



DOSE-DEPENDENT EFFECTS OF TAURINE IN ACUTE RESTRAINT STRESS-INDUCED HAEMATOLOGICAL ALTERATIONS IN WISTAR RATS



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Abstract: The aim of this study was to investigate the effects of taurine administration (100, 200 and 400 mg/kg) on haematological parameters in Wistar rats subjected to acute restraint stress (ARS) (1 hr/day for 14 days). Thirty adult Wistar rats were divided into five groups of six rats each; unstressed control group (USC) that received distilled water 1 ml/kg, restraint stressed control group (RSC) that received distilled water 1 ml/kg, restraint stressed group (RS) that received taurine 100 mg/kg, restraint stressed group (RS) that received taurine 200 mg/kg and restraint stressed group (RS) that received taurine 400 mg/kg. Taurine was administered once daily by oral gavage. At the end of the 14 days, the rats were sacrificed and blood sample was collected via intra-cardiac route; 2.5 ml blood was taken from each animal for blood analysis. Total white blood count (WBC), differential white blood cell count, platelet count, Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), erythrocyte indices, and erythrocyte osmotic fragility (EOF) test were determined. Results showed that, platelets count significantly ($P < 0.05$) increased at taurine 200 mg/kg. There was significant ($P < 0.05$) increase in WBC and neutrophil counts at taurine 400 mg/kg. However there was significant ($P < 0.05$) decreases in lymphocyte count in the taurine treated groups. The neutrophil/lymphocyte ratio showed no significant ($P > 0.05$) difference in the groups. In conclusion taurine at doses of 200 and 400 mg/kg significantly increased platelets and WBC counts respectively in restraint-stressed Wistar rats.

Keywords: Lymphocyte, neutrophil, platelets, restraint, stress, taurine.

Introduction

Taurine, also known as 2-amino ethanesulfonic acid is a conditionally essential β amino acid that is not utilized in protein synthesis. Taurine is found in abundant in mammalian tissues and is one of the three well-known sulfur-containing amino acids (Sirdah 2015). Taurine was identified almost two centuries ago and was named after the ox, *Bos taurus*, because it was first isolated from the bile of an ox. It was discovered that in the human body, taurine is distributed with high concentration in various tissues that are excitable or prone to generate free radicals, these includes: white blood cells, platelets, spleen, and liver (Nguyen *et al.*, 2013). Large numbers of taurine analogues have also provided protection against erythrocyte membrane damage induced by hydrogen peroxide, which effects may suppress inflammatory responses (Pokharel *et al.*, 2000). The high levels of taurine in phagocytes and its accumulation in inflammatory lesions suggests its role in innate immunity (Schuller-Levis and Park, 2004). It is commonly accepted that taurine plays an important role in the immune system as an antioxidant to protect cells, including leukocytes, from oxidative stress (Wang *et al.*, 2009; Garber *et al.*, 2011).

Stress has become an integral part of human life and organisms are constantly subjected to stressful stimuli that affect numerous physiological processes (Bhatia *et al.*, 2011). Emotional or physical stress can cause elevated white blood cell counts. Stress has been described as circumstances where coping with a variety of actual or perceived stimuli alters the homeostatic state of an organism, including haematological parameters. Previous work demonstrates hematopoietic changes in rodents exposed to physical and psychosocial stressors (Kiank *et al.*, 2006; Dygai and Skurikhin, 2011). This study was,

therefore, aimed at investigating the effects of three different doses of taurine on haematological parameters of Wistar rats subjected to acute restraint stress.

Materials and Methods

Taurine preparation

Taurine (TAU) (CAS No. 107-35-7; purity $\geq 99\%$) preparation of analytical grade (100 g - Sigma-Aldrich, USA) was used in this study.

Experimental animals

A total of thirty adult male 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 natural 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 (Smith, 2012). Animals were allowed free access to food and water *ad libitum*.

Experimental protocol

Wistar rats were weighed and randomly allocated into five groups, with six animals in each group.

Group A- unstressed control (USC) received 1 ml/kg of distilled water per day.

Group B- restraint-stressed control (RSC) received 1 ml/kg of distilled water per day.

Group C- restraint-stressed group (RS) received taurine 100 mg/kg per day.

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Group D- restraint-stressed group (RS) received taurine 200 mg/kg per day and

Group E- restraint-stressed group (RS) received taurine 400 mg/kg per day.

