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Research Article

Waterleaf (Talinum triangulare) Enhances Cerebral Functions in Swiss albino mice

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Abstract

The aim of this investigation was to verify the effect of aqueous extract of waterleaf commonly used as food supplement on the cerebrum of Swiss albino mice.

Forty mice of both sexes were randomly assigned into three treatments (n=30) and control (n=10) groups. The mice in the treatment groups-B, C and D respectively received 20mg, 30mg and 40mg of waterleaf extract for fourteen consecutive days, while the control mice (group A) received equal amount of normal saline. The cerebral tissues were assayed for Malondialdehyde (MDA) and catalase activities while some were fixed in 10% formol calcium for routine histological study.

The histological findings after Haematoxylin and Eosin (H&E) staining indicated that the treated sections of the cerebrum showed no degenerative changes and no intercellular vacuolations in the stroma. There was also a dose dependent reduction ($p<0.05$) in MDA activities in the treated groups demonstrating the ability of waterleaf to inhibit oxidative stress thus preventing neuronal injury.

These findings indicate that waterleaf consumption has benefiting effects on the neurons of the cerebrum and may probably enhance the cognitive ability in Swiss albino mice.

Keywords: Cerebrum, waterleaf, MDA, catalase, histology

Talinum Triangulare İsviçre Beyaz Sıçanlarında Beyin Fonksiyonlarını Arttırır

Özet

Bu araştırmanın amacı sarmaşık dalı (Talinum triangulare) ekstrelerinin sıvı şeklinin ilave bir besin kaynağı olarak İsviçre beyaz sıçanlarının beyin fonksiyonlarına yararlı etkisini göstermektir. Her iki cinsten toplam 40 sıçan üç ayrı tedavi rejimi (n=30) ve kontrol (n=10) grubu olarak gelişigüzel belirlendi. Tedavi gruplarındaki sıçanlar sırasıyla B,C,D olarak 20mg, 30mg ve 40mg sarmaşık dalı ekstresi alırken kontrol grubundaki sıçanlar (grup A) 15 gün boyunca sadece normal tuzlu su aldılar. Serebral dokular malondialdehid (MDA) ve katalaz aktiviteleri için araştırılırken bazıları %10 formol ile fikse edilip rutin histolojik incelemeye alındılar. Hematoksilen ve eosin ile (H&E) boyama sonucu elde edilen histolojik bulgularda tedavi edilen gruptaki beyin kesitlerinde dejeneratif değişikliklere, hücre içi ya da stromal vakuolleşmelere rastgelinmedi. Tedavi edilen gruplarda doza bağımlı olarak saptanan

MDA aktivitelerinde bir azalma ($p<0,05$) saptandı. Bu durum nöronal hasarlanmayı önleyici olarak sarmaşık dalının oksidatif stresi inhibe etme yeteneği ile yorumlandı.
Bu bulgular İsviçre beyaz sıçanlarında sarmaşık dalı ile beslenmenin beyindeki nöronlara olumlu etkisi ile bilişsel yeteneğin güçlendirilebileceğini göstermektedir.

Anahtar Kelimeler: Beyin, sarmaşık dalı, MDA, katalaz, histoloji

INTRODUCTION

Various nutritional supplements from plants have been implicated as causing harmful effects[11]. It is to be noted as well that nutritional supplements of the antioxidants comes from plant sources. These plants often exhibit a wide range of biological and pharmacological activities, such as anti-inflammatory, anti-fungal and anti-bacterial properties[20,24]. One of such plant source is waterleaf which is sold in most Nigerian markets. It can also be found growing in isolation or sometimes amidst some other grasses. Waterleaf (Talinum triangulare) is an herbaceous annual and perennial plant with a broad, worldwide distribution. It is mostly found in western Africa and western North America. A thorough search in to the literatures revealed that improving dietary habits can delay the onset of diseases such as heart disease and stroke. Growing consumer's demand for traditional food supplements and interest in self-medication calls for extensive research into the nutritional potential of Talinum triangulare vis-à-vis its possible side effects. Although it is extensively consumed, not much is known about its nutritive value. Waterleaf's crude protein content compares favourably with that of cowpea, peanut, millet, and cashew nuts[10]. Akachuku and Fawusi[1] investigated the crude protein content of waterleaf leaves and tender stems and found it to be as high as 29.4% and 13.4%, respectively. Sridhar and Lakshminarayana[25] also gave a report on high total lipids, essential oils, and alpha-tocopherols and beta-tocopherols in Talinum triangulare.

Brain has intrinsically moderate activities of catalase[13,14,17]. Brain is an organ in which homeostasis must be strictly maintained, based on a high dependence on oxidative phosphorylation[4].

