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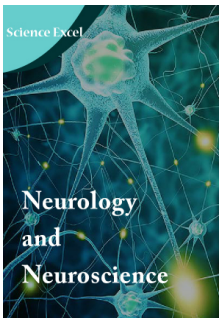

Anti-Oxidative Property of *Pennisetum Glaucum (Poaceae)* Supplement Contributes To Its Anti-Convulsant Activity In Pentylenetetrazole-Kindled Wistar Rats

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PTZ-Pentylenetetrazole, PMS-pearl millet supplement, SOD-superoxide dismutase, CAT-atalase, GSH-glutathione, MDA-malondialdehyde

Abstract

Introduction: Oxidative stress is one of the prime pathogenic factors going on in both human epilepsy and experimental epileptogenic models. PTZ-Seizure has been associated with over production of superoxide anion, reduced GSH levels, increased lipid peroxidation and protein oxidation in the hippocampus.

Methodology: Forty male wistar rats were divided into 5 groups (normal saline, 200 mg/kg sodium valporate, 25% PMS, 50 % PMS and 100 % PMS) with each group receiving PTZ (35 mg/kg) on every alternate day for 30 days. Thirty minutes after each PTZ injection rats were observed for seizure behavior using the Racine scale. The hippocampal tissues were isolated, homogenized and used to determine SOD, CAT, GSH and MDA level.

Result: This result revealed significant increase in the mean hippocampal SOD activity of PTZ-kindled wistar rats fed with PMS [25% (18.90 ± 1.08), 50% (18.90 ± 1.08, 24.90 ± 1.37) and 100 % (11.06 ± 0.58 IU/ mg protein) when compared to normal saline group (8.84 ± 0.96 IU/ mg protein).

PM Supplementation increased mean CAT activity at 25% and 50% [25% (12.68 ± 0.68), 50% (8.74 ± 0.92) and 100% (15.66 ± 1.12 µg/ mg protein)] Vs normal saline treated group (9.60 ± 0.72 µg/ mg protein).

A significant increase in mean hippocampal GSH concentration was seen in all the PMS fed wistar rats [25 % (29.52 ± 0.44), 50 % (24.42 ± 1.51) and 100 % (40.32 ± 1.43 µg/ mg protein)] when compared to normal saline (10.88 ± 0.32 µg/ mg protein).

PTZ-kindled wistar rats fed with PMS [25% (86.62 ± 2.81), 50% (73.86 ± 3.26) and 100% (73.56 ± 1.82 nmol/ mg protein) showed significant decrease in the mean concentration of hippocampal MDA when compared to normal saline (119.90 ± 2.34 nmol/ mg protein).

Conclusion: Supplementation with PG inhibits hippocampal redox imbalance and reinforces it antioxidant system.

Introduction

NEpilepsy remains a common cause of morbidity and mortality in spite of the advances in management regimens [1]. People with epilepsy are at higher risk of death than those without epilepsy with an adjusted life years (a measure of the years lost living in those with the disease) of more than 13 million globally [2]. In addition, epilepsy burden caused 14.8 million disability-adjusted life-years (DALYs; a summary measure of health loss defined by the sum of years of life lost due to premature mortality and years lived with disability) [3]. Improving access to effective treatment could reduce the burden of epilepsy [4].

Astrocytes are source of neurotrophins that via nuclear factor erythroid related factor 2 (Nrf2) mediates the formation of antioxidant enzymes such as SOD, CAT, glutathione peroxidase (GSH-Px), and reduced form of GSH (GSHred) [5]. Seizure activity during epilepsy decreases the brain antioxidant defense mechanism and induce over-production of mitochondrial free radicals [6] that could decrease mitochondrial reserve capacity of inhibitory interneurons, deplete ATP production by neuronal mitochondria, inhibits Na+/K+ ATPase activity that lower resting membrane potential, decrease inhibitory input resulting to hyper-excitability [7]. It also upregulate the expressions of redox-sensitive transcription factors, activator protein-1 (AP-1) and NFκB that in turn increase extracellular O2- production through iNOS [8].

Advances in science, increasing health care costs and interest in attaining wellness are factors rising interest in confirming the relationship between the components in diet and risk of disease or health condition [9].

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The identification of good amount of phyto-constituents like phenolics, flavonoids and phospholipids in pennisetum glaucum raises it prospective for a large number of medicinal properties and makes it one of the best food grains in the world [10]. In our previous study we found that pennisetum glaucum supplement seizure severity in PTZ-kindled wistar rats were suppressed among other seizure models used in that study [11].

Material and methods

Experimental animals

Forty (40) male wistar rats (140-180g) were purchased from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

Ethical approval

This study was carried out under the approval of the Ahmadu Bello University Ethical Committee on Animal Use and Care, ABUCAUC/2021/008.

