

Effect of Taurine on Acute Restraint Stress Induced Biochemical Alterations in Wistar Rats

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Abstract

THE aim of the present study was to investigate the effects of taurine administration on biochemical parameters in Wistar rats subjected to acute restraint stress. Twenty four Wistar rats were divided into four groups of six rats each. The control group received distilled water and the other treatment groups were administered taurine at 100, 200 and 400 mg/kg. The treatments were administered once daily by oral gavage. The rats were sacrificed brain and blood samples were collected after 14 days. The brain malondialdehyde, Acetylcholinesterase concentrations and the activities of brain antioxidant enzymes were evaluated. The serum samples were analyzed for proteins and enzymes concentration. The results indicated that acute restraint stress induced biochemical alterations in the rats. It is hypothesized that taurine might have decreased the alterations in the biochemical parameters through its antioxidant properties. It is concluded that taurine may be a useful prophylactic agent against biochemical alterations in individuals that are constantly exposed to one form of stress or another, as it counteracted the restraint stress-induced lipid peroxidation and maintained the antioxidant defense system to near normal.

Keywords: antioxidant, enzymes, malondialdehyde, taurine, peroxidation, restraint, stress

1. Introduction

Stress is one of the major contributory factors that stimulate numerous intracellular pathways, thus leading to increased free-radical generation, causing oxidative damage [1]. Free radicals are highly reactive moieties, playing an important role in health and disease. The brain is especially vulnerable to free radical-induced damage because of its high oxygen consumption, abundant lipid content and low levels of enzymatic and non-enzymatic antioxidants [2].

In stress, symphatho-adrenal (SA) and Hypothalamo-Pituitary-adrenal (HPA) systems collaborate in maintaining internal body homeostasis, resulting in simultaneous increases of catecholamines from the SA system. Restraint stress is an easy and convenient method of inducing both psychological and physical stress, resulting in restricted mobility and aggression. Restraint does not physically

harm the animal, but does activate the HPA-axis and increases the production of glucocorticoids [3], initiating the deleterious effects of stress. Taurine (2-aminoethane sulphonic acid) is a sulphonic acid which is derived from cysteine and it is one of the few naturally occurring sulphonic acids. Taurine is widely distributed in animal tissues and one of the most abundant amino acid in mammals. Taurine plays several crucial roles including modulation of calcium signaling, osmoregulation and membrane stabilization [4]. There is a growing consensus that oxidative stress is linked to mitochondrial dysfunction [5, 6], and that the beneficial effects of taurine are as a result of its antioxidant properties [7, 8], as well as its ability to improve mitochondrial function by stabilizing the electron-transport chain and the generation of reactive oxygen species [9, 10].

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2.0 Materials and Methods

2.1 Chemicals

2.1.1 Taurine Preparation

Taurine (TA) (CAS No. 107-35-7; purity \geq 99%) preparation of analytical grade (100 g - Sigma-Aldrich, USA) was

2.2 Experimental animals

A total of 24 adult Wistar rats, weighing 150-200 g were used in this study. The animals were obtained from the animal house of the Department of Human Physiology Ahmadu Bello University Zaria and were assigned randomly to three treatment groups, with the experimenter blinded to the drug treatments. The rats were housed in plastic cages under normal conditions of ambient

2.2.1 Experimental protocol

The Wistar rats were weighed and randomly allocated into four groups, with 6 animals in each group. Group A- served as control and were given 1 ml/kg of distilled water each per os through a gavage, Group B- received 100 mg/kg of taurine per os; group C- received 200 mg/kg taurine; and

2.3 Experimental Design

2.3.1 Acute Restraint Stress Induction in Wistar Rats

Restraint stress was induced according to the method of [12], with some modification. A Perspex restraint cage with dimensions of 14 cm (L) \times 5 cm (B) \times 6 cm (H) was used in this experiment. Each rat was housed individually in a multi-compartment cage for the remaining time to avoid aggression and to prevent social isolation. Unrestrained rats (control group) were left undisturbed in their home cages but without access to food or water during the same period. The Wistar Rats were exposed to acute restraint stress, 1

2.4 Preparation of brain tissue homogenates

The brain samples were rinsed immediately with physiological saline, patted dry with filter paper and weighed following their excision from the rats. Portions of the tissues were mixed with phosphate buffered saline (PBS) pH 7.4 in a 1:10 (w/v) ratio and made into