The treatments were administered once daily 60 min prior to the commencement of the stress sessions via oral gavage for 14 days.

Experimental Design

Acute restraint stress (RS) induction in wistar rats

Restraint stress was induced according to the method of (Moazzam *et al.*, 2013) with some modification. 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 control group (URC) were left undisturbed in their home cages but without access to food or water during the same period. Wistar rats were exposed to acute restraint stress, 1 h daily for 14 days (Proctor *et al.*, 2011) 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. 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. local time.

Evaluation of haematological parameters

Intra-cardiac sampling was done after 14 days exposure to acute restraint stress. Rats were placed in a closed chamber containing ether-soaked cotton. It took 3 - 5 minutes to get the rats anaesthetized. Two and half milliliters of blood were drawn with the help of 5 ml syringe, by intra-cardiac puncture into a sample bottles containing ethylenediaminetetraacetic acid (EDTA) (PLASTI Lab Sarl, Beirut, Lebanon). The haematological parameters evaluated include: Total white blood cell count (WBC), differential white blood cell count, total platelet count, total red blood cell count (RBC), erythrocyte osmotic fragility (EOF) other indices includes: Haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The evaluation was conducted with the use of an automatic haematological assay analyzer, Adva 60[®] hematology system (Bayer Diagnostic Europe Ltd, Dulbin, Ireland). The neutrophil/lymphocyte ratio was calculated according to (Faulkner and King, 1970).

Erythrocyte osmotic fragility

This was determined as described by (Novozhilov *et al.*, 2013). Sodium chloride solution (PH 7.4) was prepared at

varying concentrations, 0.0%, 0.1%, 0.3%, 0.5%, 0.7% and 0.9%. Five millilitres (5 ml) of each NaCl concentration was placed in labeled test tubes serially in a rack. Exactly 0.02 ml of the blood sample was pipette into each of the test tubes. The content of the tubes was gently mixed by inverting the tubes and allowing them to stand at room temperature (24-26°C) for 30 minutes; thereafter, the tubes were centrifuged at 1500 x g for 5 min using a centrifuge model IEC HN- SII cDamon/IEC Division, UK). The supernatant obtained from each tube was transferred to a clean glass cuvette and the absorbance of the supernatant was measured spectrophotometrically with spetronic 20 (Bausoh and Lomb, USA) at a wavelength of 540 nm. The percentage haemolysis for each sample was calculated using the following formular:

$$\text{Percentage haemolysis} = \frac{\text{Optical density of test solution}}{\text{Optical density of standard solution distilled water}} \times 100$$

Statistical analysis

All data were expressed as Mean \pm SEM and was analyzed by SPSS software (version 20) using one way analysis of variance (ANOVA) with multiple comparisons. Tukey's post-hoc test was used to determine difference between groups. Values of P < 0.05 were considered as statistically significant.

Results and Discussion

There was significant (P < 0.05) increase in the PLT counts in the RS group that received 200 mg/kg taurine when compared with the other groups, however the RS group that received 400 mg/kg taurine showed significant (P < 0.05) decrease when compared with other taurine treated groups and the USC group.

There was significant (P < 0.05) increase in WBC count in RS groups that received taurine 200 and 400 mg/kg compared to RS control group that did not receive taurine. There was significant (P < 0.05) decrease in neutrophils in the RSC and RS + Tau 100 mg/kg groups when compared with RS + Tau 200 and 400 mg/kg. However there was significant (P < 0.05) increase in lymphocyte count in the RSC group when compared with the all the RS + Tau groups. The N/L ratio did not show any difference at any level (Table 1). There was significant (P < 0.05) increase in RBC count in the RSC and RS + Tau 100 mg/kg groups when compared with RS + Tau 200 and 400 mg/kg groups respectively. There was significant (P < 0.05) increase PCV in the RSC when compared with the RS + Tau 200 and 400 mg/kg groups. There was no significant (P > 0.05) difference in HB, MCV, MCH and MCHC in the taurine treated groups when compared with the control group (Table 2).

