EFFECTS OF TAURINE ON BEHAVIOURAL, HAEMATOLOGICAL AND BIOCHEMICAL RESPONSES OF MICE AND WISTAR RATS TO RESTRAINT STRESS

By

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN HUMAN PHYSIOLOGY

DEPARTMENT OF HUMAN PHYSIOLOGY, FACULTY OF MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA

NOVEMBER, 2015

DECLARATION

I declare that the work in the thesis, entitled 'Effects of Taurine on Behavioural Haemetological and Biochemical Responses of Mice and Wistar Rats to Restraint Stress' has been performed by me in the Department of Human Physiology, Ahmadu Bello University, Zaria under the supervision of Professor J. O. Ayo, Dr. R. A. Magaji and Dr. Yusuf Tanko. The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other University.

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CERTIFICATION

This thesis, entitled 'Effects of taurine on behavioural, haematological and biochemical responses of mice and Wistar rats to restraint stress' by Ezekiel Iliya meets the regulations governing the award of the degree of Doctor of Philosophy (PhD) of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty, my help in ages past; and also to my loving wife and our daughter, Sophie. I love you all.

ACKNOWLEDGEMENTS

My deepest appreciation goes to my creator, the King of kings, the Almighty God and my help in ages past. I am really grateful to my supervisors, Prof. J.O. Ayo, Dr. R. A. Magaji and Dr. Y. Tanko, for their patience and their willingness to teach and guide me in the research. They reasoned with me, gave positive suggestions, criticized and corrected the work in spite of their tight schedules. I sincerely do appreciate my parents, Mr. and Mrs. Iliya Sanda Kwanchi, who have been by my side from the beginning to the end, sharing my triumphs and sorrows. They encouraged and supported me in every step I took. I remain grateful to you all. My unreserved thanks goes to Dr. Aliyu Mohammed, Head, Department of Human Physiology, Ahmadu Bello University, Zaria, and Dr. Agere Hemen, Head, Department of Biological Sciences, Federal University, Wukari, Taraba State, for their unswerving support and help.

My warmest regards goes to my colleagues; Goji, Kabiru, Sherif and Jumo for their unalloyed love and support. I do remember Dr. J. A Tende and Salamatu Garba for their support; and to the members of the Department of Human Physiology, the academic and administrative staff and the laboratory staff, you all constitute an enviable family, I thank you all. I thank all my brothers, Ayuba Iliya Kwanchi, Enoch Iliya Kwanchi, Demas Iliya Kwanchi, Gappeng Daniel, Habu Gambo, Gelle Daniel, John Kurma, Micah Amos, Ephraim Saidu and my sisters Mary Iliya Kwanchi, Gloria Iliya Kwanchi, Helen Iliya Kwanchi, Esther Iliya Kwanchi, Kande Iliya kwanchi, Juliana Iliya Kwanchi, Victoria Yohanna and Niniko Yohanna. Special thanks to my uncles, Yohanna Ishaku, Isa Sanda, Daniel Yusuf, Babangida Hassan. To Wilfred Habibu, Ahmed Ishaku, Obadiah Hassan,

Aminu Abba, Mr and Mrs. Habila Mamman and George Kofi; I say thank you all for showing me what true love is. My special thanks also goes to the following special friends, who have helped in making me who I am today: Umar Ben, Samuel Ahmed Ben, Sabo Yakubu, Uwa Ahmed Ben, Sani Ahmed Ben, Kabiru Mohammed Danlami, Aliyu Yusuf, Dr. Samuel Alhassan, Dr. Natsah Joseph, Elizebeth Nyam, Dr. Kuma Yandev, Patrick Ibrahim Iliyasu, Pastor Gabriel, Peter Jiya, Shedrack Kolo, Pharm. Mponan Guga Dung, Joy Michael, Comfort Dangana, Patience Samuel Nyam, Mr and Mrs Alexander Sindama, Mr and Mrs Emmanuel Okrikata. I will not fail to appreciate special people like Hon. Abba Akawu Zaggah, Hajiya Fati Kafinta, Mr and Mrs Ayuba Kwagiri, for their encouragement and love; I say thank you.

ABSTRACT

The aim of this study was to investigate the effects of taurine administration (100, 200 and 400 mg/kg) on behavioural, haematological and biochemical responses of mice and Wistar rats subjected to acute restraint stress (1 hour/day for 14 days) and chronic restraint stress (6 hour/day for 21 days). The treatments were administered once daily by oral gavage. Forty eight **(**48) Wistar rats, divided into four groups of six rats each and 48 mice divided into four groups of six mice each were used for this study. Learning and memory in both the acute and chronic restraint-stressed animals was assessed using step-down passive-avoidance test in Wistar rats and elevated plus-maze memory for mice. Exploratory activity in mice was accessed using hole-board method while motor coordination was accessed using beam-walk assay. Total and differential leucocyte counts, red blood cell count (RBC), platelet count (PLT), packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration and osmotic fragility (EOF) test were evaluated using automatic haematological assay analyzer. Serum total protein and albumin concentration was estimated using Bio-Rad protein assay kit based on the principle of Bradford. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by the method of Reitman and Frankel. Serum alkaline phosphate activity (ALP) was assayed as described by Bassey. The concentration of malondialdehyde was evaluated in the fore brain homogenate samples using the method of Draper and Hadley. The brain acetylcholinesterase (AChE) activity was conducted as decribed by Ellman. Activity of superoxide dismutase in the rat brain was assessed using the NWLSSTM SOD activity assay kit. Catalase activity was

analyzed using the NWLSSTM CAT activity assay kit. Glutathione peroxidase activity was assessed using NWLSS^{TM} cGPx (Gpx1) Enzyme-linked immunosorbent assay (ELISA) assay kit. The results showed that taurine improved learning and memory in both acute and chronic restraint-stressed Wistar rats that received 200 and 400 mg/kg taurine, in acute (216.67 ± 8.73s and 228.50 ± 9.98s) and chronic (230.67 ± 10.72s and 223.17 ± 7.84s). Elevated plus-maze showed decreased acquisition time (34.67±4.52s) and short retention time (19.50±7.29s) in group that received 400 mg/kg taurine in the acute restraint-stressed group. Short acquisition (47.50±12.50s) and retention (21.83±15.28s) time were observed in group that received 400 mg/kg taurine in chronic restraint-stressed groups. Exploratory activity in mice was increased in both acute (22.00 ± 1.24s) and chronic (18.17 ± 1.49s) restraint-stressed group that received 200 mg/kg taurine. The acute restraint-stressed group showed significant decrease in white blood cell count (11.15 ± 1.59 x10^9/L), neutrophil (17.67 ± 0.25 %) and lymphocyte (58.12 ± 1.06 %) counts at 100 mg/kg taurine. The neutrophil/lymphocyte ratio (N/L) showed increase (0.35 ± 0.69) group that received 400 mg/kg taurine in chronic restraint-stressed group. There is significant increase in PLT in the acute group that received 200 mg/kg taurine (512.83 ± 6.00 x10^9/L) and significant increase in the chronic group that received 100 mg/kg taurine (568.67 ± 92.86 x10^9/L). There is significant decrease (5.65 ± 0.72 x10^{12}/L) in RBC count at 100 mg/kg taurine in the chronic restraint-stressed group. 400 mg/kg taurine showed significant decreased in MCV (51.85 ± 0.85 fl) and MCH (17.72 ± 0.17 pg) in the chronic restraint-stressed group. There was no significant difference in the EOF test in both the acute and chronic restraint-stressed groups. AChE activity was increased (24.17 ± 0.75 nmol/mg) in the acute group 200 mg/kg taurine and in chronic

group 100 mg/kg (24.17 ± 1.17 nmol/mg). 400 mg/kg taurine showed significant increase in the activities of brain antioxidant enzymes; CAT (53.00 ± 0.93 IU/L) and GP_x (49.83 ± 1.08 IU/L) in the chronic group compared with the acute group. Also 400 mg/kg taurine in the chronic group alleviated the alterations in the activities of AST (41.17±1.19 IU/L), ALT (44.83 ± 1.49 IU/L) and ALP (71.50 ± 1.52 IU/L). There was significant increase in total protein (66.33 ± 1.36 g/dL) and albumin (37.00 ± 2.07 g/dL) in the group that received 400 mg/kg taurine in chronic restraint-stressed group. In conclusion taurine improved memory and learning and it counteracted the restraint stress-induced lipid peroxidation and maintained the antioxidant defense system at near normal. Taurine might be used as an acute or chronic anti-stress agent since it possesses antioxidant property.

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LIST OF ABBREVIATIONS

AChE	=	Acetylcholinesterase
ACTH	=	Adrenocorticotropic Hormone
ALB	=	Albumin
ALP	=	Alkaline Phosphatase
ALT	=	Alanine Aminitransferase
AST	=	Aspartate Aminotransferase;
CAT	=	Catalase
CBT	=	Core Body Temperature
CNS	=	Central Nervous System
CRF	=	Corticotropin Releasing Factor
CRH	=	Corticotrophin- Releasing Hormone
DSB	=	Duration of Stay on Bench
DW	=	Distilled Water
GABA	=	Gamma Aminobutric Acid
GAD	=	Glutamate Decarboxylase
GPx	=	Glutathione Peroxidase
Hb	=	Haemoglobin
HPA	=	Hypothalamo-Pituitary-Adrenal
MCH	=	Mean Corpuscular Haemoglobin
MCHC	=	Mean Corpuscular Haemoglobin Concentration
MCV	=	Mean Corpuscular Volume
MDA	=	Malondialdehyde

N/L	=	Neutrophil/Lymphocyte
NMDA	=	N-methyl-D-Aspartate
NS	=	Number of Shock
PCV	=	Pack Cell Volume
PLT	=	Platelet
RAS	=	Renin-Angiotensin System
RBC	=	Red Blood Cell
ROS	=	Reactive Oxygen Species
SA	=	Symphatho-Adrenal
SDL	=	Step-Down Latency
SOD	=	Superoxide Dismutase
TAG	=	Taurine Antagonist
TAUTs	=	Taurine Transporters
TP	=	Total Protein
tRNA	=	Transfer RNA
WBC	=	White Blood Cell

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

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. It plays several crucial roles in the body, including modulation of calcium signalling, osmoregulation, and membrane stabilisation (Kumari *et al*., 2013). 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. After its discovery, taurine was considered non-essential and biologically inert; however, its numerous functions have now been identified. In the human body, taurine is distributed with high concentration in various cells and tissues that are excitable or prone to generating free radicals, including retina, white blood cells, platelets, central nervous system (CNS), heart, skeletal muscles, spleen, and liver (Nguyen *et al*., 2013). High levels of taurine are found also in the cerebral cortex, basal ganglia, hippocampus, hypothalamus, cerebellum and the caudate-putamen (El-Idrissi, 2006). The phylogenetically oldest and best documented function of taurine is in its conjugation with bile acids in bile salt synthesis (Jacobsen, 1968). Taurine is present in diets, especially in meat and seafood, and it is widely used as an ingredient in some nutraceuticals and energy drinks (Dombovy-Johnson, 2012).

High levels of taurine in the brain have been observed to protect brain tissues from cerebral ischaemia. Taurine has been reported to have neuroprotective properties

(Wu and Prentice, 2010). It increases acetylcholine concentration in the brain of animals; and in patients with Alzheimer's disease taurine concentration decreases (Birdsall, 1998). There are suggestions which support that taurine affects brain chemicals and also improves cognitive function (Irfan *et al*., 2013). Previous studies reported that its administration suppresses and delays the ability of learning and memory ability in rats (Ito *et al*., 2012), while other studies reported no effect on learning and memory following taurine administration (Ito *et al*., 2009). Taurine improves memory in rats exposed to ozone 839, but fails to do so in healthy untreated mice. However, taurine administration is effective in attenuating the amnesia produced by ethanol, pentobarbital, cycloheximide and sodium nitrite (Vohra and Hui, 2007). A taurine-containing drink has been reported to improve performance in endurance athletes. Taurine and caffeine-containing drinks stimulate cognitive performance and well-being (Dombovy-Johnson, 2012). A drink containing taurine, caffeine and vitamins improves aerobic endurance, anaerobic and mental performance, concentration and memory (Alford *et al*., 2001). Caffeinated taurine beverage improves attention and verbal reasoning, but does not affect memory. It is not clear, however, whether caffeine alone underlies the improvements or whether they are also due to taurine (Dombovy-Johnson, 2012).