2.4.1 Determination of brain Malondialdehyde (MDA)

The concentration of malondialdehyde (MDA) as an indication of lipid peroxidation was evaluated in the fore brain homogenate samples with the method [14]. The principle of the method was based on the spectrophotometric measurement of the colour developed during the reaction of thiobarbituric acid (TBA) with MDA. The procedure was conducted for the brain homogenate of baths for 15 min. The solutions were cooled under tap water and the absorbance was measured with a UV spectrophotometer (T80+ UV/VIS Spectrophotometer®, PG Instruments Ltd., Liicestershire, LE 175BE, United Kingdom) at 532 nm. The concentration of MDA in the samples were calculated by

2.4.2 Evaluation of brain acetylcholinesterase (AChE) activity

obtained for this study. Taurine was reconstituted as 40 % stock solution in distilled water.

temperature in a 12 h light/dark cycle in the animal house of the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria. The study was conducted in accordance with the guidelines of the National Institute of Health Guide for Care and Use of Laboratory animals [11]. Animals were allowed free access to food and water *ad libitum*.

group D- received 400 mg/kg taurine. The treatments were administered once daily 60 minutes prior to the commencement of the stress sessions by oral gavage for 14 days.

hour daily for 14 days [13], by keeping them in a Perspex restraint cage, restraining up to 6 rats simultaneously without food and water during the restraint stress. The rats were pretreated with the various doses of taurine according to their groups 60 minutes prior to the commencement of the restraint. The stress procedure was carried out at the animal house of the Department of Human Physiology, Ahmadu Bello University Zaria, throughout the experimental period between 9 a.m. and 4 p.m.

homogenates with pestles and mortars. The mixtures obtained were kept cold with ice baths and were centrifuged afterwards for the evaluation of the activities of brain malondialdehyde, acetylcholinesterase and the concentration of brain antioxidant enzymes.

the rats as follows: 2.5 ml of 100 g/L trichloroacetic acid solution was added to 0.5 ml of the samples in centrifuge tubes that were placed in boiling water baths for 15 min. After cooling under tap water for 5 min, each mixture was centrifuged at $1000 \times g$ for 10 min. Subsequently, 2 ml of each supernatant was added to 1 ml of 6.7 g/L TBA solution in test tubes placed in boiling water

using the absorbance coefficient of MDA-TBA complex $1.56 \times 10^5/\text{cm}/\text{M}$ and expressed as $\mu\text{mol}/\text{L}$ (in the serum) nmol/mg protein (in the brain homogenate).

The brain homogenates of the rats were evaluated for acetylcholinesterase assay. The method is based on the reaction of acetylcholine with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to yield 5-thio-2-nitrobenzoate anion which is yellow in colour. The rat brain tissues were dissected on petri dishes chilled on crushed ice, weighed and homogenized in 0.1 M sodium phosphate buffer (pH 8). Subsequently 0.4 ml of the aliquots of the brain tissue homogenates were added to cuvettes containing 2.6 ml phosphate buffer and 5,5'-dithiobis-nitrobenzoic acid (200 μ l; final concentration = 0.33 Mm). The contents of the cuvettes were mixed thoroughly by bubbling air and the absorbance was measured at 412 nm with a Shinadzu UV

spectrophotometer (Model UV 160; Kyoto, Japan). The changes in the absorbance were recorded for a period of 10 minutes at intervals of two minutes after the addition of acetylthiocholine (30 μ l); final concentration = 0.5 mM) to the mixture. Thus, the change in the absorbance per minute was determined. The enzyme activity was calculated by using the formula:

$$R = \frac{5.74 \times 10^{-4} \times A}{CO}$$

Where 5.74×10^{-4} is a dissociation coefficient, R = rate in moles of substrate hydrolyzed/g tissue, A = change in absorbance/min and CO = original concentration of the brain tissues.

2.4.3 Assessment of activities of antioxidant enzymes in the brain

Superoxide dismutase (SOD) activity was assessed with the NWLSSTM SOD activity assay kit and the principle of the method was based on autoxidation of haematoxylin. Catalase (CAT) activity was analyzed with the NWLSSTM CAT activity assay kit and the method used was based on the consumption of H_2O_2 substrate as described by Beers

and [15]. The activity of glutathione peroxidase (GPx) was evaluated with the NWLSSTM GPx activity assay kit and the procedure was based on the oxidation of reduced GSH to form oxidized GSH [16]. The assays were conducted according to the manufacturer's (Northwest Life Science Specialities, LLC, Vancouver, WA 98662) instructions.

