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TAURINE PREVENTED BIOCHEMICAL ALTERATIONS IN CHRONIC RESTRAINT-STRESSED WISTAR RATs

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Abstract: Stress has the potential of altering physiological homeostasis. The body's ability to cope under stressful stimuli is a vital determinant of health and disease conditions. In this study, Wistar rats were weighed and randomly allocated into four groups (n=6): group A: control (1 ml/kg of distilled water), group B: (100 mg/kg of taurine), group C: (200 mg/kg taurine) and 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 21 days. During the experiment, concentration of malondialdehyde (MDA) and activity of acetylcholinesterase were evaluated in the fore brain homogenate samples. Other biochemical parameters assayed in the serum were the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The result revealed significant ($p < 0.05$) increase in the brain AChE activity in taurine 100 mg/kg when compared to the control group. Also there was significant ($p < 0.05$) increase in total protein in all the taurine treated groups but a significant ($p < 0.05$) decrease in albumin in the group that was administered 100 mg/kg taurine when compared with 200 and 400 mg/kg groups. There was no significant ($p > 0.05$) difference in the serum MDA concentration and activities of SOD, CAT and GPx in all the taurine treated groups when compared with the control group. The results of this study suggested that, taurine has immense potential as an anti-stress agent of great therapeutic relevance.

Keywords: Stress, Taurine, Anti-stress, Malondialdehyde, Antioxidant enzymes, Acetylcholinesterase

Introduction

Restraint stress is a well-known method used by many researchers to induce chronic stress that results to alterations in various behavioral and biochemical parameters (Nade and Yada, 2010). Studies have shown that stress and other stress-related disorders form a significant cause of disease in recent times, contributing to perhaps 75% of all illnesses. Stress has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases ranging from psychiatric disorders such as anxiety and depression, immunosuppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension and ulcerative colitis (Singh and Yadav, 2014). During stress condition, symphatho-adrenal (SA) and Hypothalamo-Pituitary-adrenal (HPA) systems collaborate to maintain internal body homeostasis, resulting in simultaneous increases of catecholamines from the SA system.

An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, is termed 'oxidative stress'. Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. Antioxidant defense involves several strategies, both enzymatic and non-enzymatic (Sies, 1997).

The unregulated oxidative modification of lipids, proteins, and nucleic acids induced by multiple oxidants has been implicated in the pathogenesis of many diseases. Antioxidants with diverse functions exert their roles either directly or indirectly in the physiological defense network to inhibit such deleterious oxidative modification of biological molecules and resulting damage. The efficacy of antioxidants depends on the nature of oxidants. Therefore, it is important to identify the oxidants which are responsible for modification of biological molecules (Niki 2018).

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 (Kumari *et al*., 2013). There is a growing consensus that oxidative stress is linked to mitochondrial dysfunction (Das *et al*., 2012; Perfeito *et al*., 2012), and that the beneficial effects of taurine are as a result of its antioxidant properties (Chen *et al*., 2012; Marcinkiewicz and Kontny, 2012) as well as its ability to improve mitochondrial function by stabilizing the electron-transport chain and the generation of reactive oxygen species (Schaffer *et al*., 2009; Jong *et al*., 2012). The aim of this study was to investigate the effects of taurine administration on some biochemical parameters in Wistar rats subjected to chronic restraint stress.

Materials and Methods

Taurine Preparation

Taurine (TA) (CAS No. 107-35-7; purity ≥ 99%) preparation of analytical grade (100 g - Sigma-Aldrich, USA) was obtained for this study. Taurine was reconstituted as 40 % stock solution in distilled water.

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 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 (Garber *et al*., 2011). Animals were allowed free access to food and water *ad libitum*. All experiments were performed using the same timing on the day of every experiment (from 9:00 a.m. to 4:00 p.m.) during the light period.

Experimental protocol

The Wistar rats were weighed and randomly allocated into four groups (n=6) and treated for 21 consecutive days. 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 group D: received 400 mg/kg taurine. The treatments were administered once daily 60 min. prior to the commencement of the stress sessions by oral gavage for 21 days.

Chronic Restraint Stress Induction in Wistar Rats

Restraint stress was induced according to the method of (Smith *et al*., 2011) as modified by (Ezekiel *et al*., 2015). A perspex restraint cage with dimensions of 14 cm (L) x 5 cm (B) x 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 (group A) were left undisturbed in their home cages but without access to food or water during the same period. The Wistar Rats were exposed to chronic restraint stress, 6 hour daily for 21 days (Moazzam *et al*., 2013) with slight modification by keeping them in a purpose-designed 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, Faculty of Medicine Ahmadu Bello University Zaria, throughout the experimental period between 9 a.m. and 4 p.m.

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 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.

