occidentalis*. Comparisons: a: PEM vs. all, b: PG vs PTG and TG

Table 4: Effect of different dietary treatments on the level of lipid peroxidation

Groups	Mda levels ($\times 10^{-5}$)	SIG
PEM	1.18±0.20	
Control	0.08±0.03	‡a
PTG	0.26±0.05	‡ab
PG	0.57±0.12	‡a
TG	0.38±0.13	‡ab

Values are Mean±SD of nine rats. Data were analyzed by students t- test to examine difference between average means. †: $p<0.05$, ‡: $p<0.01$, #: $p>0.05$. PEM: Group fed protein malnourished diet, Control: Group fed normal rats pellets, PTG: Group fed recovery diet protein+*T. occidentalis*, PG: Group fed recovery diet protein, TG: Group fed recovery diet *T. occidentalis*, Comparisons: a: PEM vs all, b: GroupPG vs. group PTG and groupTG

Table 5: Effect of different dietary treatments on antioxidant enzymes

Groups	SOD	SIG	CAT	SIG
PEM	0.79±0.1		5.3±0.3	
Control	1.73±0.1	‡a	11.9±1.5	‡a
PTG	1.48±0.2	‡a, †b	10.6±1.2	‡a, #b
PG	1.08±0.4	#a	11.9±2.6	‡a
TG	0.87±0.2	#ab	10.9±1.8	‡a, #b

Values are Mean±SD of nine rats. Data were analyzed by students t-test to examine difference between average means.†: $p<0.05$, ‡: $p<0.01$, #: $p>0.05$. PEM:Group fed protein malnourished diet, Control: Group fed normal rats pellets, PTG: Group fed recovery diet protein+*T. occidentalis*, PG: Group fed recovery diet protein, TG: Group fed recovery diet *T. occidentalis*. Comparisons: a: PEM vs all, b: PG vs. PTG and TG

The Superoxide Dismutase (SOD) and Catalase (CAT) activities in the brain are presented in Table 5. Compared with the normal control group , the SOD and CAT activities were markedly reduced ($p<0.01$) in the PEM animals. In the recovery groups, Group PTG had the highest level ($p<0.01$) of SOD activity compared with PEM, PG and TG groups. However, for CAT activity, Group PG animals on protein supplement alone had the highest activity ($p<0.01$), which was however not significantly ($p>0.05$) different from activities for Groups PTG and TG.

DISCUSSION

The rats fed protein deficient diet appeared to lose appetite about the eighth day of the experiment and their general food intake was less than those of rats on the control diet. The phenomenon of appetite loss is characteristic of amino acid imbalance as reported by Harper and co-workers (Harper *et al*., 1970) and would be expected to cause generalized reduced protein synthesis and enzyme function. We observed that the PEM rats became less active, urinated less and defecated less and their stool was watery, showing evidence of diarrhea compared with the control group. The watery stool or diarrhea observed agrees with previous reports on severe protein malnutrition when rats developed edema, diarrhea, mucosal lesions, sparse hair and staggering gait after eating a protein-free diet for about two weeks (Andrew-Lin, 2003).

The significant reduction in brain weight observed in the PEM animals in our present study is consistent with the report by Tuula (1995) that children who had been severely malnourished in the first year of life had a small head circumference, reflecting reduced brain growth and a lower intelligence quotient than a control group. The animals on recovery diets of protein and *T. occidentalis* however showed considerable increase in brain weight compared to the other groups fed either protein or *T. occidentalis* alone, hence, establishing a likely recovery of mental vigor following the administration of this recovery mixture. This is not in support of previous report by Ramdath and Golden (1993) who found that malnutrition imposed during early life not only reduces the growth of the brain but may leave

it permanently smaller in size. Compared with the control group, Malondialdehyde (MDA) levels were found to be significantly increased in the PEM animals indicating an over production of ROS which is capable of damaging a wide range of essential cellular biomolecules such as proteins, enzymes, DNA, RNA, membrane lipids and carbohydrates through oxidative modification, giving rise to different pathological conditions. Lipid peroxidation is assessed by maximal rate of Malondialdehyde formation (Child, 1999) and it is an index of oxidative damage to membrane lipids during oxidative stress. All the recovery diets groups showed significant decrease in the MDA levels with the highest level in PTG, the co-administration of the protein and *T.occidentalis leaves* successfully depressed the PEM induced lipid peroxidation thus suggesting an efficient antioxidative role of the diet that is more significant ($p<0.01$) than both the protein only and the *T. occidentalis* only. This effect of the *T. occidentalis* leaves may be attributed to their inherent flavonoids which are known to be potent oxygen free radical scavengers and metal chelators (Oboh, 2005).

Antioxidant enzymes Superoxide Dismutase (SOD) and Catalase (CAT) in the present study was significantly suppressed in the PEM animals as compared to the control rats. It has been suggested that many of the of the clinical and pathologic manifestations of PEM results from an imbalance between free radical defense and free radical production (Ramdath and Golden, 1993). The reduction in SOD and CAT activities may in part be due to the reduction in the rate of the synthesis of these enzymes (Jimoh *et al*., 2005). The disorder in the supply of amino acid that occurs in PEM could also result in reduced synthesis of these enzymes (Jimoh *et al*., 2005). The significant reduction in activity show that low protein diet affects the activity of these enzymes, the implication of this finding is that there could be accumulation of free radicals when there is inadequate protein levels in diet and free radicals are known to have deleterious effect on the biological system.

The activities of brain SOD and CAT, the enzymes whose natural interaction constitute the most effective system of free radical control in the body (Gilbert, 1981) were significantly increased in the recovery diet groups of protein and *T. occidentalis*, This increase in activity of SOD and CAT following the administration of the recovery diets may be imputable to the replenishment of protein obtained from *T. occidentalis* leaves and the protein diet which is essential for the supply of amino acids necessary for the synthesis of the enzymes (Kayode *et al*., 2009). It therefore propose the recovery diet of protein and *T. occidentalis* as an excellent choice in the recovery of the brain tissue from PEM induced oxidative stress.

A short term therapy that selectively elevates antioxidant status in target organ combined with long term treatment of nutritional support may significantly, increase the chances of survival and total recovery from PEM.

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