2.5 Evaluation of serum enzymes and proteins

The rats from each group were sacrificed after light ether anaesthesia by cardiac puncture at the end of the 21 days dosing period. Two and half millimeters of blood sample were collected from each rat into a centrifuge test tube that was devoid of anticoagulant. The blood samples were allowed to clot and were incubated at room temperature for 60 min. Subsequently, the blood samples were centrifuged at $1000 \times g$ for 5 min to obtain a clear straw coloured serum. This was used to evaluate biochemical parameters such as concentrations of total protein, albumin and globulin. Other biochemical parameters assayed in the serum were the activities of alanine aminotransferase (ALT), aspartate

aminotransferase (AST) and alkaline phosphatase (ALP). The biochemical parameters were assayed with Bayer Express Plus Clinical Chemistry Autoanalyser (Bayer® Germany). Serum total protein concentration was estimated based on the Biuret method [17], serum albumin concentration was determined as described by [18]. Serum globulin was obtained by the deduction of serum albumin concentration from total serum protein concentration, and the albumin/globulin ratio was also calculated. AST and ALT activities were evaluated using the same auto-analyzer as stated. ALP concentration was estimated based on the enzymatic hydrolysis method [19].

2.6 Statistical analysis

Data were presented as mean \pm standard error of the mean (SEM). The biochemical parameters were analyzed with one-way analysis of variance followed by Tukey's multiple

comparison post-hoc test. Statistical analysis was conducted with SPSS software version 20. Values of $P < 0.05$ were considered significant.

3.0 Results and Discussion

3.1 Results

Table 1: Effect of taurine treatments on MDA, AChE and enzymatic antioxidant in the brain of acute restraint-stressed Wistar rats

TREATMENTS	MDA (NMOL/MG PR-)	AChE (NMOL/MG PR-)	SOD (I.U/L)	CAT (I.U/L)	GPx (I.U/L)
CONTROL (DW)	1.30±0.08	24.17±1.17	2.23±0.08	52.33±1.05	48.33±1.12
TAURINE:					
100 MG/KG	1.27±0.10	22.67±1.02	2.23±0.11	52.67±0.95	47.17±3.06
200 MG/KG	1.15±0.08	24.17±0.75	2.35±0.19	51.67±1.33	47.50±0.62
400 MG/KG	1.12±0.11	23.00±1.37	2.25±0.08	51.67±1.36	47.50±1.38

Note: Values are mean ± SEM, n = 6. DW (Distil water), MDA (Malondialdehyde), AChE (Acetylcholinesterase), SOD (Superoxide dismutase), CAT (Catalase) and GPx (Glutathione peroxidase)

There is no significant ($p > 0.05$) difference in the MDA, AChE, SOD, CAT and GPx in the taurine treated groups when compared with the control groups (table 1).

Table 2: Effects of taurine on serum protein concentration in acute restraint-stressed Wistar rats

TREATMENTS	TP (G/DL)	ALB (G/DL)	GLB (G/DL)	ALB/GLB
CONTROL (DW)	63.67±1.28	35.00±1.29	28.67±0.01	1.22±1.29
TAURINE:				
100 MG/KG	64.00±1.00	35.33±1.12	28.67±0.12	1.22±0.93
200 MG/KG	65.00±1.21	35.33±1.12	29.67±1.00	1.19±1.12
400 MG/KG	65.17±1.42	37.67±1.05	27.50±0.37	1.37±2.83

Note: Values are mean ± SEM, n = 6. DW (Distil water), TP (Total protein), ALB (Albumin), GLB (Globulin), ALB/GLB (Albumin Globulin ratio)

There is no significant ($p > 0.05$) in the TP, ALB, GLB and ALB/GLB ratio when the taurine treated groups where compared with the group (table 2).

Table 3: Effects of taurine treatments on the activities of serum enzymes in acute restraint-stressed Wistar rats

TREATMENTS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
CONTROL (DW)	42.00±2.44 B	47.83±1.74B	68.17±1.14
TAURINE:			
100 MG/KG	42.50±1.15 B	48.00±0.82B	68.17±2.43
200 MG/KG	45.83±1.25B	51.17±2.04A	68.83±2.36
400 MG/KG	38.17±0.79A	45.50±3.78B	64.83±1.66

Note: values are mean ± SEM, n = 6. DW (Distil water), AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) and ALP Alkaline phosphatase). a,b = values with different superscript differ significantly ($p < 0.05$)

There is significant ($p < 0.05$) increase in activities of ALT in the group that received taurine 200 mg/kg but, there is significant ($p < 0.05$) decrease in the AST activities of the taurine 400 mg/kg group when compared with control group (table 3).