Determination of brain Malondialdehyde (MDA)

The concentration of malondialdehyde (MDA) as an indication of lipid peroxidation was evaluated in the fore brain homogenate samples as previously described by Draper and Hadley (1990). 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 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 × 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 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 using the absorbance coefficient of MDA-TBA complex 1.56 × 105/cm/M and expressed as µmol/L (in the serum) nmol/mg protein (in the brain homogenate).

Evaluation of brain acetylcholinesterase activity

The brain homogenates of the rats were evaluated for acetylcholinesterase activity according to the method of Ellman *et al*. (1961). 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, weight 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 cuvattes containing 2.6 ml phosphate buffer and 5,5'-dithiobis-nitrobenzoic acid (200 µl; final concentration = 0.33 Mm). The contents of the cuvattes 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 µl); 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 formular:

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

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

Assessment of antioxidant enzymes activities 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 (Martin *et al*., 1987). 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 Sizer, 1952). The activity of glutathione peroxidase (GPx) was also evaluated with the NWLSSTM GPx activity assay kit and the procedure was based on the oxidation of reduced GSH to form oxidized GSH (Paglia and Valentine, 1967). The assays were conducted according to the manufacturer's (Northwest Life Science Specialities, LLC, Vancouver, WA 98662) instructions.

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 × 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 (Henry *et al*., 1974), serum albumin concentration was determined as described by (Spencer and Price, 1977). 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 based on the enzymatic hydrolysis method (King and Armstrong 1934). ALP concentration was estimated using the same auto-analyzer as stated.

Statistical analysis

Data were presented as mean ± 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 version 20. Values of $P < 0.05$ were considered significant.

Results and Discussion

As presented in table 1, treatment with 100 mg/kg of taurine significantly ($p < 0.05$) increase AChE activity in the rat brain, however there was no significant ($p > 0.05$) difference in MDA concentration, SOD, CAT and GPx activities of the taurine treated groups when compared to control group (table 1).

Table1: Effects of taurine treatments on malondialdehyde concentration, acetylcholinasterase activity and antioxidant enzymes (SOD and CAT) in the brain of chronic restraint-stressed Wistar rats

Treatments	Control (DW)	**Taurine** (100mg/kg)	**Taurine** (200mg/kg)	**Taurine** (400mg/kg)
MDA (nmol/mg pr)	1.25 ± 0.10^{ns}	1.18 ± 0.09^{ns}	1.13 ± 0.10^{ns}	1.18 ± 0.09^{ns}
AChE (nmol/ml pr)	21.33 ± 0.71^{a}	24.17 1.17^{b}	22.17 ± 0.65^{a}	22.33 ± 1.17^{a}
SOD (IU/L)	2.30 ± 0.13ns	2.33 ± 0.08ns	2.37 ± 0.10ns	2.22 ± 0.05ns
CAT (IU/L)	51.83 ± 0.95ns	52.67 ± 0.99ns	53.33 ± 1.15ns	53.00 ± 0.93ns
GPx (IU/L)	47.00 ± 1.03ns	47.67 ± 0.88ns	48.33 ± 1.31ns	49.83 ± 1.08ns

Note: values are mean ± SEM, n = 6
DW, distil water; MDA, Malondialdehyde; AChE, Acetylcholinesterase; SOD, Superoxide dismutase; CAT, Catalase; and GPx, Glutathione peroxidase a,b = means with different superscript letters are significantly different ($P < 0.05$), ns = no significant

There was also a significant ($p < 0.05$) increase in ALP values in all the taurine treated groups, but a significant ($p < 0.05$) decrease in ALT values in the group that were administered Taurine 400 mg/kg compared with other treated groups and the control group. The AST, there is no significant ($p > 0.05$) difference in the taurine treated groups when compared to control group (table 2).

Table 2: Effects of taurine treatments on activities of serum enzymes in chronic restraint-stressed Wistar rats

Treatments	Control (DW)	**Taurine** (100mg/kg)	**Taurine** (200mg/kg)	**Taurine** (400mg/kg)
AST (IU/L)	42.50 ± 0.99^{ns}	42.33 ± 1.33^{ns}	41.17 ± 1.40^{ns}	41.17 ± 1.19^{ns}
ALT (IU/L)	46.67 ± 1.17^{ns}	47.17 ± 1.64^{ns}	45.17 ± 1.30^{ns}	44.83 ± 1.49^{ns}
ALP (IU/L)	65.00 ± 1.41^{a}	71.00 ± 1.37^{b}	71.50 ± 3.51^{b}	71.50 ± 1.52^{b}

Note: values are mean ± SEM, n = 6
DW, distilled water; AST, Aspartate aminotransferase); ALT, Alanine aminotransferase; and ALP, Alkaline phosphatase a, b = means with different superscript letters are significantly different ($P < 0.05$), ns = no significant

Continuous treatment with taurine (200 and 400 mg/kg) shows a significant ($P < 0.05$) increase in the TP and ALB values when compared with the taurine 100 mg/kg and the control group. However, no significant ($p > 0.05$) difference in the GLB and ALB/GLB ration of taurine treated groups when compared with control groups (table 3).