Table 1: Effect of taurine on platelets and white blood cell indices in acute restraint stress wistar rats

Treatments	USC	RSC	RS + Tau 100 mg/kg	RS + Tau 200 mg/kg	RS + Tau 400 mg/kg
PTL (x10 ⁹ /L)	456.33 \pm 82.40 ^b	405.45 \pm 34.00 ^a	459.17 \pm 22.22 ^b	512.83 \pm 6.00 ^c	403.42 \pm 87.90 ^a
WBC (x10 ⁹ /L)	13.80 \pm 1.45 ^b	10.33 \pm 1.48 ^a	11.15 \pm 1.59 ^a	13.75 \pm 1.33 ^b	14.13 \pm 1.28 ^c
Neutrophil (%)	26.59 \pm 0.40 ^b	21.48 \pm 0.33 ^a	21.67 \pm 0.25 ^a	23.00 \pm 0.39 ^b	24.39 \pm 0.14 ^b
Lymphocyte (%)	70.14 \pm 1.23 ^b	77.18 \pm 0.23 ^a	71.12 \pm 1.06 ^b	72.18 \pm 1.15 ^b	72.22 \pm 0.85 ^b
N/L ratio	0.38 \pm 0.33	0.35 \pm 0.43	0.30 \pm 0.24	0.31 \pm 0.02	0.33 \pm 0.16

USC (unstressed control); RSC (restraint stress control); RS (restraint stress); Tau (taurine); PTL (Platelet); WBC (White blood cell); N/L (neutrophil-lymphocyte ratio); ^{a,b,c} = Values with different superscript letters are significantly (P < 0.05) different, while those with the same superscript letters are not significant (P > 0.05).

Table 2: Effect of taurine on erythrocyte indices in acute restraint stress wistar rats

Treatments	USC	RSC	RS + Tau 100 mg/kg	RS + Tau 200 mg/kg	RS + Tau 400 mg/kg
RBC (x10 ¹² /L)	6.19±0.40 ^a	7.40±0.34 ^b	7.07±0.18 ^b	6.90±0.15 ^c	6.87±0.15 ^c
Hb (g/dL)	12.13±0.82	14.28±0.15	13.40±0.54	13.23±0.27	13.03±0.24
PCV (%)	33.73±2.09 ^a	40.22±0.67 ^b	39.12±0.97 ^b	37.17±0.89 ^c	36.73±0.97 ^c
MCV (fl/cell)	54.70±0.75	56.65±0.92	55.45±1.02	53.90±0.11	53.53±0.68
MCH (Pg)	19.57±0.19	18.79±0.56	18.87±0.40	19.13±0.10	18.95±0.16
MCHC (g/dL)	35.87±0.75	34.32±0.54	34.13±0.79	35.57±0.21	35.58±0.30

USC (unstressed control), RSC (restraint stress control), RS (restraint stress) Tau (taurine), RBC (red blood cell), Hb (haemoglobin), PCV (packed cell volume), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration) ^{a,b,c} = Values with different superscript letters are significantly (P < 0.05) different, while those with the same superscript letters are not significant (P > 0.05).

The restraint-stressed group that received no intervention, showed increased haemolysis. The haemolysis appears to be faster in the restraint-stressed group that received no any intervention when compared with the control and the treatment groups. The red blood cells were hemolyzed at 0.5% saline and were completely broken down at 0.35% saline. However, there was no significant difference in haemolysis when the treated groups were compared with the control group (Fig. 1).

The result of the present study revealed significant (P < 0.05) increase in platelet counts in group that received taurine 200 mg/kg. Though there was decrease in the platelet counts of the taurine 400 mg/kg but this was not significant (P > 0.05) when compared with the control group. The significant increase in the platelet count in the taurine 200 mg/kg could be due to the ability of taurine to protect platelet from oxidative damage which could be through the reduction of lipid peroxidation within the platelets membrane (Akande *et al.*, 2014). The significant increase in WBC at dose of 200 and 400 mg/kg taurine compared to 100 mg/kg taurine group might be considered as a defensive mechanism by the immune system. This finding corroborates with other work who reported that when an antigen is introduced into an organism, antibodies are produced in response to the antigen. Taurine may have protected the WBC's at 200 and 400 mg/kg through its antioxidant properties (Patrick-Iwuanyanwu *et al.*, 2007).