3.2 Discussion

In the present study, there were no significant ($p > 0.05$) difference in the serum MDA concentration in the acute restraint-stressed Wistar rats in all the taurine treated groups when these were compared with the control group. One of the major oxidation products of peroxidized polyunsaturated fatty acids is MDA. Increase in MDA content is an indicator of oxidative stress and lipid peroxidation [20]. Lipid peroxidation will lead to oxidative degradation of poly-unsaturated fatty acids and its occurrence in biological membranes engenders impairment of membrane fluidity and inactivation of several membrane-bound enzymes that are crucial for numerous biological processes [21]. Administration of taurine may have attenuated tissue lipid peroxidation either by inhibition of ROS formation or by binding Fe^{2+} like a chelator [22]. Studies also have shown that taurine offsets lipid peroxidation either through scavenging ROS directly or by binding to ferrous ion or copper ion through its sulphonic acid group [23]. These mechanisms of action of taurine may have contributed to the mitigation of lipid peroxidation in the brain of the restraint-stressed Wistar rats. In the present study, there is no significant ($p > 0.05$) difference in the activity of brain AChE activity in the acute restraint-stressed groups when compared with the control. AChE is a crucial enzyme for cholinergic neurotransmission and it plays an important role in the regulation of numerous vital functions including neurobehavioural processes. It was demonstrated that AChE inhibition induces oxidative stress and this results in neurotoxicity that may be manifested as cognitive impairment in rodents [24]. Taurine has been shown to enhance AChE activity in some studies [25], and this was confirmed in this study.

In the present study there is no significant ($p > 0.05$) difference in the activities of the brain antioxidant enzymes (SOD, CAT and GPx) in the taurine treated restraint-stressed groups as compared with the control group both. Antioxidant enzymes comprise the antioxidant defense system of the body against oxidative stress. SOD catalyzes the conversion of superoxide radicals to H_2O_2 while CAT converts H_2O_2 into H_2O [26]. Besides, GPx converts H_2O_2 into H_2O in the presence of oxidized GSH [27]. It has been shown that taurine exhibits its antioxidant capacity by enhancing the antioxidant system, forming chloramines with hypochlorous acid and replacing glutathione (GSH) in biological systems during oxidative stress [28]. Although taurine is a poor scavenger of ROS, complex

formation between sulphonic acid group (SO_3^-) to free metal ion species such as Fe^{2+} , Cu^+ or oxidant metalloproteins has been reported [29]. It was proposed that taurine attenuated the alterations in the biochemical parameters partly by alleviating oxidative stress, augmenting the activities of the antioxidant enzymes and by exhibiting protective effects on the liver and kidneys [30]. Taurine may also inhibit lipid peroxidation by inducing GPx and SOD. Taurine could protect tissues against reduced glutathione pool depletion by preventing a decrease of glutathione reductase activity. The observed effects of taurine in the present study could be attributed to its ability to resist cell damage in a non-specific way either by membrane stabilization or by osmoregulation. Our result showed that the antioxidant taurine prevented oxidative stress and loss of cellular antioxidants and suggested that taurine protected forebrain from restraint stress-induced oxidative damage.

In serum protein concentration, there is no significant ($p > 0.05$) difference in the taurine treated groups when compared with the control group. It is noteworthy that albumin (an antioxidant) is usually depleted during oxidative stress and its cysteine participates directly in the scavenging of free radicals in biological systems [31]. Taurine slightly increased total protein, albumin, globulin and albumin/globulin concentration in the present study. Studies have shown that taurine protects the immune system from oxidative stress by preventing DNA damage and apoptosis in lymphocytes [32, 33], and this may have contributed to its ability to normalize the total protein, albumin, globulin and albumin/globulin concentration in the restraint-stressed groups. Taurine also exhibits hepatoprotective [34], and nephroprotective [35], effects; these may have contributed to the normalization of the serum protein parameters in the restraint-stressed groups.

The taurine 400 mg/kg showed significant ($p < 0.05$) decrease in AST concentration. There is significant ($p < 0.05$) increase in ALP activity in the group that received taurine 200 mg/kg. It is notable that the hepatoprotective property of taurine is due to its ability to decrease oxidative stress, enhance mitochondrial function and amend cytoplasmic and mitochondrial Ca^{2+} homeostasis in biological systems [36]. The significant decrease in AST may not necessarily be due to liver disease. Changes in AST activity is less specific for liver disease, they may also signal damages to the cardiac and skeletal muscles, RBC, kidney and brain tissue [37].

4.0 Conclusion

The antioxidant taurine also possesses anti-stress activity as; it counteracted the restraint stress-induced lipid peroxidation and maintained the antioxidant defense system. Taurine have alleviated the alterations in the activities of the enzymes (AST, ALT, and ALP) and protein

in plasma of acute restraint-stressed rats. It is concluded that taurine may be a useful agent against biochemical alterations in individuals that are constantly exposed to stress.

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