Although there hasn't been any local or global report on the harmful or injurious effect of this highly esteemed plant to the body, laboratory testing has shown that high intakes of a food supplement and additive from certain plant source have an adverse effect[11]. We however wish not to assume waterleaf is safe to all the visceral organs and therefore aim to explore its effect on the cerebrum which is the centre of intelligence and learning and also provide useful information that will guide the proper application of this food supplement (Talinum triangulare).

METHODS

Plant Materials

The leaves of Waterleaf were procured from a local market in Ile-Ife, Osun-State, Nigeria. It was identified in the Department of Botany, Igbinedion University, Okada, Nigeria, were a voucher was deposited at the Herbarium. The leaves were oven dried at 40^{o}C for 6 days and then grounded to a fine powder.

Preparation of extract

The powdered material (100g) was extracted with distilled water. The aqueous extract was completely dried under vacuum and then weighed and the residue was used in testing (a yield of 35.23%).The dried extract was dissolved in normal saline before gavages.

Animal treatment

Forty Swiss male albino mice (27.9-30.2g) were used for the experiment. They were maintained under standard laboratory conditions in the Animal Holdings of Igbinedion University, Okada, Nigeria, and fed with standard pelleted diet and water ad libitum. The animals were randomly assigned into groups A, B, C and D. Groups B, C and D (n=30) were respectively administered with 20, 30 and 40mg/kg doses of the extract; an equivalent volume of normal saline was given to group A (control group; n=10) for fourteen consecutive days. Twenty four hours after the last administration, five mice in each group were sacrificed by cervical dislocation, the cerebrum excised and assayed for Malondialdehyde (MDA) and catalase activities while the other five mice were sacrificed following whole body perfusion. The brain was carefully dissected out and fixed in 10% formol calcium for routine histological study. All experimental procedures followed the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health[19].

Assay for Malondialdehyde (MDA)

Samples of the brain (cerebrum) were excised and blotted dried on a filter paper and weighed. A 10% homogenates of each cerebrum in chilled phosphate buffer was immediately prepared with a Potter-Elvhjem homogenizer. The homogenates were centrifuged (3000 rpm for 10 min) and the supernatant immediately stored in the freezer (-20^{o}C) and assayed within 48 h. MDA an index of lipid peroxidation was determined using the method earlier described by Ofusori et al. [23].

Determination of catalase activity

This was determined adopting the methods of Aksenes and Njaa[2]. Hydrogen peroxide was prepared with phosphate buffer; 0.2ml of sample was added to 1.8 ml of 30 mM of hydrogen peroxide (H_20_2) substrate in a 2 ml curvette. The phosphate buffers were used as a blank. The absorbance for the test sample, blank and standard was read against a blank at 240 nm at 30s interval for 1 min. The enzyme activity was calculated using the molar extinction coefficient of 40.00 per M per CM expressed as unit per ml.

Determination of total protein

This was determined using Biuret method[12]. 5.0 ml of blank Biuret reagent prepared by dissolving $CuSO_4$ $5H_20$ crystal in 500 ml of distilled water was added to sample blank. These were mixed well and allowed to stand for 20 min at room temperature 25 - 27^{o}C. Absorbance was read for one test and standard against a blank at 540 nm. The concentration of protein was calculated using: optical density for standard × concentration of standard.

Histological procedure

The cerebral cortices were trimmed from the excised brain and fixed in 10% formol calcium for histological studies. After fixation, the cerebral tissues were embedded in paraffin; serial sections were cut at 5 μm and stained with Haematoxylin and Eosin (H & E). The sections were examined under a light microscope and photomicrographs taken using Olympus Research Microscope.

Statistical Analysis

Data were statistically evaluated with SPSS/10 software. Hypothesis testing methods included one-way analysis of variance (ANOVA). A value of $p<0.05$ was considered to indicate a significant difference between groups. All the data were expressed as the Mean ± Standard Error of Mean (SEM).

RESULTS

The control and treated sections of the cerebrum showed normal histological features with the neurons appearing distinct and the glial cells normal without vacuolations in the stroma (Figures 2, 3, 4, 5). These observations were much more

prominent in the treated groups (Figures 3, 4, 5). There was a significant increment in the weight difference of the treated animals from the commencement of the experiment to the end of the experiment as shown in Figure 1. The relative weight of the brain presents a dose dependent increment in the treated animals (Table 1). The activities of MDA was significantly lowered in the treated groups just as the catalase activities was increased in a dose dependent manner (Table 2)