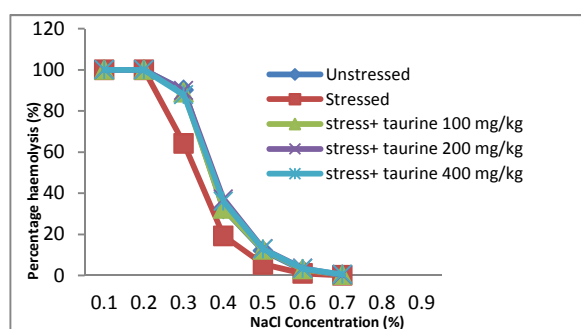


Fig. 1: Effect of taurine on erythrocyte osmotic fragility (EOF) of Wistar rats subjected to acute restraint stress

The result showed significant (P < 0.05) decreases in the neutrophils and lymphocytes counts in the groups that received taurine 100 mg/kg, however, the 200 and 400 mg/kg taurine showed relative increase in the values of neutrophils and lymphocyte counts though not significant (P > 0.05) when compared to the control group. Increase in neutrophils and lymphocyte counts in the taurine 400 mg/kg group represent the typical development of the stress reaction (Redmond *et al.*, 1998). It was reported that taurine had a protective effect on human lymphocytes.

Further studies showed that exogenous taurine maintained human lymphocytes viability through its membrane stabilizing capacity (Frick *et al.*, 2009). Circulating neutrophils are committed to apoptosis by reactive oxygen species which is produced by activated cells. It is recognized that taurine could protect neutrophils from apoptosis by its antioxidant activity. Previous studies have demonstrated that physical restraint decreases peripheral blood lymphocyte and mitogen-induced proliferation in rats and produces changes in T cell functional capacity (Takagi *et al.*, 2011).

Neutrophils/lymphocyte (N/L) ratio showed significant (P < 0.05) decrease in the group that received taurine 200 mg/kg compared with the control. Neutrophil/lymphocyte ratio has recently been reported as a predictor of hepatocellular carcinoma (Xiao *et al.*, 2014). It is also known that N/L ratio provides an indication of the inflammatory status in patients and it can be used to predict outcome of diseases (Halazun *et al.*, 2008). It is known that taurine protected the leukocytes from destruction by inhibiting lipid peroxidation and neutrophil activation (Kim *et al.*, 1996). The increase in neutrophil/lymphocyte ratio observed in the 400 mg/kg in the present study is in agreement with other findings (Minka and Ayo, 2007; Minka *et al.*, 2009) that the ratio increased in stress situations, especially in those induced via free-radical mechanism. Thus, the administration of taurine decreased the ratio in the present study at taurine 100 mg/kg. We may therefore postulate that taurine ameliorates the risk of adverse effects due to free-radical induced cell damages and destruction in Wistar rats.

The RBC's counts showed no significant (P > 0.05) difference when the treated groups were compared to the control group, though the group of taurine 100 mg/kg showed slight elevated erythrocytes count as compared to the control group. In a different study, they found that immobilization-induced appearance of stored erythrocytes and reticulocytes in circulation and they suggested that immobilization-induced stress leads to release of stored erythrocytes from the spleen depot. Taurine attenuates the anemic response produced by restraint stress in the rats; this may be due to its ability to improve the erythrocyte membrane integrity by mitigation of oxidative damage to the erythrocyte membrane.

The erythrocyte osmotic fragility did not show any significant (P > 0.05) difference when the taurine treated groups were compared with the control group. Studies have shown that oxidative stress increases haemolysis (Bernabucci *et al.*, 2002). In a different study, it was discovered that taurine can suppress hemolysis through osmoregulation and biomembrane stabilization (Ayo *et al.*, 2014). Frequent exposure to ROS can cause erythrocytes

to be sensitive to redox imbalance which may alter its mechanical properties thereby resulting into physiological and pathological oxidative stress (Pendav and Rizvi, 2009; Anand *et al.*, 2010). The current study may suggest that, taurine prevented erythrocyte osmotic fragility in the treated groups through its action as antioxidant. We therefore hypothesized that taurine has improved the erythrocyte parameters in the restraint-stressed groups using this mechanism. Taurine improved PCV, and Hb concentration in the restraint stress groups. This result agrees with the findings of (Akanke *et al.*, 2014) who reported that taurine augmented PCV, Hb concentration and RBC counts in Wistar rats.

Conclusion

Taurine prevented blood cells from destruction in acute restraint-stressed Wistar rats at taurine 200 and 400 mg/kg. It also improved PCV and Hb concentration in the restraint stress groups. We therefore concluded that taurine may be a useful agent against haematological alterations in individuals that are constantly exposed to stress.

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