A large number of taurine analogues protect the erythrocyte membrane againt damage induced by hydrogen peroxide; the effects of which may suppress inflammatory responses. The high levels of taurine in phagocytes and its accumulation in inflammatory lesions suggests its role in innate immunity (Schuller-Levis and Park, 2004). As taurine is present at high concentrations in leucocytes, it may be hypothesized that taurine

deficiency affects immune functions. However, there is no clear evidence on the association between taurine deficiency and immune system deficiency in humans. On the other hand, it is commonly accepted that taurine plays an important role in the immune system as an antioxidant to protect cells, including leucocytes, from oxidative stress (Schaffer *et al*., 2009; Wang *et al*., 2009). Oxidative stress plays an important role in memory deterioration and taurine has been found to protect the neurones from the deterioration. Since taurine has an antioxidant property, it has protective functions such as scavenging reactive oxygen species (ROS), attenuating lipid peroxidation and, thus, involved in membrane stabilisation (Wu *et al*., 1999). An increase in glutamic acid decarboxylase expression, increased levels of neurotransmitters such as Gamma Aminobutric Acid (GABA), glutamate and a rise in levels of somatostatin and neurons, which are somatostatin-positive, have been reported to be induced by taurine supplementation (El-Idrissi, 2006).

Stress is one of the major contributory factors that stimulate numerous intracellular pathways; thus leading to increased ROS generation causing oxidative damage (Matsumoto *et al*., 1999). The ROS are highly reactive moieties, playing an important role in health and disease. The brain is especially vulnerable to ROS-induced damage because of its high oxygen consumption, abundant lipid content and low levels of enzymatic and non-enzymatic antioxidants (Liu *et al*., 1994). In stress, symphatho-adrenal (SA) and hypothalamo-pituitary-adrenal (HPA) systems collaborate in maintaining the body homeostasis, resulting in simultaneous increases in 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 (Harris *et al*., 2004), initiating the deleterious effects of stress. Since restraint is a relatively easy method of stress induction, researchers have used restraint in order to investigate the effects of stress on learning and memory (Ito *et al*., 2012; Irfan *et al*., 2013).

1.2 Statement of Research Problem

Studies have shown that restraint stress results in the imbalance of antioxidant status, which ultimately leads to increased oxidative stress and consequently, oxidative damage (Radak *et al*., 2001). The overproduction of ROS and the body's inability to stem the accumulation of highly reactive free radicals have been implicated in cardiovascular disease (Chen *et al.*, 2012), diabetes-induced renal injury (Das *et al.*, 2012), inflammatory disease (Marcinkiewicz and Kontny, 2012), light-induced lipid peroxidation in photoreceptors, ischaemia-reperfusion injury and many major CNS disorders (Crompton and Andreeva 1993). Thus, taurine may play an important role in ameliorating behavioural, biochemical and haematological responses of the body to restraint stress by scavenging the increasesd ROS produced during the restraint.

1.3 Justification of the Study

Taurine has many physiological functions, including antioxidation, conjugation of bile acids, osmoregulation, stabilisation of membrane and calcium signalling modulation. It has many different effects, possibly because of its interaction at the GABA, glycine and

"unknown" taurine receptors (El-Idrissi *et al*., 2006). The best known role of this amino acid is its involvement in the synthesis of taurine bile acid conjugates (Clauson *et al*., 2008). However, its function in improving activity level and mental performance has been controversial. Taurine is involved in physiological mechanisms such as chemo-protection, neurotransmission (Olive, 2002), and calcium homeostasis. 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 thus, decrease ROS generation (Schaffer *et al*., 2009; Jong *et al*., 2012).

In vivo studies in various species have shown that taurine is essential in certain aspects of mammalian development. It has been demonstrated that low levels of taurine are associated with various pathological lesions, including cardiomyopathy, retinal degeneration, and growth retardation, especially if deficiency occurs during development (Sturman, 1993). Considering its broad distribution, cytoprotective properties, (Wu *et al*., 1999), cellular development, nutrition and survival, taurine may play an important role in many physiological and behavioural responses of the body, including responses to stress.

Despite the large body of evidence supporting the beneficial effects of taurine supplementation in many diseases, the underlying modifying action of taurine with respect to either molecular or biochemical mechanisms is still relatively unknown (Della *et al*., 2002). There is dearth of information regarding the effects of taurine on restraint

stress in the available literature. The present study was designed to investigate effects of taurine on learning and memory, oxidative stress and haematological biomarkers in restrained-stressed Wistar rats.

1.4 Research Hypothesis

Taurine does not exert significant effects on behavioural, and haemato-biochemical responses of mice and Wistar rats subjected to restraint stress.

1.5 Aim of the Study

The aim of the study was to investigate effects of taurine on behavioural, haematological and biochemical responses in mice and Wistar rats subjected to restraint stress.

1.5.1 Objectives of the study

The specific objectives of the study are to evaluate effects of taurine on:

i. Learning and memory using step-down passive-avoidance test in acute and chronic restraint stress Wistar rats, elevated plus-mace memory for mice in acute and chronic restraint-stressed mice. Exploratory activity using Hole-board model in acute and chronic restraint-stressed mice and motor coordination using Beam-walk assay, in both acute and chronic restraint-stressed mice.

ii. Haematotological parameters using automatic haematological assay analyzer in acute and chronic restraint-stressed Wistar rats.

iii. Activities of Malondialdehyde, Acetylcholinesterase, Superoxide dismutase, Catalase, Glutathione peroxidase, Aspertate aminotransferase, Alanine

aminotransferase, Alkaline phosphatase, total protein, Albumin and Globulin concentrations in acute and chronic restraint-stressed Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Stress Concept and Definition

Stress is an integral part of human life. Stressful events exert deleterious effects on normal (physiological) functions, leading to pathogenesis of diseases. Many of the diseases of the modern life like hypertension, diabetes mellitus, behavioural disorders have been associated with deteriorated effects of stress. Stress is an important factor of depression that causes changes in various body systems. Animal and human health are affected adversely by the stressful events of life inducing situations which alter cognition, learning, memory and emotional responses, resulting in mental disorders like depression and anxiety (Bhatia *et al*., 2011: Kondam *et al*., 2013). Many negative factors exert effects on the body, which are mediated by stress-induced neurochemical and hormonal abnormalities, often associated with oxidative stress (Kumar *et al*., 2010).

Stress is also defined as physical and psychological modifications that disrupt the homeostasis and the balance of organisms (Ganesan *et al*., 2011). The first definition of stress was put forward by Seyle, an endocrinologist, who defined stress as the state manifested by a specific syndrome, which consists of all the non-specifically-induced changes within a biologic system. Thus, stress has its own characteristic form and composition, but no particular cause. The elements of its form are the visible changes due to stress, which addictive indicators are expressing the sum of all the different adjustments, occuring on in the body at any time (Selye, 1975).

Stress has been described as circumstances where coping with a variety of actual or perceived stimuli alters the homeostatic state of an organism, including behavioural, immunological and endocrine systems. Neurophysiological responses induced by different stressors activate the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis (Malvandi *et al*., 2010). The effects of various kinds of stressors on the plasma levels of stress hormones, behavioural and neural responses have been studied in many animal species (Mantsch and Katz, 2007). Due to the wide range of glucocorticoid-mediated effects on organ and cellular activities, it is not surprising that stress may enhance the probability of genomic damage and alter the transcriptional regulation of the cell cycle in dividing cells (Flint *et al*., 2005). Stress may alter the expression of genes for cell cycle regulators, heat-shock proteins, protooncogenes, and metabolic enzymes. The effects of stress on cellular function via glucocorticoids and induction of malfunction in proliferation and cell division may cause genetic abnormalities in descendant daughter cells (Dickmeis *et al*., 2007).

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). Stressful events activate the HPA axis (Kvetnansky *et al*., 2002) and increase the release of corticotrophin- releasing hormone (CRH) from the hypothalamic paraventricular nucleus, causing the secretion of adrenocorticotropic hormone (ACTH) from anterior pituitary which in turn stimulates the secretion of glucocorticoids from the adrenal cortex. In humans, glucocorticoids are synthesized by the adrenal cortex and released following activation of the HPA axis, and play an important role in the maintenance of resting and

stress-related homeostasis (Nicolaides *et al*., 2014). Named for their effects on glucose metabolism, glucocorticoids are involved in almost every cellular, molecular and physiologic network of the organism. They regulate a broad spectrum of physiologic functions essential for life, including growth, reproduction, cognition, behaviour, cell proliferation and survival, immune, central nervous system (CNS) and cardiovascular functions (Nicolaides *et al*., 2010). Stress has been postulated to be involved in the aetiopathogenesis of a variety of disease states, including hypertension, coronary heart disease, gastric ulcers, diabetes mellitus, immuno-suppression, mental depression, memory loss, and host of other diseases. The resultant disturbances may vary depending upon type, intensity and the duration of a particular stressor, and the strain\sex differentiation of the subjects (Kondam *et al*., 2013).

Stress has its own characteristic form and composition, but no particular cause. The elements of its form are the visible changes due to stress, and the addictive indicators of stress express the sum of all the different adjustments taking place in the body at any time. Stress exerts detrimental effects on several cellular functions through impairment of antioxidant defences; leading to oxidative damage, a process central to many diseases (Torres *et al*., 2004). Selye's definition of stress has enabled researchers to incorporate all of the biological mechanisms influenced by stress and not just activation of the HPA-axis. Stress is the plaque of the modern society as well as unavoidable consequences of life. A moderate amount of stress may have positive effects, making individuals more alert, motivated and thereby allowing for better performance. However, too much pressure causes physical damage to the gastro-intestinal tract, endocrine system, skin and

cardiovascular system (Chrousos and Gold, 1992). Stress may also cause the feelings of distrust, rejection, anger and depression, which in turn lead to health problems such as headache, stomach upset, ulcers, liver disease, rashes, insomnia, hypertension, heart disease, stroke, diabetes mellitus, immune and sexual disorders (Dimsdale *et al*., 2000).

The concept of stress has become popular in explaining a wide range of behaviours that appear to defy explanation. Indeed, it has become fashionable in the Nigerian society to attribute any erratic or unexplainable behaviour of people to the fact that they are under stress (Omoniyi and Ogunsanmi, 2011). In common language, stress is associated with distress; that is any kind of demand, burden, pressure or hardship on the individual. Biologically speaking, stress means anything that constitutes a threat, either real or imagined, which may damage the body. Stress is, therefore, a process in which events or forces threaten the well-being of an individual in the society. It is a disruption of the emotional stability of the individual that induces a state of disorganisation in personality and behaviour (Omoniyi and Ogunsanmi, 2011).

2.2 The Physiology of Stress

Stress elicits a variety of physiological responses and the magnitude of which may be decreased or increased by prior stress history (Grissoma *et al*., 2008). Although stressors vary widely, their physiological responses are relatively non-specific. Whether the stressor involves an approaching lion or an unhappy life partner, physiological responses are designed to cause many important changes that will enhace the body responses. Initiated by the brain and largely mediated by stress hormones, the changes include an

increase in oxygen intake, redirecting blood flow to favour the muscles, an increase in blood sugar levels to provide energy and a behavioural urgency to act. Thus, digestion, tissue repair and growth, reproductive, and immune functions are all inhibited by the stress response (Sapolsky, 1998). The stress response also acts on the brain to presumably affect certain cognitive operations and predispose certain types of behaviour. Thus, to understand the effects of stress on cognition, it is critical to understand the physiological stress response. Although a number of hormones are released during stress, there are two primary systems that provide a "fast" and "slow" response, which are the sympathetic (catecholamine) and the glucocorticoid responses, respectively.

2.2.1 Sympathetic adreno-medullary system

The first system involved in response to a stressor is the sympathetic adreno-medullary system (Nelson, 2000). The sympathetic response is the "fast" system, in which the hypothalamus directly stimulates internal organs via the sympathetic nervous system to initiate physiological arousal, or the "fight or flight" response. This is accomplished via a two-neuronal pathway: a hypothalamic neurone synapses on a post-ganglionic neurone in the peripheral nervous system (the "sympathetic chain"); the post-ganglionic neurone innervates target organs, releasing adrenaline, which binds to adrenergic receptors on the organ. Sympathetic activation has a wide-spread effect on the body and is intended to prepare the body for action. Physiological changes include an increase in heart rate and vasoconstriction (thereby increasing blood pressure), dilation of respiratory pathways to maximize oxygen intake, and vasodilation of heart and skeletal muscle vessels to deliver oxygen and glucose to the muscles (Sherwood, 1997). The sympathetic response also

involves inhibiting maintenance activities such as digestion and immune function while the body confronts the stressor (Sapolsky, 1998).