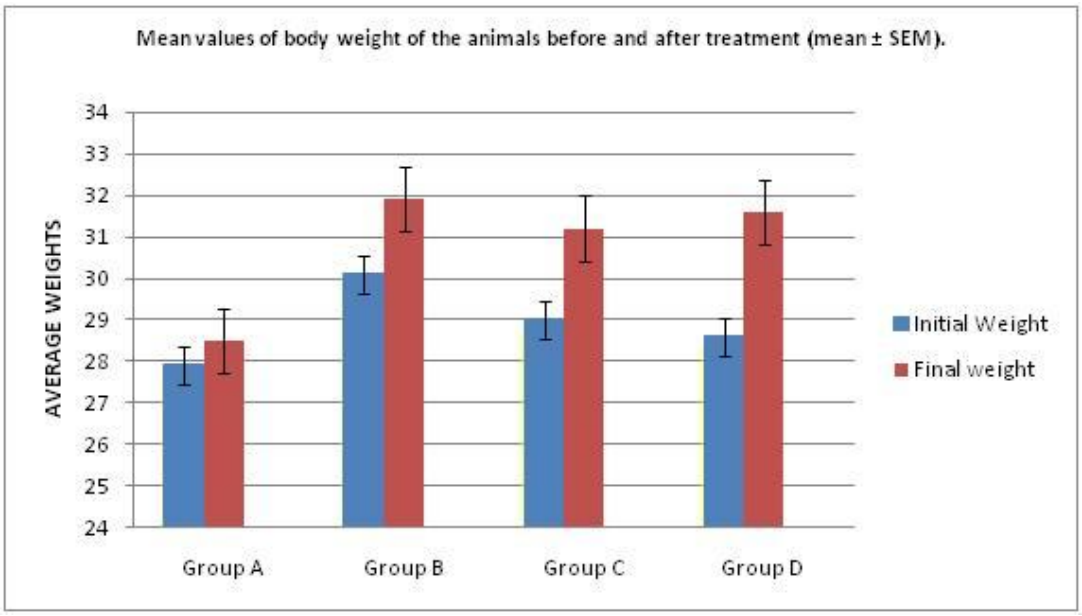

Figure 1: *Chart showing the average weights of the mice at the commencement and the end of the experiment. Note the gradual increment in the average weight of the mice at the beginning of the experiment (Initial weight) to the end of the experiment (final weight).*

Figure 2: *Photomicrograph of the cerebrum of group A (control). Note the normal appearance of the neurons and glial cells (H&E, x100)*

Figure 3: *Photomicrograph of the cerebrum of group B. Note the distinct and better organization of the neurons and glial cells vis-à-vis group A (H&E, x100)*

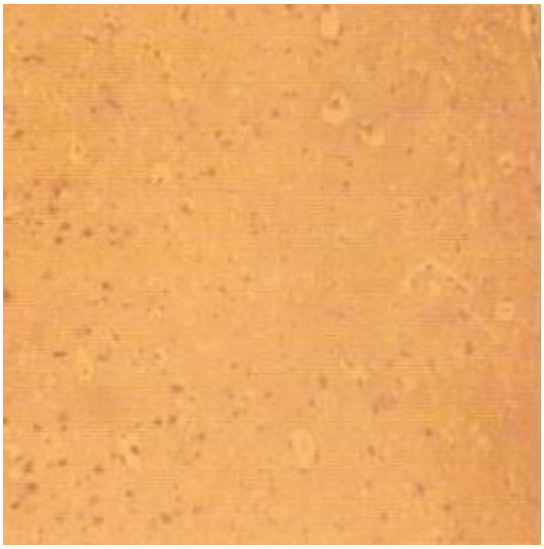

Figure 4: *Photomicrograph of the cerebrum of group C. Note the distinct and better organization of the neurons and glial cells vis-à-vis group A & B (H&E, x100)*

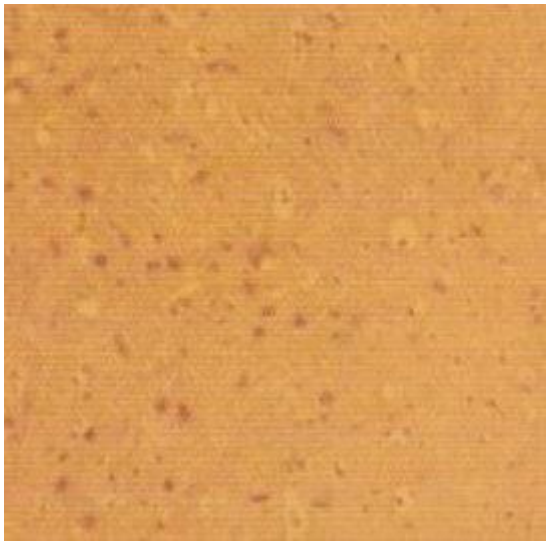

Figure 5: *Photomicrograph of the cerebrum of group D. Note the distinct and better organization of the neurons and glial cells vis-à-vis group A, B & C (H&E, x100)*

Table 1. Relative weights of the cerebrum to the absolute body weight (%).