Drugs and reagents

Pentylenetetrazole (Sigma-Al¬drich, St. Louis, USA), Sodium valproate (Epilim) (Sanofi aventis Riells, Spain), 0.05 M carbonate buffer, 0.3 mM adrenaline, 0.2 M phosphate buffer, trichloroacetic acid, Ellman's reagent, thiobarbituric acid

Plant material

Millet was purchased from Samaru Market, Sabon Gari Local Government Area, Kaduna State, Nigeria, in June, 2020. Identified and authenticated as the pennisetum glaucum (PG) variety by taxonomist at Herbarium unit of the Department of Biological Sciences, ABU, Zaria, Nigeria where it was given the voucher number (1824).

Preparation of supplement

The identified PG was soaked in water for 12 hours, paste grinded, sieved with muslin cloth, residue discarded and filtrate allowed to settle in a container. It was then decanted, drained with muslin, shade dried into powder [12], measured and added to pellets grower mash at 25 %, 50 % and 100 %.

Pentylenetetrazole induced kindling seizures in wistar rats

Forty rats were divided into 5 groups of 8 each with groups 1 and 2 fed on standard diet (SD) only while 3-5 with 25 %, 50 % and 100 % PGS respectively. On every alternate day, groups 1 and 2 were orally administered normal saline and sodium valporate an hour before all the 5 groups are injected with freshly prepared PTZ (35 mg/kg) [13] and observed for 30 minutes for the presence/ absence seizure and it severity using the scale of [14] for 30 days.

Group 1-SD + normal saline (1mg/kg, Oral) + PTZ (35mg/kg, I.p)

Group 2-SD + sodium valporate (200mg/kg, Oral) + PTZ (35mg/kg, I.p)

Group 3- PGS (25 %) + PTZ (35mg/kg, I.p)

Group 4- PGS (50 %) + PTZ (35mg/kg, I.p)

Group 5- PGS (100 %) + PTZ (35mg/kg, I.p)

Rats were considered fully kindled when they showed stages 4 and 5 on two consecutive trials which happened on 15 administration of PTZ (30th day of the experiment).

Sample collection and preparation

All rats were cervically dislocated, decapitated, brains dissected, cortices exposed and hippocampi isolated while placed over ice. Five hippocampal tissues from each group were washed with phosphate buffer, homogenized, collected into labelled vials, freezed and used within two weeks for the determination of SOD by the method described by [15], CAT [16], GSH concentration [17] and MDA [18].

Procedure for superoxide dismutase assay

Hippocampal homogenate of 0.1 ml was diluted in 0.9 ml of distilled water to make 1:10 dilution of microsome. An aliquant mixture of 0.2 ml of the diluted microsome was added to 2.5 ml of 0.05 M carbonate buffer. The reaction was started with the addition of 0.3 ml of 0.3 mM Adrenaline. The reference mixture contained 2.5ml of 0.05 M carbonate buffer, 0.3 ml of 0.3 mM Adrenaline and 0.2 ml of distilled water. The absorbance was measured within 2 minutes at 480 nm.

Calculations:

Increase in absorbance per minute= A2-A1/2.5

%Inhibition=100-{absorbance for sample/ absorbance of blank × 100}

1 unit of SOD activity is the quantity of SOD necessary to elicit 50 % inhibition of the oxidation of Adrenaline to adenochrome in 1 minute.

Procedure for catalase assessment

About 10 µl of hippocampal homogenate was added to a test tube containing 2.80 ml of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 ml of freshly prepared 30 mM H2O2 and the decomposition rate of H2O2 was measured at 240 nm for 5 minute on a spectrophotometer. A molar extinction coefficient (E) of 0.041mM¯1 -cm¯1 was used to calculate the catalase activity.

Catalase Concentration = Absorbance / E

Catalase Activity = Catalase Concentration (mg) / Protein Concentration (ml)

Glutathione concentration assay procedure

To 150 µl of tissue homogenate (in phosphate - saline buffer pH 7.4), 1.5 ml of 10 % TCA was added and centrifuge at 1500 ×g for 5 minute. 1 ml of the supernatant was treated with 0.5 ml of Ellman's reagent and 3 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. The quantity of GSH was obtained from the graph of the GSH standard curve.

Malondialdehyde assay procedure

Exactly 2 ml of 15 % trichloroacetic acid was measured into a test tube, 2 ml of thiobabitutric acid were added and 100 µl of the hippocampal homogenate was as well be added. The mixture was incubated at 80^{0}C using DHP-9035A heating incubator for 30 minute in a water bath and allowed to cool for some time, followed by centrifugation at 3000 rpm for 10 minute. A clear supernatant was collected and the absorbance determine at 535 nm in a spectrophotometer.