An important target organ for sympathetic activation is the adrenal medulla, which releases the catecholamine hormone adrenaline. Hormones are released into the bloodstream and must find their target tissues in sufficient concentration; therefore their effect is relatively slower than direct neural stimulation, but lasts longer. Adrenaline reinforces and maintains the sympathetic responses; that is, promoting cardiovascular changes which redirect blood flow away from peripheral sites to skeletal muscles and the heart, and inhibiting competing functions. Adrenaline also plays a critical metabolic role by increasing blood glucose levels in order to provide energy for the body (Nelson, 2000). Catecholamines (both adrenaline and noradrenaline) also have an effect on the brain.

In general, adrenaline increases alertness as part of the arousal response. More recent research has focused on the role of catecholamines in memory formation (Roozendaal, 2000). Since catecholamines do not readily pass across the blood-brain barrier, they exert their effect on the brain indirectly. Adrenaline released peripherally activates the β-adreneric receptors on vagal nerve cells, which are part of the sensory autonomic nervous system. The vagal afferents project to the solitary nucleus in the brainstem (medulla), and cause the release of noradrenaline in the baso-lateral amygdala. Experimental research suggests that the baso-lateral amygdala plays an important role in modulating hormonal influences on memory consolidation in the hippocampus. The solitary nucleus and the

baso-lateral amygdala also have glucocorticoid receptors, and are, thus, directly affected by adrenal steroid release during stress (Roozendaal *et al*., 1997).

2.2.2 Hypothalamus-pituitary-adrenal axis

The second hormonal system to be activated during the stress response is the HPA axis. The HPA stress response relies exclusively on the relatively slower action of adrenal hormones to exert their effect. HPA activity, thus, maintains and builds upon the sympathetic response. The hypothalamus first releases corticotropin releasing factor (CRF), which in turn stimulates the pituitary to release ACTH into the bloodstream. ACTH acts on the adrenal glands, causing the adrenal cortex to release adrenocortical hormones, which are steroids (lipids derived from cholesterol). There are three classes of hormones produced and released from the adrenal cortex: mineralocorticoids, involved in maintenance of electrolyte balance, sex hormones, and glucocorticoids (Sapolsky, 1998).

The most important glucocoticoid in humans is cortisol, while in rodents it is corticosterone. As the name implies, glucocorticoids, such as cortisol, play a critical role in raising circulating levels of glucose in the blood to provide muscles and the brain energy for the stress response. Cortisol does this by stimulating the liver to convert glycogen into glucose, which is then released into the blood. Cortisol inhibits the secretion of insulin, which takes up glucose for storage and promotes hepatic gluconeogenesis converting amino acids into glucose when carbohydrate sources are depleted. Cortisol also promotes the break-down of protein (muscle) into amino acids for

later gluconeogensis, and fat into fatty acids to provide an additional source of energy for some tissues, although the brain can only use glucose (Sherwood, 1997).

While cortisol is involved in energy release, it also contributes to the shut-down of bodily activities that compete for longer-term or maintenance activities that can be delayed until after the emergency. These include immune function, tissue repair, digestion and energy storage, and certain reproductive activities. Although, cortisol exerts anti-inflammatory effects and is the drug of choice for treating excessive or damaging inflammation. There has been an increasing interest in the effect of cortisol on immune-suppression. Cortisol inhibits the production of new lymphocytes (T-helper cells, T-suppressor cells), mobilises lymphocytes from the circulation, and can also induce lymphocytes self-destruction. It also inhibits the activity of interleukins and interferons, dampening the immune alarm response. Clearly, if such acute effects of the stress response continue under conditions of chronic stress, the result may be very damaging to the body (Sapolsky, 1998).

2.3 Stages of Stress

During stress, the responces of the body is called the general adaptation syndrome (GAS). The GAS occurs in three stages - alarm, resistance, and exhaustion stages. The fight or flight response is the most common type of alarm stage. However, the body may or may not pass through all the stages of stress, depending on the events that triggers the occurrence (Seyle, 1936).

2.3.1 Alarm stage

The first reaction of the body to stress is to know there is danger and prepare to deal with the threat; that is fight or flight response. Activation of the HPA axis, the nervous system and the adrenal glands take place. During this phase, the main stress hormones, cortisol, adrenaline, and noradrenaline, are released to provide instant energy. If this energy is repeatedly not used by physical activity, it may become harmful. Too much adrenaline results in a surge of blood pressure that can damage blood vessels of the heart and brain, a risk factor in heart attack and stroke. The excess production of the cortisol hormone causes damage to cells and muscle tissues. Stress-related disorders and disease from cortisol include cardiovascular conditions, stroke, gastric ulcers, and high blood sugar levels (Seyle, 1936).

2.3.2 Resistance stage

The body shifts into this second phase with the source of stress being possibly resolved. Homeostasis begins restoring balance and a period of recovery for repair and renewal takes place. Stress hormone levels may return to normal, but may reduce defences and adaptive energy left. If a stressful condition persists, the body adapts by a continued effort in resistance and remains in a state of arousal. Problems begin to manifest when this process repeats itself too often, with little or no recovery. Ultimately, this moves the body into the final stage (Seyle, 1936).

2.3.3 Exhaustion stage

At this phase, the stress has continued for some time. The body's ability to resist is lost because its adaptation energy supply is depleted. This is often referred to as overload, burn-out, adrenal fatigue, maladaptation or dysfunction. Stress levels rises and stays up. The adaptation process is over and not surprisingly; this stage of the GAS is the most hazardous to health. Chronic stress may damage nerve cells in tissues and organs. Particularly vulnerable is the hippocampus section of the brain. Thinking and memory are likely to become impaired, with tendency towards anxiety and depression. There may also be adverse function of the autonomic nervous system that contributes to high blood pressure, heart disease, rheumatoid arthritis, and other stress related illness (Seyle, 1936).

2.4 Restraint stress and physiological changes

Restraint is considered a psychological stressor for animals. It does not physically harm the animal, but does activate the HPA-axis and increases the production of glucocorticoids (Harris, *et al*., 2004). Due to the fact that restraint is a relatively easy way to induce stress, many researchers have used restraint in order to examine the effects of stress on learning and memory. However, as with many stressors, intensity and timing of restraint stress strongly influence learning in animals. Physiological and psychological changes occur in response to activation of the body's stress mechanism. Like other kinds of stress, restraint stress stimulates an immediate increase in plasma levels of glucocorticoids (Bowers *et al*., 2008). Restraint stress also suppresses the biosynthesis of testosterone (Weissman *et al*., 2007). At the molecular level, the stress may cause

changes in expression pattern of genes, which are responsible for cell cycle regulation and apoptosis in mouse T cells (Flint *et al*., 2005). However, very intense stressors that induce the fight-or-flight response drastically increase the production of glucocorticoids and prevent them from providing the negative feed-back signal to regulate the HPA-axis (Mackin and Young, 2004). Physiological and psychological processes are disturbed during periods of stress, therefore, alterations in both corticosteroid and glucocorticoid levels have been under intense investigation over the past decade. However, the results are often conflicting (Bowers *et al*., 2008).

2.4.1 Effect of restraint stress on learning and memory

Restraint stress impairs spatial learning and memory (Sunanda and Raju, *et al*., 2000). It inhibits performance on an 8-arm (radial arm maze), compared to non-restrained animals. Furthermore, when restraint animals were paired with a second stressor, such as cold-water immersion, the deficit in performance was worse than animals that only received the restraint stressor. Restraint plus cold-water immersion stress also produce a deficit on a passive-avoidance task. If animals receive restraint plus cold-water immersion 1 hour prior to training on the passive avoidance task, and are tested 24 hours after training; then the latency to cross scores decrease and total time on the safe-side scores increase, compared to controls (Klenerova *et al*., 2002). Restraint stress applied over 21 days prior to maze training caused a similar impairment in spatial memory. Evidence suggests that the impaired memory following chronic stress is related to reduced hippocampal plasticity, rather than neuronal loss (Bodnoff, *et al*., 1995).

In another study, restraint stressed animals showed memory impairment, evident in passive avoidance task as well in the Morris water maze test (Kumar *et al*., 2007). Chronic stress causes memory impairments (Sunanda and Raju, 2000). Neural basis for such impairment is the neuronal injury in the hippocampal region due to excitotoxicity and alterations in the neurotransmitter levels in the brain regions. Alternatively, neuronal damage in this region may be mediated through increased glucocorticoids (McEwen, 2007). Stress results in increased oxidative stress and decreases antioxidant defence status in the brain (Zaidi and Banu, 2004), resulting in increased of ROS generation in the body which may form the basis for decreased memory. Antioxidant treatment has been shown to modulate the effects of ROS on the brain in restrained rats (Zaidi *et al*., 2003).

2.4.2 Restraint stress and immune responses

Physical and psychological stressors modify multiple aspects of immune responses, which include decreasing natural killer (NK) cell activity (Wu *et al.*, 2000). It is also well accepted that, as a consequence of the modulatory effects of stress on immune responses, physical or psychological stress modulate host defences against invading pathogens and alter susceptibility to infectious diseases (Yang and Glaser, 2000). Studies on the neuroendocrine regulation of the mucosal immune system have focused on the quantity of IgA secreted in saliva (Proctor and Carpenter, 2002), the synthesis and secretion of the secretory component of the tear glands (Lambert *et al*., 1995), and the regulation of the synthesis of the secretory component in the female reproductive apparatus. On the other hand, studies done on the effects of stress on the mucosal immune system have focused almost exclusively in relation to inflammatory diseases of the intestine and the secretion

of IgA in saliva. In relation to IgA levels in human saliva, both reductions and increases have been reported, depending on the type of psychological and/or physical stress protocol employed. The contradictions make it difficult to reach any conclusion about the effects of stress on the humoural immune response of the mucosa, which is represented by secretory IgA (sIgA) levels (Teeuw *et al*., 2004).

2.4.3 Restraint stress and pain sensitivity

Restraint stress alters pain sensitivity (Costa *et al*., 2005). A single exposure of restraint rats increased tail-flick latencies, when tested with the hot-plate test. The opposite effect occurred when the animals were chronically restrained; they had decreased tail-flick latencies compared to controls. In another studies, morphine alone produces analgesia; but if animals are restrained prior to receiving morphine, the analgesic response is enhanced. Stress may exert bilateral effects on pain-related phenomena. First, acute stress can produce analgesia in animals and humans (stress-induced analgesia). On the other hand, stress has also been reported to produce hyperalgesia; that is, increased sensitivity to painful stimuli or allodynia (pain triggered by innocuous stimuli). It appears that the repetitive nature of the stress favours the induction of hyperalgesia/allodynia in rodents. Hence, repeated exposure to loud sound, cold environment, restraint, swim stress or sleep deprivation potentiates pain perception in rats. Acute stress induced by non-noxious stimuli including hand-holding, vibration and novel environment may also trigger hyperalgesia (Imbe *et al*., 2006).

2.4.4 Restraint stress and core body temperature

Activation of the sympathetic nervous system in response to perceived threat, or generalised psychological arousal, causes an increase in core body temperature in animals and humans that is similar in both warm and cold environments, and not related to increased locomotor activity (Hansel and Kanel, 2008). Many animal models of psychological stress employ restraint as a method to induce hyperthermia because of the control this paradigm provides over the timing and duration of the stress as well as the reliability of the response (Bouwknecht *et al*., 2007; Vinkers *et al*., 2008). However, interpretation of stress-induced hyperthermia in restrained rodents can be complicated by a hypothermic component that precedes the hyperthermic stress response in some studies (Johnson *et al*., 2004; van Eijl *et al*., 2006). This hypothermic component is generally considered to be part of anxiogenic response (Stamp and Herbert, 2001; Johnson *et al*., 2004).