Measured Parameter	Group A 0 mg/kg	Group B 20 mg/kg	Group C 30 mg/kg	Group D 40 mg/kg
Relative Weight	0.55	0.58	0.69	0.78

Table 2. Activities of MDA and Catalase in the cerebrum of Swiss albino mice (mean ± SEM).

Measured Parameters	Group A 0 mg/kg	Group B 20 mg/kg	Group C 30 mg/kg	Group D 40 mg/kg
MDA (mol/mg protein)	0.66 ± 0.00	0.59 ± 0.02*	0.53 ± 0.05*	0.49 ± 0.04*
Catalase (U/mg protein)	106 ± 0.00	113 ± 2.23*	115 ± 2.31*	119 ± 2.25*

*P < 0.05 (significantly different) vs. control; n = 5

DISCUSSION

The results revealed that oral administration of waterleaf showed no histological derangement, degenerative changes and vacuolations both in the treated and control sections (Figures 2, 3, 4, 5). The sections which conform to the normal histological outline were observed to be better organized in the treated groups' vis-à-vis the control. This is indicative of non-toxic and non-interference of waterleaf with the cellular integrity and normal metabolic processes in agreement with the earlier reports of Ofusori et al. [21]. The histological features of the cerebrum present a distinct appearance of neurons and the glial cells without any vacuolations in the stroma of the treated animals (Figures 3, 4, 5). It was reported that cell death in response to neurotoxins might trigger an apoptotic death pathway within brain cells[27]. Cell death in response to neurotoxins occurs as a controlled event involving a genetic programme in which cascade enzymes are activated [27]. In some cases brain cells are damaged by oxidized compounds called free radicals generated in the body by the oxidation of food substances and other chemical reactions in the cells[3]. To cope with the damaging actions of these free radicals, organisms have developed multiple systems of antioxidant defense. Some are low-molecular weight antioxidants which include metabolites such as glutathione, ascorbic acid, tocopherol, uric acid, etc., others are high-molecular weight defenses such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione-S-transferase (GST) enzymes[9,13,18]. These enzymes that deal directly with radical species and the damage caused by them to macromolecules constitute the first line of antioxidant enzymatic defense, whereas other enzymes including glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH) contribute to the renewal of reducing power[4]. The greater the severity of an insults the more rapid the progression of neuronal injury[15]. Beal et al. [6] revealed in their investigation that chronic ingestion of sugarcane results in damage to the striatum. In a related vain, Zhang et al. [29] found from their investigation that oxidative stress generated by the systemic presence of neurotoxins are the principal cause of deterioration of mental activity and aging. The non-toxic effect of waterleaf was demonstrated by the dose dependent significant decrement in the activities of MDA (Table 2). MDA was used in this investigation as an index of lipid peroxidation. The significant reduction of MDA in the treated groups as compared with the control demonstrates the ability of waterleaf to inhibit oxidative stress. This may be connected with the dose dependent increment observed in the relative brain weight (Table 1). The mechanism of action may probably be as a result of the induction of the catalase activities which is sufficient enough to interrupt lipid peroxidation. Lipid peroxidation is one of the consequences of oxidative stress, a situation that usually occurs when the production of reactive oxygen species (ROS) exceeds that of the antioxidant defense systems. The ROS can be inactivated through the action of antioxidant enzymes or other unspecific antioxidants[7]. An increase in the activities of catalase in the treated compared with the control shows that waterleaf increases antioxidant enzyme catalase, which probably make the cerebrum more fortified for any biochemical injury in accordance to earlier reports[26]. Antioxidants have been known to enhance brain functions[5,20]. The vitamin C, vitamin E, and Beta-carotene, minerals (such as calcium, potassium, and magnesium), and soluble fiber (pectin) contribute to waterleaf's highly-elevated antioxidant values and its total biological effect[1]. Although our observation is a bit contradictory to the earlier report[16]; this

may however be attributed to the disparity in the concentration of the extract administered. It is worthy of note at this juncture that the production of antioxidants in the body declines with age which therefore necessitates the need for nutritional supplement[20,22]. Consumption of waterleaf going by this investigation can be recommended as food supplement to protect the brain cells and provide numerous other functions that are beneficial to the body. Prior studies have shown that consumption of vegetables and other food supplements rich in polyphenols can reduce age-related neurological disorders[8,20,28]. In this investigation, we demonstrated the beneficial potential of waterleaf in the enhancement of the brain activities in Swiss albino mice and by extension, may be recommended as pro-cognitive and neuroprotective agent. It is recommended that further studies be carried out to corroborate these findings.

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