Table 3: Effects of taurine on serum protein concentration in chronic restraint-stressed Wistar rats

Treatments	**Control (DW)**	**Taurine (100mg/kg)**	**Taurine (200mg/kg)**	**Taurine (400mg/kg)**
TP (g/dL)	63.50 ± 1.11^{b}	62.17 ± 1.14^{b}	66.00 ± 1.57^{a}	66.33 ± 1.36^{a}
ALB (g/dL)	35.50 ± 1.43^{b}	33.50 ± 0.85^{b}	37.83 ± 1.76^{a}	37.00 ± 2.07^{a}
GLB (g/dL)	28.00 ± 0.32^{ns}	28.67 ± 0.29^{ns}	28.17 ± 0.1	29.33 ± 0.7^{ns}
A/G	1.26 ± 0.46^{ns}	1.16 ± 0.29^{ns}	1.34 ± 0.92^{n}	1.26 ± 0.29^{ns}

Note: values are mean ± SEM, n = 6
DW, distilled water; TP, Total protein; ALB, Albumin; GLB, Globulin; A/G, Albumin: Globulin ratio a,b = means with different superscript letters are significantly different ($P < 0.05$), ns = no significant

Discussion

Oxidative stress occurs when there is imbalance between the antioxidant defence and the production of ROS culminates in oxidative damage to biomolecules such as DNA, lipid and proteins (Halliwell and Gutteridge, 2007). This present study investigated the impact of Taurine in prevented biochemical alterations in chronic restraint-stressed Wistar rats. The results from this study showed that there were no significant ($p > 0.05$) difference in the serum MDA concentration in the chronic restraint-stressed Wistar rats in all the taurine treated groups when compared with the control group. One of the major oxidation products of peroxidazed polyunsaturated fatty acids is MDA. Increase in MDA content is an indicator of oxidative stress and lipid peroxidation (Demir *et al*., 2011). 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 (Goel *et al*., 2005). Administration of taurine may have attenuated tissue lipid peroxidation either by inhibition of ROS formation or by binding Fe^{2+} like a chelator (Wu *et al*., 1999). 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 (Franconi *et al*., 2004). These mechanisms of action of TA may have contributed to the mitigation of lipid peroxidation in the brain of the restraint-stressed Wistar rats. The brain AchE activity showed significant ($p < 0.05$) increase in the taurine 100 mg/kg group compared to other taurine groups and the control group. 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 (Kazi and Oommen, 2012). Taurine has been shown to enhance AChE activity in some studies (Das et al., 2009; Akande *et al*., 2014b) and this was confirmed in this study especially in the group that received taurine100 mg/kg. The results of the current study did not show any 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. 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 (El-Demerdash 2011). Besides, GPx converts H_2O_2 into H_2O in the presence of oxidized GSH (Kanbur *et al*., 2009). 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 (Devi and Anuradha, 2010). 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 (Trachtman *et al*., 1992). 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 (Akande *et al*., 2014a). 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 restrain stress-induce oxidative damage. There was significant ($p < 0.05$) increase in total protein and albumin concentrations in the group that received taurine 200 and 400 mg/kg 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 (El-Nekeety *et al*., 2009). Taurine significantly increased total protein and albumin concentrations. There was no significant ($p < 0.05$) difference in globulin concentration and albumin/globulin ratio in the present study. Studies have shown that taurine protects the immune system from oxidative stress by preventing DNA damage and apoptosis in lymphocytes (Schuller-Levis and Park, 2004; Sokól *et al*., 2009), 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 (El-Sayed *et al*., 2011) and nephroprotective (Das and Sil, 2012) effects; these may have contributed to the normalization of the serum protein parameters in the restraint-stressed groups. Taurine alleviated the alterations in the activities of the serum liver enzymes (AST, ALT and ALP) in chronic restraint-stressed groups. While the ALT activity revealed significant decrease (400 mg/kg) of taurine, there is no significant difference in the AST activity. However the ALP showed significant increase in all the taurine treated groups when compared to the control group. 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 (Asha and Devadasan, 2013). The differences in the results were not well understood. However, it may be due to differences in duration of exposure to the stress. 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 (Rochling 2001).

Conclusion

The antioxidant taurine also possesses anti-stress activity as; taurine counteracted the restraint stress-induced lipid peroxidation and maintained the antioxidant defense system. Taurine also alleviated the alterations in the activities of liver enzymes and protein in serum of chronic restraint-stressed Wistar rats. This study therefore indicates the beneficial role of taurine for the treatment of restraint stress-induced disorders.

Conflict of Interests: The authors declare that they have no conflict of interests.

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