2.4.5 Restraint stress and renin-angiotensin system

Accumulated evidence supports the viewpoint that renin-angiotensin system (RAS) and stress phenomenon are interrelated. Stress is accompanied by an increase in the plasma renin activity and the circulation of angiotensin II, thereby contributing to the modulation of HPA and cardiovascular response to stress. Angiotensin II receptors and, in particular AT1 receptor subtype, are widely spread throughout the brain and in areas responsible for the regulation of stress responses (Saavedra *et al*., 2005). Various studies have shown a

distinct involvement of angiotensin receptors in the effects of angiotensin II in restraint stress (Kiss *et al*., 2001) Restraint stress increases the expression of AT1A mRNA receptors and AT1 receptor binding in a variety of brain areas. It is known that brain RAS, particularly AT1 receptors, are involved in acute restraint stress-induced analgesia and impaired retention. Different stressful manipulations, including restraint stress, induce anticonvulsant effects in rodents (Homayoun *et al*., 2004). A putative role of angiotensin peptides in the seizure phenomenon and the nociception has been suggested (Tchekalarova and Georgiev, 2006).

2.4.6 Restraint stress and oxidants

Several studies have reported the effects of stress on the antioxidant system and induction of lipid peroxidation in brain after immobilisation-induced stress model (Madrigal *et al*., 2001; Zaidi *et al*., 2003; Sahin and Gumuslu, 2004). The balance disturbance in favour of activation of ROS processes occurs with maximal changes in the brain after immobilisation stress. Stress decreases the level of glutathione (GSH) and antioxidant vitamin (vitamin C), involved in protection of tissues against oxidative damage. In order to scavenge ROS, the body uses enzymatic (copper, zinc-superoxide dismutase, catalase and selenium-dependent GSH peroxidase) and non-enzymatic (reduced GSH, vitamins and certain compounds) antioxidant (Sahin and Gumuslu, 2004). Thus, antioxidants reduce the oxidative stress by scavenging ROS generated in the body (Zaidi *et al*., 2005).

2.5 Behavioural Responses to Restraint Stress and their Significance

Activation of the response to restraint stress induces cognitive alterations in humans and animals. Either cognitive enhancements or deficits results after restraint stress. Deficits in visual tasks and general day-to-day memory occur in response to corticosteroid treatments or to high stress levels in humans. Data obtained in the literature on animals, which are more inconsistent than those of humans, demonstrates that stress produces either deficits or enhancements in animal memory (Joels *et al*., 2006). The exact reason for the inconsistencies in animals is unknown. Variations in type of learning task, stressor, and duration of stressor administration, from one experiment to the next, may be contributing factors.

The exact mechanism of generation and progression of stress response remains unknown (Bhatia *et al*., 2011). Immobilization by restraint stress is an aversive stimulus which disturbs homeostasis and is reflected on a variety of biological systems of the body (Kumar *et al*., 2010). Exposure of animals to restraint stress and psychosocial stress in humans has been implicated in the pathophysiology of anxiety and mood disorders (Walesiuk *et al*., 2006). Research also reported that sub-acute immobilisation stress in mice worsens animal behaviour through inflammatory mechanisms in the brain and causes neurobehavioural changes (Caso *et al*., 2008). Restraint stress causes wide range of anxiety-like behaviour, which alters cognition, learning, memory and emotional responses (McLeod *et al*., 2001). Stress, either emotional or social, has damaging effects on cellular integrity in tissues, particularly in the brain (Caso *et al*., 2008). Sub-acute restraint stress may affect the locomotor activity and increase the anxiety-like behaviour. It may be due to immobilisation by restraint stress which is one of the explored models of

stress that is combined with emotional stress (escape reaction), Physical stress results in both restricted mobility and aggression. Physiological response to stress is the activation of the HPA axis which causes release of corticosterone, which in turn accelerates ROS generation (Kondam *et al*., 2012).

2.6 Taurine as a Neurotransmitter

Generally, a substance is considered as a neurotransmitter, if that substance has fulfilled the following criteria: First, the substance and/or its synthesizing enzyme has to be present in the suspected neurone, preferably concentrated at the nerve terminal; second, it is released upon stimulation in a calcium-dependent manner; thirdly, it elicits proper physiological response; fourthly, a specific receptor is present and fifthly, an inactivation mechanism is present to terminate the action of the suspected neurotransmitter (Wu and Prentice, 2010). The following lines of evidence have supported the notion that taurine is a neurotransmitter in the mammalian central nervous system.

1. The presence of a specific enzyme responsible for taurine biosynthesis in the brain, namely, cysteic/cysteine sulphuric acid decarboxylase (CAD/CSAD), which is distinctly different from the GABA-synthesizing enzyme, L-glutamate decarboxylase (GAD) has been reported (Wu, 1982). Immunocytochemical studies have revealed the localisation of CAD/CSAD in the cell body, dendrite as well as in the nerve terminal (Magnusson *et al*., 1989).
2. Release of taurine has been shown to be either calcium-dependent or calcium independent (Lin *et al*., 1985).

3. Taurine has been shown to elicit neuronal hyperpolarisation, presumably through its action by opening the chloride channels in the cerebellum (Okamoto *et al*., 1983).
4. The presence of a specific taurine receptor, has been demonstrated. Previously, reports have shown the presence of specific taurine receptors, which have K_d in nM range and are distinctly different from $GABA_A$, $GABA_B$ and glycine receptors since the agonists or antagonists of these receptors have little effect on the binding of taurine to taurine receptors (Frosini *et al*., 2003).
5. The presence of a taurine transporter system for inactivation of its function has also been reported. In fact, taurine transporters have been cloned and taurine transporter knock-out transgenic mice have been established (Warskulat *et al*., 2006). In summary, taurine has fulfilled most, if not all, of the criteria to be accepted as a neurotransmitter in the mammalian CNS.

2.6.1 Biosynthesis and catabolism of taurine

The enzymatic machinery for taurine biosynthesis from its precursor amino acids (methionine and cysteine) is contained in the brain. In two major synthesis pathways, cysteine is either first oxidized by cysteine dioxygenase to 3-sulphinoalanine (cysteinesulphinate). Thus, is then decarboxylated by sulphino decarboxylase (cysteinesulphinate decarboxylase) resulting into hypotaurine or transformed by a more devious route via co-enzyme A to pantetheine and then to cysteamine, which is converted to hypotaurine by means of cysteamine dioxygenase (Coloso *et al*., 2006). However, taurine biosynthesis *in situ* may be slower than supply from other organs, particularly

from the liver (Tappaz, 2004). Some species exibit an inadequate capability to synthesize taurine to meet the body demands. For such animals, for example, carnivorous cats, taurine is an essential nutrient and its deficiency results in grave structural malformations and functional deficits (Maar *et al*., 1995).

In primates and humans, taurine is also an essential nutrient for babies, its deficiency results in similar developmental problems. Taurine is not broken down in mammalian organisms. It is conjugated with bile acids in the liver and the kidneys excrete taurine into the urine, the rate of excretion reflecting the dietary intake and plasma levels. Taurine supplementation increases brain taurine only moderately and only temporarily (Lallemand and De Witte, 2004). The slow break-down and elimination of taurine signify that these factors cannot play any role in the termination of its possible synaptic action in the CNS (Tappaz, 2004).

2.6.2 Taurine uptake

The uptake is saturable, concentrative, energy-, Na^+- and Cl^--dependent, and structurally specific for β-amino acids; that is, the acidic group and basic groups reside at the opposite ends of the molecule, separated by two or three carbon atoms. Two or three Na^+ ions are required for the transport of one taurine molecule. Both neurones and glial cells actively take up taurine from the extracellular spaces. The uptake comprises two components, low and high affinity. The estimated Km constants for the low-affinity transport have been within the micromolar range, and those for high-affinity transport are within the

millimolar range. These constants are generally smaller in preparations from the developing brain than the adult brain (Huxtable, 1989).

There are now taurine transporters (TAUTs) that have been cloned from different central nervous system (CNS) preparations. The transporters exhibit marked homology with one another and also with the transporters of established neurotransmitters. Research findings have shown that distribution of mRNA for the rat TAUT is entirely within the CNS and among different types of cells, both glial cells and neurones (Olson and Martinho, 2006); but the two different clones TAUT-1 and TAUT-2 are differently localized. TAUT-1 is found in the rat pituicytes, cerebellar Purkinje cells, and in photoreceptors and bipolar cells in the retina. TAUT-2 is mainly found in high concentration in the CNS and is predominantly associated with Bergmann glial cells in the cerebellum. It is also associated with astrocytes in other brain areas; and at lower levels, with some neurones such as CA1 pyramidal cells in the hippocampus (Pow *et al.*, 2002). Base on the presence of TAUT in the mouse cerebral cortex, TAUT is believed to be responsible for uptake of taurine (Fujita *et al.*, 2006).

Electrophysiological research proved that TAUT is present in Bergmann glia. It is also shown to exhibit the same electrogenic stoichiometry as seen in GABA transporter GAT1. Its gene expression is under the control of two antagonistic regulations, osmolarity-induced up-regulation and taurine-induced down-regulation (Bitoun and Tappaz, 2000), but the regulation in the brain is a partially open matter. The most potent inhibitors of cloned TAUTs are hypotaurine and β-alanine, which also efficiently inhibit

taurine transport in brain slices and cultured cells. The transporters also accept GABA, albeit with lesser affinity. On the other hand, cloned GABA transporters also transport taurine, but their affinity for taurine is much lower than that for GABA (Melamed and Kanner, 2004).

2.6.3 Purported taurine receptors

Neurotransmitter function of taurine presupposes the existence of membrane receptors. It has been difficult to determine whether the physiological functions of taurine are mediated by GABA and or glycine receptors, or whether specific taurine receptors are involved in certain brain areas, at particular developmental stages and in different animal species. Taurine binding to brain membranes has likewise proved difficult to demonstrate. Kontro and Oja, (1989) were the first to report taurine binding to mouse brain synaptic membranes. The binding proved to be Na^+-independent and shows sigmoidal dependence on the ligand concentration. Taurine antagonist TAG, is an effective displacer of this binding. It is also affected by the glycine receptor antagonist, strychnine, and by GABA antagonists. Therefore it is not possible to delineate the binding to glycine and GABA receptors from the possible binding to taurine receptors (Kontro and Oja, 1987).

Two taurine receptor subtypes are surmised in the frog spinal cord to exist, based on their pharmacological properties, which differ from the responses to GABA and glycine. Putative taurine receptor proteins have been made in the olfactory organ of the lobster.

Binding of taurine is displaced by TAG, 2-aminoethylarsonate, 2-hydroxyethanesulfonate, and (±) cis-2-aminocyclohexanesulfonate characterization of [^{3}H] taurine binding to washed and detergent-treated synaptic membranes from the rabbit brain. The effectors, therefore, do not interact with $GABA_A$ and $GABA_B$ receptors, taurine and GABA uptake systems, or GABA transaminase. Thus, taurine binding does not represent binding to GABA receptors, but the effects of glycine agonists and antagonists on this taurine binding have not been tested. Detergents can specifically bind to taurine. Furthermore, the endogenous muscimol-binding inhibitor in brain tissue has been claimed to be a more potent inhibitor of taurine binding than that of muscimol binding, though this observation has not been further explored. It is also known that only minor changes in the amino acid chains may alter the relative affinities of receptors for glycine and taurine, and there exist, marked species differences and alterations during development. The matter of the independent identity of taurine receptors is, thus, not yet settled (Frosini *et al*., 2003).

2.6.4 Interactions of taurine with glycinergic systems

Taurine interacts with glycinergic systems only at the receptor level, since it is not a preferred substrate for glycine transporters. It displaces glycine from its strychnine-sensitive binding sites in the mouse brain stem. It acts also as an agonist at glycine receptors in Xenopus oocytes, injected with mouse brain mRNA in the rat supraoptic magnocellular neurones, and in dopaminergic neurones in the ventral tegmental area in young rats (Ye *et al*., 2004). In mammalian receptors, glycine is more effective than taurine, but in *Hydra vulgaris* glycine and taurine are almost equipotent. Taurine in some

instances affects both $GABA_A$ and glycine receptors simultaneously. For example, it controls hormone release from the rat neurohypophysis and prevents the ammonia-induced accumulation of cGMP in the rat striatum in this manner (Hilgier *et al*., 2005).