TBARS concentration = Sample Absorbance (nmol) / 1.5×10^{-5} x Protein Conc. (mg)

Statistical analysis

Data collected were expressed as mean + SEM. It was analyzed using one way analysis of variance, ANOVA, and *Tukey's post-hoc* test was used to compare the level of significance between the control and treatment groups using SPSS version 23.0. Values of $p \leq 0.05$ were considered significant

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***Table 1**: Effect of Pennisetum Glaucum Supplement on Mean Hippocampal Oxidative stress biomakers in PTZ-kindled Wistar Rats*

Groups	SOD (IU/ mg protein)	CAT (µg/ mg pro)	GSH (µg/ mg pro)	MDA (nmol/ mg protein)
N. Saline + PTZ	8.84 ± 0.96	9.60 ± 0.72	10.88 ± 0.32	119.90 ± 2.34
NaVal + PTZ	17.56 ± 1.85a	15.76 ± 1.75	9.72 ± 0.39	109.16 ± 8.75
25% PGS + PTZ	18.90 ± 1.08 a	12.68 ± 0.68	29.52 ± 0.44c	73.86 ± 3.26d
50% PGS + PTZ	24.90 ± 1.37 a	8.74 ± 0.92	24.42 ± 1.51c	73.56 ± 1.82d
100% PGS + PTZ	11.06 ± 0.58	15.66 ± 1.12b	.32 ± 1.43c	73.56 ± 1.81d

Results

The values were expressed as Mean ± SEM, superscripts a,b,c,d indicate statistically significant difference a [F(4,20)=12.04, p<0.01], b [F(4,20)=8.77, p<0.01], c [F(4,20)=17.03, p<0.01], d [F(4,20)=21.30, p<0.01] respectively compared to normal saline using One-way ANOVA followed by Tukey's post hoc test using SPSS 23. PTZ: Pentylenetetrazole (35 mg/kg), mg pro: mg protein, PGS: pennisetum glaucum supplement, NaVAL: sodium valporate (200 mg/kg), SOD: superoxide dismutase, CAT: catalase, GSH: reduced glutathione, MDA: malondialdehyde.

Discussion

The inhibition of free radicals by antioxidants increasing the levels and/ activities of SOD, CAT, GSH or GSH peroxidase could prevent abnormal neuronal discharge and in turn generation of seizure [19].

Seizures stimulate excessive production of O2- radicals [7], H2O2 causing a deficiency in CAT enzyme [6] and low levels of SOD and CAT are associated with cellular damage from oxidative stress [20]. In addition, a significant increase in MDA concentration couple with a decrease in GSH levels indicate antioxidant imbalance [6].

The recorded significant increase in the hippocampal levels of SOD, CAT except for 50 % and GSH coupled with the dose dependent decrease in the hippocampal level of MDA in PGS supplemented groups suggest that PGS possesses a free radical scavenging activity that could exert beneficial effect against pathological alterations caused by oxidants.

It was further observed in this study that SOD activity was much higher in 50 % PGS followed by 25 % PGS and least in 100 % PGS meanwhile 50 % PGS showed the least CAT activity and GSH level followed by 25 % while the highest was seen with the 100 % this could be that PGS at the tested doses are able to reduce oxidative stress by modulating the activities of more specific antioxidant enzymes (SOD by 50 %, CAT and GSH by 100 %). However, the decrease in MDA concentration was found to dose dependent with highest level recorded in 25 % followed by 50 % and least in 100 % PGS supplemented group.

At the tested doses PGS seems to be more beneficial in terms of handling peroxidation of lipid when compared to valporate-a standard AED as shown by the significant decrease in MDA concentration and increase GSH level in comparison to valporate. Hence, the observed antioxidant activity could have been due to PGS pro-activity on endogenous SOD synthesis in addition to the presence of antioxidant nutrients like zinc present in PG [9]. Similarly, [21] affirms that oxygen free radical are scavenged mainly via decreasing MDA level by polyphenol and flavonoids which are reported present in PG by [11,22] among other researchers.

Moreover, one of the ways by which PTZ produces convulsion is pro-oxidation of reactive species that result to increase MDA concentrations. Accordingly, the decrease offered by PGS could be through direct mopping out of the reactive species hindering the progression of lipid peroxidation. Flavonoids and isoflavonoids have the potential of reacting with and inactivating various oxidative processes like superoxide anions, oxygen lipid peroxide radicals, and/or stabilize free radicals through hydrogenation or complexing with oxidant species as they are rich in hydroxyl groups [6] and these compound are found in significant quantities in PG [23]. Tannins and phytic acid present in PG [9] could be responsible for the free radical quencing activity [24] since phytic acid from grains form chelates with pro-oxidant transition metals like Fe [25] preventing their interaction with oxygen atoms that may form free radicals [26] and suppresses lipid peroxidation thereby protects cells from damages [27]. In addition to phenols that are known of having the ability of disrupting cellular oxidative processes in the CNS [6].

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