At low concentrations, taurine activates glycine receptors in CA1 neurones in the immature rat hippocampus, whereas at high concentrations both glycine and GABA receptors are activated. In mouse the target of taurine shifts from glycine receptors to GABA receptors during postnatal development (Yoshida *et al*., 2004). Taurine may be an important endogenous ligand for glycine receptors in certain parts of the CNS. For instance, the glycine-receptor clusters and the glial fibrillary acidic protein-positive astroglial processes, which contain high levels of taurine in the rat supraoptic nucleus, are anatomically associated. The activation by taurine of glycine receptors in neurohypophyseal nerve terminals participates in the osmoregulation of vasopressin secretion. It acts mainly on non-synaptic glycine receptors in the ventral tegmental area, and their dopaminergic neurones could be exposed tonically to taurine. Taurine has been shown to inhibit the glycine responses of oocytes, injected with rat a1 subunits of the glycine receptor or spinal cord poly (A) RNA. It is a full agonist of the glycine receptors in the ventral tegmental area (Wang *et al*., 2005).

Taurine more efficiently gates glycine a1 receptors than a2 receptors, but glycine is more effective than taurine in both receptor subtypes. Human and rat a2 and a3 glycine receptors display rather low responses to taurine, and the amino acid 167 in the a2 subunit is essential for taurine effects. On the other hand in the mammalian a1 subunits,

isoleucine in position 111 and alanine in 212 have been considered important determinants of taurine activation, and valine in these positions in a2 may reduce the efficacy of taurine. The glycine receptor a1 subunit of the zebra fish (aZ1) is very similar to the rat a1 receptor in both length and amino acid residue composition, but the homomeric aZ1 receptor exhibits exceptionally high sensitivity for taurine, almost comparable to glycine sensitivity. It contains valine instead of isoleucine in position 111 and this infer that the natural substitution Ile111Val may not be a critical determinant for taurine selectivity. Sequence differences in the immediate vicinity of Val 111 may rather underlie the high efficacy of taurine (Imboden *et al*., 2001).

In other experiments, taurine has also acted as a full agonist in aZ2 glycine receptors in spite of the presence of Val 111 and Val 212. The more recently cloned homomeric a4 subunit from chicks, containing valines in the equivalent positions is also potently activated by taurine. Two separate point mutations at the same base pair in the gene encoding the human glycine receptor a1 subunit, resulting in arginine at position 271 being substituted by leucine (R271L) or glutamine (R271Q), have been observed to convert taurine from a full agonist to a competitive antagonist. The same changes occur with the Y279C and K276E mutations (tyrosine to cysteine and lysine to glutamate, respectively). It is assumed that these mutations either simply disconnect the binding site of taurine from the Cl^- channel activation site or selectively disrupt a common agonist recognition subsite, and thereby unmask the antagonist subsite for taurine (Imboden *et al*., 2001).

2.6.5 Interactions of taurine with other transmitter systems

There are only scattered data on the effects of taurine on other neurotransmitter systems. Taurine modulates noradrenaline uptake and release in rat cerebral cortical slices, interferes with the binding of labeled spiperone (serotonin 5-HT2A and dopamine D2 receptor antagonist) to cerebral cortical membranes and interacts with dopaminergic neurotransmission in the striatum. The age-related decline in striatal taurine is correlated with a loss of dopaminergic markers. Intranigrally-injected taurine modulates striatal dopaminergic transmission and reduces extracellular dopamine. Intracerebroventricular and intraperitoneal taurine and taurine administered directly into the striatum significantly increased extracellular dopamine. At variance, in other studies direct administration into the rat striatum did not markedly affect extracellular dopamine, but reduced extracellular dopamine metabolite, dihydroxyphenylacetic acid accentuated its NMDA-induced decrease (Anderzhanova *et al*., 2006). Taurine inhibits the synthesis and release of serotonin in rat rostral, but not in caudal, rhombencephalic raphe cells. It also affects the binding of phencyclidine (which blocks NMDA receptor activation) in the mouse cerebral cortex. Finally, taurine reduces K^+-stimulated adenosine release in slices from the hippocampus in the developing, but not in adult mice (Saransaari and Oja, 2003).

2.7 Physiological Roles of Taurine

A considerable body of evidence has been gathered on the pharmacological actions of taurine. These studies have largely focused on the cytoprotective activity of taurine. However, they provide little information on the physiological actions of taurine. Until

recently, the physiological actions of taurine were studied using a model of taurine depletion mediated by taurine transport inhibitors or a model of nutritional depletion in cats. Although these studies have uncovered new actions of taurine, in the case of the transport inhibitors it is unclear if the reported actions are related to taurine (Schaffer *et al*., 2010).

The biological actions of a compound are inevitable consequence of its physicochemical properties. Taurine is an amino acid that differs from many familiar substances of that class because it contains sulphuric rather than a carboxylic amino acid. It is also a β-amino acid, rather than α-amino acid. Compared with carboxylate groups, the sulphonate group is a strong acid, having an acidic dissociation constant (pKa) equivalent to that of a mineral acid, like hydrochloric acid. This high acidity makes taurine almost completely zwitterionic over the physiological pH range. This is a contrast because significant fractions of carboxylic amino acids exist unionized over this range. At pH 7.4, the fractions of α-alanine and GABA having unionized acid functions are 125 and 340 times greater than the fraction of taurine (Huxtable, 1992).

The zwitterionic nature of taurine gives it high water solubility and low lipophilicity. Consequently, compared with carboxylic amino acids, diffusion through lipophilic membranes is slow for taurine. The impermeability of biological membranes to taurine probably underlies the extraordinarily high concentration gradients that may be maintained across such membranes. The poor permeability of taurine also permits renal regulation of the whole body content in mammals, as reuptake from the tubular fluid is a

function of a hormonally controlled active transport system. To maintain such concentration gradient high capacity, high-affinity uptake systems in the face of significant diffusion out of the cell would place an unacceptable energy demand on the cell. Depending on the taurine system and the observer, for each taurine molecule transported, between 1 and 3 Na^+ are carried. This Na^+ is pumped back out via the Na^+-K^+- ATPase, hydrolyzing 1 ATP per 3 molecules of Na^+. As a result of its zwitterionic nature, taurine has a high dipole (Huxtable, 1992).

The membrane modulatory actions of taurine and its interactions with Ca^{2+} and other cations probably stem from its unique ionic characteristics. The overriding significance of the ionic properties of taurine is supported by the inability of isosteres of taurine to substitute for it in various biological phenomena. Amino-ethane phosphonate and β-alanine are two compounds that sterically resemble taurine. The sulphur in taurine is in the form of a sulphonate and may be further oxidized to sulphate. The lowest oxidation state for sulphur is -2 and the highest is +6. The sulphur in taurine, at +4, has a free energy content of ~260 kJ/mol relative to sulphate (Huxtable, 1992).

Taurine was once considered an end-product of sulphur amino acid metabolism, a presumption based on the +4 oxidation state of the sulphur (present as a sulphoric acid moiety). Although it has been suggested that taurine can serve as an antioxidant, the sulphoric acid is incapable of scavenging the common oxidants, namely, superoxide, hydrogen peroxide and hydroxyl radical. Nonetheless, the amino group of taurine can neutralize hypochlorous acid, one of the reactive species generated by neutrophils. In that

reaction, taurine is converted to taurine chloramine, which is less toxic than hypochlorous acid, and serves as a modulator of the immune system (Kirino *et al*., 2005). The amino group of taurine is commonly involved in conjugation reactions. One potentially important reaction is the interaction of taurine with uridine to form 5-taurinomethyluridine, a modified base located in the wobble position of some mitochondrial t-RNAs (Suzuki *et al*., 2002).

This reaction has attracted considerable attention because of its potential involvement in the regulation of mitochondrial protein synthesis (Kirino *et al*., 2005; Schaffer *et al*., 2009). Other taurine-associated conjugation reactions play central roles in the elimination of toxins from the body, the metabolism of lipids (via bile acid conjugation) and the activity of glycolipids. However, the relative chemical inactivity of taurine makes the b-amino acid attractive as a modulator of basic processes, such as osmotic pressure, cation homeostasis, enzyme activity, receptor regulation, cell development and cell signaling (Schaffer *et al*., 2009).

2.7.1 Effects of taurine on membrane ion conductance

Taurine inhibits neuronal firing by producing hyperpolarisation through alteration of membrane permeability to ions in different brain areas like the cerebral cortex, diencephalon, striatum, hippocampus, cerebellum, brain stem, and spinal cord (Huxtable, 1989). The actions of taurine are mostly blocked by antagonists of GABA and/or glycine receptors, depending on the brain area. An experiment has shown that taurine effects were generally abolished by GABA antagonists in the higher brain areas and by glycine

antagonists in the brain stem and spinal cord. Since there are no sufficiently specific taurine antagonists available, except for 6-aminomethyl-3-methyl-4H-1, 2, 4 benzothiadiazine-1, 1-dioxide hydrogen maleate (TAG), the effects of taurine per se are difficult to demarcate from those mediated via GABA or glycine receptors. It has been shown that taurine antagonist, TAG blocks taurine-induced hyperpolarisation in Purkinje cell dendrites in the guinea pig cerebellum. At low concentrations, taurine inhibits GABA-induced Cl^- fluxes into synaptic membrane microsacs, prepared from different brain areas; but at higher concentrations, taurine enhances the fluxes, apparently due to its activatory properties.

Taurine is found to increases Cl^- conductance in cerebellar Purkinje cell dendrites *in vitro* in rat *substantia nigra pars reticulate* neurones, and in mitral and tufted cells in slices from the rat main olfactory bulb. As noticed in the cells, the currents evoked by GABA are fast and those induced by taurine are slow (Belluzzi *et al*., 2004). Taurine may, thus, cause prolonged reduction in the input resistance and sustain a prolonged and modest inhibition of these cells. Taurine and glycine activate the same Cl^- conductance and apparently, act at least predominantly at the same recognition site. This occurs in freshly isolated neurones from the *medulla oblongata* and hippocampus, the rat sacral dorsal commissural neurones and in acutely dissociated rat hippocampal neurones (Yu *et al*., 2003). Together with β-alanine, taurine activates glycine receptors in cultured rat hippocampal slices (Mori *et al*., 2002).

Taurine increases the amplitude of spontaneous inhibitory postsynaptic currents in dopaminergic neurones in the ventral tegmental area in young rats, in which GABA is excitatory due to variations in the extracellular content of Cl^-, having potency of one tenth that of glycine (Imboden *et al*., 2001). By reason of developmental changes in the extracellular concentration of Cl^-, the activation of Cl^- channels by taurine during cortical circuit formation in the post-natally developing mouse alters its physiological effects from excitatory to inhibitory (Yoshida *et al*., 2004). Within the same time-frame the target of taurine shifts from glycine receptors to GABA receptors (Yoshida *et al*., 2004).

2.7.2 Neuromodulatory functions of taurine

In synaptic structures, taurine displaces GABA and muscimol binding to the GABA-benzodiazepine receptor complex and interferes with ligand binding to its benzodiazepine site allosterically. Taurine activates $GABA_A$ receptors, but less effectively than GABA itself. Taurine binds to the $GABA_A$ receptors in hippocampal and cortical neurones and prevents b-amyloidpeptide neurotoxicity (Paula-Lima *et al*., 2005). Taurine also attenuates 1-methyl-4-phenylpyridinium neurotoxicity in coronal slices from the rat brain by means of activation of $GABA_A$ receptors. The $GABA_A$ antagonists bicuculline and picrotoxin block taurine effects on $GABA_A$ receptors, solubilized from the rat brain, and on these receptors in the mitral and tufted cells in the rat main olfactory bulb (Belluzzi *et al*., 2004).

At the $GABA_A$ receptor complex taurine is a full agonist of GABA, but preferentially only in the b2 subunit-rich areas of $GABA_A$ receptors, enriched in the dentate gyrus,

substantia nigra, cerebellar molecular layer, median thalamic nuclei, and hippocampal CA3 field. Differences in taurine sensitivity may stem from the different subunit composition of $GABA_A$ receptors. In the *nucleus accumbens* of young rats and in cultured cerebellar granule cell, taurine is only a partial agonist. Treatment of cultured cerebellar granule cells with taurine induces low-affinity GABA receptors, but chronic supplementation to mice induces a down regulation of expression of the $GABA_A$ receptor b-subunit, the key subunit present in virtually all $GABA_A$ receptors. On the other hand, glutamate decarboxylase-positive neurones increase in number in the cerebral cortex of taurine-fed mice (El-Idrissi, 2006).

In some instances taurine has been reported to affect $GABA_B$ receptors. It has proved an even more potent displacer at $GABA_B$ than at $GABA_A$ receptors in rat brain membranes. In the frog olfactory bulb *in vivo*, taurine suppresses the spontaneous firing of mitral cells and acts mainly on $GABA_B$ receptors (Chaput *et al*., 2004) by increasing the signal to-noise ratio. In the rat, taurine has likewise suppressed olfactory nerve-evoked monosynaptic responses of mitral and tufted cells and blocked chloride conductance, probably owing to the $GABA_B$ receptor-mediated inhibition of glutamate release (Belluzzi *et al*., 2004). Taurine may also regulate GABA release from rat brain olfactory bulb synaptosomes via activation of presynaptic $GABA_B$ receptors. The paucity of reports may signify that taurine affects $GABA_B$ receptors only in limited cases (Chaput *et al*., 2004).

2.7.3 Neuroprotective actions of taurine

Taurine is a neuroprotective agent. It is effective in a rat hypoxic model, possibly due to its anti-acidity and membrane-stabilising actions (Mankovskaya *et al.*, 2000). Exogenously applied taurine alleviates neuronal damage, evoked by a variety of pathological impacts, including ischaemia (Kingston *et al*., 2004). For example, taurine improves the recovery of neural functions in brain slices after hypoxic conditions. Taurine could attenuate the excessive neuronal accumulation of Ca^{2+} ions, which predisposes cells to damage and protects neurones from glutamate-induced excitotoxicity, thus preventing or reducing the glutamate-induced elevation of intracellular Ca^{2+} (Chen *et al*., 2001). Taurine also inhibits the reverse mode of the Na^{+}/Ca^{2+} exchanger and protects cultured rat astrocytes against reperfusion injury. It inhibits the glutamate-induced release of Ca^{2+} from the internal pools, regulates cytoplasmic and mitochondrial calcium homeostasis, and inhibits the glutamate-induced Ca^{2+} influx through L-, P/Q-, and N-types of voltage-gated calcium channels (El Idrissi *et al.*, 2003).

On the other hand, Ca^{2+} increase the accumulation of $45Ca^{2+}$ in mitochondria of the rat cerebral cortex, thus being able to attenuate the cytosolic free Ca^{2+} concentration, which in turn inhibits specific protein phosphorylation and phosphoinositide turn-over. On the whole, taurine may have an essential role in the modulation of intracellular calcium homeostasis. The glutamate and glutamate-receptor-agonist evoked taurine release from neural cells might be neuroprotective in balancing glutamate activity under cell-damaging conditions. It efficiently counteracts the glutamate-induced Ca^{2+} uptake and forestalls cell damage, evoked by overactivation of ionotropic glutamate receptors. Over-activation of NMDA receptors induces ROS generation. It is thought that a part of the neuroprotective

action of taurine depends on its antioxidant properties. Co-infusion of taurine with ammonia or NMDA virtually abolishes both the ammonia and NMDA-induced accumulation of cGMP and markedly attenuates accumulation of hydroxyl radicals (Anderzhanova *et al*., 2006).

The effects of taurine on the accumulation of cGMP are mediated by interactions with $GABA_A$ and glycine receptors, since they could be blocked by co-infusion of the $GABA_A$ receptor antagonist, bicuculline; and the glycine receptor antagonist, strychnine. Taurine prolongs rat survival and reduces striatal damage and partially protects the hippo-campus from damage caused by 3-nitro-propionic acid (Rodríguez-Martínez *et al*., 2004). Systemic intravenous administration of taurine protects the rabbit spinal cord against ischaemia in both normothermia and hypothermia. On the other hand, dietary depletion of brain taurine by β- alanine results in cerebellar and retinal damage in adult cats (Anderzhanova *et al*., 2006). There has been extensive research demonstrating that taurine has a unique protective role and that it can down-regulate several stress-associated proteins, and increase neuronal survival under conditions of glutamate-induced cytotoxicity, mitochondrial stress and endoplasmic reticulum stress (Kumari *et al*., 2013).

2.7.3.1 *Role of taurine in mitochondrial dysfunction*

Mitochondria are highly sensitive to oxidative damage and there is much evidence of a cytoprotective role of taurine towards mitochondria in addition to other organelles within the cell. In mitochondria, taurine has been shown to be a key regulator of levels of superoxide production and of oxidative phosphorylation, since taurine deficiency results

in oxidative stress in mitochondria through respiratory chain impairment (Kumari *et al*., 2013). Many taurine-conjugated products are functionally involved in energy metabolism and cholesterol metabolism. One of the key products is 5-taurinomethyluridine-tRNA leucine (UUR), which is involved in stabilization of U-G pairing in an anti-codon loop transfer RNA (tRNA), responsible for efficient decoding of UUG (Kurata *et al*., 2008). Taurine deficiency lowers the taurinomethyluridine-tRNA leucine encoded protein synthesis, disabling efficient assembly of respiratory chain components (Schaffer *et al*., 2009).

Superoxide generation is the result of the diversion of electrons to acceptor oxygen from the respiratory chain (Jong *et al*., 2011). Mitochondrial DNA mutations can cause mitochondrial dysfunction found to be the primary cause of several kinds of mitochondrial disease. Some key studies have demonstrated that these mutated mitochondrial tRNAs are the result of the absence of post-transcriptional taurine-dependent modifications, leading to the molecular pathogenesis of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS); and a second disease, myoclonus epilepsy, associated with ragged red fibers (MERRF). In an investigation of the protective role of taurine in a rat model of stroke it was shown that taurine preserves mitochondrial function and prevents the cell death mediated by the mitochondrial pathway of apoptosis (Sun *et al*., 2011).

2.7.3.2 *Role of taurine in endoplasmic reticulum stress*

The accumulation of misfolded proteins leading to endoplasmic reticulum stress interferes with neuronal signaling and induces neuronal cell death (Kumari *et al*., 2013). Under such conditions of neuronal stress, the unfolded protein response (UPR) becomes activated to restore normal cellular function by activating three signaling systems: PKR-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE 1), activation transcription factor 6 (ATF 6), which then regulate protein synthesis during endplasmic reticulum stress at both the transcriptional and posttranscriptional levels (Harding *et al*., 2003). In addition to misfolding of proteins, calcium overload and oxidative stress all can lead to endoplasmic reticulum stress.

The accumulation of unfolded proteins within the endplasmic reticulum lumen leads to dissociation of glucose-regulated protein 78 (GRP 78) from PERK, IRE1, and ATF 6, respectively, which is the initiating step in activation of these signaling molecules and their down-stream signaling counterparts. The primary function of PERK, IRE1 and ATF 6 is to activate signaling events to overcome endplasmic reticulum (ER) stress, but under severe conditions of stress when the UPR system fails to restore correct protein folding and processing capacity, PERK, IRE 1 and ATF 6 can also activate apoptosis. This activation is done not directly, but by activating downstream pro-death components, including CHOP, JNK and caspases (Higo *et al*., 2010; Anand and Babu 2012). Taurine is effective in reducing endoplasmic reticulum (ER) stress in a number of neural systems, including PC12 cell cultures, primary neuronal cultures, and human neuroblastoma cell lines. Taurine exerts its neuprotective function in part by restoring the integrity of the structure and function of the endplasmic reticulum. Treatment with taurine under

conditions of oxidative stress, excitotoxicity, or hypoxic stress results in a decrease in levels of expression of a number of endplasmic reticulum stress proteins including Grp 78, CHOP/GADD153, p-IRE and p-eI0046–2 alpha protein, and caspase-12. It also occurs as a decreased ratio of cleaved ATF6 and full-length ATF6, and a decreased ratio of Bax/Bcl2 (Pan *et al*., 2011).

2.7.3.3 *Role of taurine in apoptosis*

Taurine has the ability to down-regulate several molecules that trigger apoptosis (Kumari *et al*., 2013). In a recent study on neuronal cell cultures, taurine treatment resulted in decreased levels of the Bax to Bcl-2 ratio after exposure to glutamate. Taurine was able to prevent the decline in Bcl-2 expression in these cultures in the presence of glutamate. A major route by which taurine can regulate apoptosis is by decreasing intracellular free calcium through inhibiting different types of calcium channels (Leon *et al*., 2009) and also by increasing Ca^{2+} levels in mitochondria. Furthermore, beyond its reported antioxidant role there is substantial evidence that taurine acts on apoptotic components and helps to restore the cellular Bcl-2 pool. Previous studies have shown that taurine prevents the release of cytochrome c from mitochondria and also suppresses the assembly of the Apaf 1/caspase- 9 apoptosome complex, preventing caspase-9 activation. Further studies investigating this pathway revealed that taurine regulates the interaction of Apaf 1 and caspase-9 through Akt. In an investigation of morphine-induced toxicity in C6 glioma cells, taurine reversed the depletion of Bcl-2 levels in conjunction with increasing the activities of superoxide dismutase, catalase, and glutathione peroxidase. Thus, taurine

contributes to preventing the oxidative insult that results in morphine-induced apoptosis (Zhou *et al*., 2011).

2.7.4 Antinociceptic effects of taurine

Taurine has been shown to be antinociceptive. It is effective in the acetic acid test, reduces nociceptive behaviour in the mouse formalin test, in rat tail-flick test and autonomy after neurectomy in rats. It inhibits the biting and scratching behaviours, elicited by intrathecal administration of substance P and strychnine-induced scratching and self-biting in rats. This inhibition occurs possibly by competing for strychnine binding, and intrathecal NMDA and kainate-induced nociception in mice (Hornfeldt *et al*., 1992). The analgesic effects manifest themselves at the spinal cord level, where taurine modulates nociceptive impulses generated by the action of substance P. The analgesic effects are dose-dependent and at least opioid and GABAergic mechanisms are involved.

2.7.5 Anticonvulsive actions of taurine

The inhibitory nature of taurine has led to the concept that this molecule may function as an endogenous anti-convulsive agent. Experiments with various animal seizure models have proved the efficacy of taurine. Clinical trials with human patients in general have shown that about one third of epileptic patients benefit by administration of taurine, at least temporarily (El-Idrissi *et al*., 2003). In a rat model of penicillin-induced seizures, the behaviour, frequency and amplitude of EEG was improved markedly by taurine administration, while diminished brain taurine levels markedly lowered the threshold for

seizure activity. Taurine has also inhibited the wet-dog shakes produced by [D-Ala2, Met3] enkephalinamide, seizures in amygdaloid kindled rats, and convulsions induced by hypoxia (Yang *et al*., 2006).

2.7.6 Role of taurine in thermoregulation

Taurine administered by intracerebroventricular injection induces dose-related hypothermia in rabbits, accompanied by depression of gross motor behaviour, whereas the taurine antagonist TAG increases the core temperature (Sgaragli *et al*., 1994). It is assumed that a specific taurine recognition site; that is, a taurine receptor in the rabbit brain is responsible for the taurine effects on thermoregulation. The involvement of brain calcium metabolism in the action of taurine in mammalian thermoregulation has also been suggested (Sgaragli *et al*., 1994).

2.7.7 Role of taurine in osmoregulation

Osmoregulation is a phylogenetically oldest function of taurine that is next to bile salt synthesis. This is clearly an important function in many invertebrates and fishes. There is increasing evidence that it may be a similarly important function of taurine in mammals. Membrane excitability is mainly derived from ionic imbalances across the membrane. If the consequential osmotic imbalances are left uncorrected, alterations in cell volume results due to the free permeability of plasma membranes to water (Huxtable, 1992). Increased cell volume leads to membrane rupture, while both increases and decreases in cell volume cause concentration changes in a plenitude of cell constituents and disruption of the biochemical processes sustaining viability (Huxtable, 1992). An area of progress in

the taurine field over the past decade arised from the realisation that cell osmoregulation is probably a significant function of taurine in mammals also. In mammalian heart and brain, taurine can be the organic osmolyte present in highest concentration. The largest shift in osmolar equivalents within a cell in response to osmotic stress is contributed by taurine. This is as true of mammalian cells, responding to a change in cell environment as of marine organisms, responding to a change in salinity (Trachtman *et al*., 1988). Thus, dilution of the incubation medium results in a release of taurine by a Na^+ - independent mechanism.

Taurine concentration is typically high in mammalian sperm and seminal fluid, and it is possible that taurine is serving an osmoprotective function. In hamsters, fluids in the reproductive tract are hyper-osmolar, having values up to 400 mosmol/kg. Hyposmotic conditions kill sperm, but chimpanzee sperm are protected by the addition of 2 mM taurine. The high-affinity transport system for taurine in mice myocytes responds sensitively to changes in osmolarity. Water deprivation leads to increased taurine concentrations (per g protein) in muscle, brain, and platelets, suggesting an osmoregulatory function for the compound. The loss of taurine from isolated retinas varies inversely with osmolarity. The excitotoxin-induced release of taurine from retina is stimulated by both K^+ and Cl^- (Pasantes-Morales *et al*., 1989).

As the oedema produced by excitotoxins is thought to be a consequence of passive influx of Cl^- accompanying increased Na^+ conductance, Cl^--enhanced taurine efflux may be a link between cell volume changes and the initiation of an osmoregulatory response.

Taurine has often been shown to protect various functions of retinal rod outer segments. Rod cells are one of two types of photoreceptors in the retina, the other type being cone cells. The outer segments of rod cells can be readily isolated. Each outer segment consists of from l, 000-2,000 disks, stacked like dinner plates, surrounded by a membrane (Pasantes-Morales *et al*., 1989). The rod, outer segment is biochemically hyperactive. New disks are continuously biosynthesized at one end of the rod and are continually shed from the other end in groups of 8-30. The protective effect of taurine is complex and appears to involve not only osmoregulatory, but antioxidant and ion regulatory activities (Pasantes-Morales *et al*., 1989).

Light exposure causes an increase in diameter in rods outer segment, probably secondary to alterations in ionic fluxes. Taurine, which is found in these structures in high concentration, may be a regulator of these volume changes. Under certain conditions, ferrous sulphate causes peroxidation, swelling, and disruption of rod outer segments. Swelling can occur secondarily to lipid peroxidation. Taurine and zinc sulphate together protect against the swelling without affecting malondialdehyde formation, an index of degree of peroxidation. β-alanine also protected against the swelling, suggesting an osmoregulatory action of these β-amino acids. The brain is particularly vulnerable to osmotic disturbances (Olney *et al*., 1986). Cerebral oedema is a serious condition, leading to seizures and other sequelae, that is hard to treat. Taurine has anticonvulsant actions in a wide variety of experimental seizure states. These actions may stem, in part, from its osmoregulatory action, although an endogenous anticonvulsant action has not been demonstrated for taurine. The mammalian brain alters cell amino acid concentrations in

response to hypo- and hyper-osmotic conditions, decreasing them in the former and increasing them in the latter condition (Baxter *et al*., 1986). During the regulatory phase, astrocytes under hyposmolar conditions show a marked and unique increase in taurine efflux, which depletes cell taurine content by up to 64 % (Olney *et al*., 1986).

The toxicity of taurine is usually assumed to be negligible, but such findings suggest that exposure to high amounts of taurine may carry cryptic risks. The swelling of astrocytes leads to a proportional depolarization of membrane potential. This is an indication that the osmoregulatory and electrical activities of cells are interdependent phenomena. The frequently studied effects of taurine on ion currents may, thus, be closely connected with its osmotic actions. Taurine is also toxic to cultured cerebellar cells from kittens, lowering survival rate (Trenkner and Sturman, 199l). In mice cells, however, taurine supports cell survival. Water intoxication both increases extracellular taurine concentrations and decreases intracellular levels (Solis *et al*., 1988). A decrease in extracellular osmolality of as little as 10 mosmol/kg leads to detectable increases in taurine concentrations. A 15-fold increase occurs at a 105-mosmol/kg decrement. Micro-dialysis of the brain with hyposmotic solutions resulted in marked increases in extracellular taurine concentrations. No other amino acid was affected at decreased osmolalities of up to 50 mosmol/kg. If osmolality is decreased further, then an increased release of other amino acids is found (Trenkner and Sturman, 199l).

2.8 Taurine as an Antioxidant and Antistress Agent

The antioxidant role of taurine has been attributed to its ability to scavenge ROS, to reduce the production of lipid peroxidation end-products, and to stabilize biomembranes (Schaffer *et al*., 2003; Franconi *et al*., 2004). It has become increasingly apparent that oxidative stress plays a major role in a broad range of human diseases. The overproduction of ROS and the body's inability to stem the accumulation of highly reactive ROS have been implicated in cardiovascular disease (Chen *et al*., 2012), diabetes-induced renal injury, inflammatory disease, light-induced lipid peroxidation in photoreceptors, reperfusion injury and several of the major CNS disorders. In each case, taurine, by virtue of its antioxidant activity plays a crucial role as a cytoprotectant and in the attenuation of apoptosis. Despite this diversity of pathophysiology in so varied a group of seemingly unrelated disorders, there is a growing consensus that oxidative stress is linked to mitochondrial dysfunction and that the beneficial effects of taurine are as a result of its antioxidant properties (Chen *et al*., 2012), and ability to improve mitochondrial function by stabilizing the electron transport chain and inhibiting ROS generation (Schaffer *et al*., 2009; Jong *et al*., 2012).

The neurotransmitter taurine, similar in structure to GABA, is an excellent stress-relieving option. Taurine inhibits the release of adrenaline, the "stress" neurotransmitter, and is effective for a range of problems, including migraine, agitation, irritability, insomnia, and depression. Found in animals and fish, vegetarians are susceptible to deficiency, and could benefit from supplementing their diet with it. 100-500 milligrams two times daily of taurine was suggested between meals. In another study, it was proposed that taurine attenuated the alterations in the biochemical parameters, partly by

alleviating oxidative stress, augmenting the activities of antioxidant enzymes and exhibiting protective effects on the liver and kidneys (Akande *et al*., 2014b).

CHAPTER THREE

5.0 MATERIALS AND METHODS

3.1 Drugs and Chemical Preparation

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

3.2 Experimental Animals

A total of 48 adult male Wistar rats, weighing 150-200 g, and 48 adult male mice, weighing between 20-25 g, were used in this study. The animals were obtained commercially and assigned randomly to four groups as follows: Group A - served as control and was given 1 mL of distilled water; Group B - received 100 mg/kg taurine; group C - 200 mg/kg taurine; and group D - 400 mg/kg taurine. There were twenty four (24) Wistar rats in acute group and 24 Wistar rats in the chronic group. Also there were twenty four (24) mice in acute group and 24 mice in the chronic group. The animals were

housed in groups of six animals per cage. Regiments were administered orally via gavage (Akande *et al*., 2014a) and all the animals were allowed free access to food and water *ad libitum*. They were maintained under normal room conditions of ambient temperature in the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. All experiments were performed on the day of every experiment (from 0900 hour to 1600 hour) during the light period.

3.3 Methods

3.3.1 Acute restraint stress induction in Wistar rats

Restraint stress was induced according to the method of Smith. (2012), with some modification. Briefly a Perspex restraint cage with dimensions of 14 cm (L) x 5 cm (B) x 6 cm (H) was used in the experiment. Each rat was housed individually in a multi-compartment cage to avoid aggression and prevent social isolation. Unrestrained rats (control group) were left undisturbed in their cages, but without access to food or water for the same restraint period. Acute restraint stress was induced in the rats for 1 hour daily for 14 days (Moazzam *et al*., 2013) by keeping them in a Perspex restraint cages, immobilising up to 6 rats simultaneously without access to food and water during the restraint stress. The rats were pretreated with the various dose rates of taurine depending on the group an hour prior to the commencement of restraint. The stress procedure was carried out daily for 2 weeks in the acute group and 3 weeks daily for chronic group at

the Animal house of the Department of Human Physiology, Ahmadu Bello University Zaria, throughout the experimental period between 0900 hour and 1600 hour of the day.

3.3.2 Acute restraint stress induction in mice

Restraint stress was induced according to the method of Smith. (2012) with some modification. Briefly a Perspex restraint cage with dimensions of 5 cm (L) x 3 cm (B) x 3 cm (H) was used in the experiment. Each mouse was housed individually in a multi-compartment cage for the induction of the restraint, to avoid aggression and to prevent social isolation. Unrestrained mice (control group) were left undisturbed in their cages, but without access to food or water during the restraint period. The mice were exposed to acute restraint stress, 1 hour daily for 14 days by keeping them in a Perspex restraint cage, immobilising up to 6 mice simultaneously without access to food and water (Kondam *et al*., 2013). The mice were pretreated with various dose rates of taurine depending on the group an hour prior to the commencement of the restraint. The stress procedure was carried out daily between 0900 hour and 1600 hour at the Animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria, throughout the experimental period.

3.3.3 Chronic restraint stress induction in wistar rats

Chronic restraint stress was induced according to the method of Smith. (2012), with some modification. Briefly, a Perspex restraint cage with dimensions of 14 cm (L) x 5 cm (B) x 6 cm (H) was used in the experiment. Each rat was housed individually in a multi-compartment cage during the restraint period 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. Rats were induced with chronic restraint stress, 6 hour daily and for 21 days (Moazzam *et al*., 2013) in a Perspex restraint cage, restraining up to 6 rats simultaneously without food and water during the restraint period. The rats were pretreated with various dose rates of taurine according to their groups, 1 hour 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, during the experimental period between 0900 hour and 1600 hour

3.3.4 Chronic restraint stress induction in mice

Restraint stress was induced according to the method of Smith. (2012) with some modification. Briefly a Perspex restraint cage with dimensions of 5 cm (L) x 3 cm (B) x 3 cm (H) was used in the experiment. Each mouse was housed individually in a multi-compartment cage for the induction of the restraint, to avoid aggression and prevent social isolation. Unrestrained mice (control group) were left undisturbed in their home cages, but without access to food or water during the restraint period. The mice were exposed to chronic restraint stress, 6 hour daily for 21 days (Kondam *et al*., 2013) by keeping them in a Perspex restraint cage, immobilising up to 6 mice simultaneously without access to food and water. The mice were pretreated with taurine at the dose rate depending on group, an hour 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, during the experimental period between 0900 hour and 1600 hour.

3.4 Neurobehavioural Studies

Neurobehavioural studies were assessed in both the acute and chronic restrained-stressed groups as follows:

3.4.1 Learning (Step-down passive-avoidance test)

The effect of restraint stress on learning task in rats and the possible ameliorative effect of antioxidant taurine were assessed 48 hours to the termination of the study using the step-down inhibitory avoidance learning task as described by Zhu *et al*. (2001). The apparatus used for the learning test was a 40 × 25 × 25 cm acrylic chamber, consisting of a floor made of parallel 2-mm calibre stainless steel bars, spaced 1 cm apart. An electric shock was administered through the floor bars. A 2.5-cm-high, 8 × 25 cm wooden platform was placed on the left extreme of the chamber. Each animal was gently placed on the platform. Upon stepping down, the rat immediately received a single 80-volt foot-shock. If the animal did not return to the platform, the foot-shock was repeated every 5 seconds. A rat was considered to have learned the avoidance task, if it remained on the platform for more than 4 minutes. The number of foot-shocks applied before the animal learned the avoidance task was recorded as an index of learning acquisition.

3.4.2 Short-term memory

Short-term memory was assessed in individual rat from each group using the step down avoidance inhibitory task as described by Zhu *et al*. (2001). The apparatus used for the memory test consisted of 40 × 25 × 25 cm acrylic chamber, with the floor made of parallel 2-mm-calibre stainless steel bars spaced 1 cm apart. A 2.5-cm-high, 8 × 25 cm wooden platform was placed on the left extreme of the chamber. In this case, the rat was again placed gently on the platform 24 hours after performing the learning task. The time an animal remained on the platform was recorded as an index of memory retention. Staying on the platform for 2 minutes was counted as maximum memory retention (ceiling response).

3.4.3 Elevated plus-maze

The elevated plus maze apparatus was made up of smooth brown opaque platforms with two open arms (50 x 10 cm) and two closed arms of the same size. The wall of the chamber was 40 cm high and the whole apparatus was elevated 50 cm above the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer-latency (TL) was taken as the time taken by mouse to move into one of the covered arms with all its four legs. The TL was recorded on the first day. Cut-off time observed was 90 s. The mouse was allowed to explore the maze for another 10 s and then returned to its home cage. Memory retention was examined 24 hours after the 1st day trial on the 2nd day.

3.4.4 Test for exploratory activity in mice (hole-board test)

The exploratory activity in mice pretreated with taurine was determined using the hole-board test (File and Wardil, 1975). The apparatus used consisted of a brown wooden board (40 x 40 cm) with four equidistant holes (1 cm diameter x 2 cm depth). Each mouse was placed singly at one corner of the board. It was allowed to move about and dip its head into the holes. Poking the nose into a hole is a typical behaviour of the mouse, indicating a certain degree of curiosity. The number of dips in five minutes (enough time to exhibit curiosity otherwise) was recorded. The test was carried out 1 hour after oral treatment with taurine at the doses 100, 200 and 400 mg/kg.

3.4.5 Mouse beam-walking assay

The method offered improved sensitivity over the mouse Rota rod in determining motor coordination deficits, induced by psychotropic agents (Stanley *et al.*, 2005). Mice were allowed to walk from a start platform along a ruler (80 cm long and 3 cm wide) elevated 30 cm above the bench by metal supports to a goal box (enclosed Hamster house). Several trials were performed for each mouse and designed such that the mice tested were aware that there was a goal box that could be reached. A ruler was used because the mouse found this easy to cross and at the same time, it induced minimum anxiety. Once the animals have been tested on the ruler, they were moved immediately to the beam. The beam was made of a wood, 8 mm in diameter, 60 cm long and elevated 30 cm above the bench by a metal support. The animals were placed at one end of the beam and allowed to

walk to the goal box an hour after treatment with the taurine. Mice that fell were returned to the position they fell from, with a maximum time of 60 seconds allowed on the beam. The number of foot slips (one or both hind limbs slipping from the beam) were recorded with the aid of tally counter. The number of foot slips is a measure of motor coordination deficit (Stanley *et al.*, 2005).

3.5 Evaluation of Haematological Parameters

Rats from each group were sacrificed after light ether anaesthesia by severance of the jugular veins at the end of the acute and chronic restraint stress. Two-and-half milliliters (2.5 ML) of blood sample were collected from each rat into a sample bottle containing ethylenediamine tetraacetic acid (EDTA) (Plasti Lab Sarl, Beirut, Lebanon). The samples were evaluated for haematological parameters; haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) mean corpuscular haemoglobin concentration (MCHC), total white blood cell (WBC) count and platelet count. The evaluation was conducted with an automatic hematological assay analyzer, Adva 60® Hematology System (Bayer Diagnostic Europe Ltd., Dulbin, Ireland). Blood smears were stained with Geimsa for the differential WBC counts. The neutrophil-lymphocyte ratio (N/L) ratio was also calculated.

3.5.1 Neutrophil/lymphocyte ratio

The N/L ratio was determined according to the method of Proctor *et al.* (2011), by first estimating the total and differential leucocyte counts and by applying the following formulae:

$$\text{Absolute count of neutrophils} = \frac{\text{TLC x \% Neutrophils}}{100}$$

$$\text{Absolute count of lymphocytes} = \frac{\text{TLC x \% Lymphocytes}}{100}$$

Where TLC = Total Leucocyte Count

3.5.2 Erythrocyte osmotic fragility

The erythrocyte osmotic fragility (EOF) was determined as described by Faulkner and King (1970). Briefly 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 mililitres (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 pipetted 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 ^{o}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 formula:

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

3.6 Preparation of Brain Homogenate

The rats from each group were sacrificed after light ether anaesthesia by severance of the jugular veins at the end of the acute and chronic restraint stress. The brain tissues were excised from each rat in the four groups, and were rinsed immediately with physiological saline and patted dry with filter paper. Subsequently, a known weight of one-half of the brain samples from each rat in the experimental groups were homogenised in a known volume of ice-cold phosphate-buffered saline (PBS, pH 7.4) in a 1:10 (w/v) ratio into the homogenates with pestle and mortar. The brain homogenates were kept cold with ice baths and were centrifuged at 3000 x g for 10 minutes to obtain the supernatant. The supernatants were used for the evaluation of brain malondialdehyde (MDA) concentration, and activities of antioxidant enzymes and acetylcholinesterase.

3.7 Estimation of Lipid Peroxidation Biomarker, Malondialdehyde

The concentration of MDA as an indication of lipid peroxidation was evaluated in the fore brain homogenate samples using the method of 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. Briefly 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 bath 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, United Kingdom) at 532 nm. The concentrations 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).

3.8 Assessment of Activities of Antioxidant Enzymes in the Brain Homogenate

A total of 48 Wistar rats were used for estimation of biomarkers of oxidative stress.

3.8.1 Superoxide dismutase (SOD)

Activity of superoxide dismutase in the rat brain homogenate was assessed using the NWLSSTM SOD activity assay kit (Product NKW-SOD02, Specificty: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/Ml). The assay kit was based on the principle of inhibition of auto-oxidation of haematoxylin (Martin *et al*., 1987).

Procedure

Plasma (0.5 Ml) was diluted to 1.0 mL with distilled water, and 250 μL of chilled ethanol and 150 μL of chilled chloroform were added. The mixture were shaken and centrifuged. The supernatant were used for the assay of enzyme activity. To 1.2 mL of the supernatant

was added 1.5 mL of 0.1 mol/L of carbonate-bicarbonate buffer, pH 10.2, containing 0.2 mmol/L EDTA. The contents were mixed, and the reaction was initiated by adding 200 μL of adrenaline (pH 3.0, 3 mMol/L) to the buffered reaction mixture. The change in optical density per minute was measured at 470 nm. The enzyme activity were then expressed as unit per milligram of protein, where 1 .U. was defined as the enzyme concentration required for the inhibition of 50% of adrenaline auto-oxidation in 1 minute under the assay conditions.

3.8.2 Catalase (CAT) activity

Catalase activity was analyzed using the NWLSSTM CAT activity assay kit (Product NWK-CAT01), Specificity: Catalase, Sensitivity: 6.0 U Catalase/mL). Catalse activity was measured; based on the principle of catalase consumption of H_2O_2 substrate at 240 nm (Beers and Sizer, 1952).

Procedure

0.1 mL of plasma and 1.5 mL of phosphate buffer were added. To this, 0.4 mL of hydrogen peroxide was added and the reaction was arrested after 30 and 60 second by the addition of 0.2 mL dichromate acetic acid reagent. A control was also carried out simultaneously. All the tubes were heated in a boiling bath for exactly 10 minutes, cooled and absorbance read at 620 nm. Standard in the range of 2-10 mMol were taken and

processed as the test. The activity of catalase was expressed as IU/L of hydrogen peroxide consumed/ min/ mg of protein.

3.8.3 Glutathione peroxidase (GPx)

Glutathione peroxidase activity was assessed using NWLSS^{TM} cGPx (Gpx1) Enzyme-linked immunosorbent assay (ELISA) assay kit (Product NWK-GPX02, Specificty: Glutathione peroxidase, Sensitivity: 12.5 pg/mL). The NWLSS^{TM} cGPx assay was based on a sandwich ELISA, where GPx concentration was determined by comparing the 450 nm absorbance of sample wells to that of known standards (Takebe, 2002). The assays were conducted according to the manufacturer's (Northwest Life Science Specialities, LLC, Vancouver, WA 98662) instructions

3.9 Evaluation of Brain Acetylcholinesterase Activity

The brain homogenates of the rats were evaluated for acetylcholinesterase assay (Ellman *et al*., 1961). The method was 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, weighted 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 µL; 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 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 formula:

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

Where 5.74×10^{-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.

3.10 Evaluation of Activities of Serum Liver Enzymes

3.10.1 Alanine aminotransferase and aspartate aminotransferase

The Alanine aminotransferase and aspartate aminotransferase activities were determined by the method of Reitman and Frankel (1957). This method uses a coupled enzyme assay to measure ALT and AST activities in serum or plasma. The AST catalyses the transfer of an amino group from L-aspartate to 2-oxoglutarate to form oxaloactate and L-glutamate. Oxaloacetate spontaneously decarboxylates to form pyruvate under the strongly acidic conditions. The ALT catalyses the transfer of an amino group from L-alanine to 2-oxoglutarate to form pyruvate and L-glutamate. An increase in pyruvate concentration corresponds with the levels of AST and ALT activities. The pyruvate concentration was determined spectrophotometrically in the form of hydrazone, which was produced by reaction with 2,4-dinitrophenylhydrazine in an alkaline medium. The pyruvate hydrazone absorbs at 510 nm more than 2-oxoglutarate hydrazone.

Procedure

Two mililitre of reaction medium was added to 0.2 mL of 2-oxoglutarate. After pre-incubation for 15 minutes of 0.3 mL serum, without or with supplementary pyridoxal-5'-phosphate, the reaction was started by adding serum or serum + coenzyme to the otherwise complete reaction medium. The decrease in absorbance at 340 nm was measured every minute for 10 minutes. Enzymes activities were calculated from the mean absorbance change from the fourth to the ninth minute.

3.10.2 Alkaline phosphatase

Serum alkaline phosphate activity was assayed as described by Bassey *et al*. (1946). In the presence of magnesium ions, *p*-nitrophenylphosphate is hydrolysed by phosphatases to phosphate and *p*-nitrophenol. The rate of *p*-nitrophenol liberation was proportional to the ALP activity and measured photometrically.

Procedure

Serum samples (5 cm) were transferred to the bottom of 6 x 50 mm tubes in a wire rack. The rack was immersed in a shallow pan of ice water and to each tube 50 cm of ice-cold reagent was rapidly added, with a constriction pipette. Each tube was mixed by tapping with the finger. Care was taken not to warm the tube by so doing. The whole rack of tubes was then immersed in a water bath at 38^{o}C at 2 depths sufficient to cover the bottom half of the tubes. After exactly 30 minutes the rack was again placed in the pan of ice water and 0.5 mL of 0.02 NaOH was added to each tube with sufficient force to mix the sample. This addition stops the reaction and the samples which were now transferred

to calorimeter tubes were read at 400 to 420 tnp = R. After the initial reading, 2 to 4 c.mm of concentrated HCl was added with a 0.1 mL graduated pipette (drawn out tip) and a second reading, R1, was made. R1 and R2 are converted into optical densities (- log transmission or 2 –log percent transmission) = D1 and D. Then D, - D, = D, is the corrected density. Standard and blanks were provided by treating 5 c.mm volumes of the standards and of distilled water exactly as though they were serum samples.

The corrected densities (CD) were used to construct a standard curve from which the serum values were calculated. Since sera and standards undergo the same dilution, it is unnecessary to take into account the exact volumes of the various plates. It is to be noted that D, is the density corrected for possible residual absorption after acid addition, but this is not corrected for the D, of the blank analysis. This second necessary correction is automatically provided by the standard curves. A "million unit" is defined as the phosphatase activity which will liberate 1 Mm of nitrophenol per liter of serum per hour. Therefore, since the standard incubation time is only 30 minutes, the 1, 2, 4, and 6 mM standards are equivalent to sera with activities of 2, 4, 8, and 12 mM units.

3.11 Determination of Total Protein

Serum total protein concentration was estimated using Bio-Rad protein assay kit based on the principle of Bradford (Bradford, 1976). The procedure is a dye-binding technique in which a different colour change of a dye occurs according to the concentrations of protein in the samples. The colour change, which has a maximum absorbance at 595 nm, was

measured spectrophometrically. Serum albumin concentration was determined by the method of 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.

Procedure:

The standard ptotocol can be performed in three different formats, 5 mL and a 1 mL Cuvette assay and a 250 µL microplate assay. The linear range of these assays for Bovine serum albumin (BSA) is 125-1,000 µg/mL, whereas with gamma-globulin the linear range is 125-1,500 µg/mL. The 1x dye reagent was removed from 4^{o} C storage and allowed to warm to ambient temperature. The 1x dye reagent was inverted for a few times before use. Each standard and unknown sample solution was pipetted into separate clean test-tubes or microplate wells (the 1 mL assay may be performed in disposable cuvettes). The 1x dye reagent was added to each tube (or cuvette) and vortex (or inverted) and samples were mixed with the microplate mixer. Incubate at room temperature for atleast 5 minutes. Samples should not be incubated longer than 1 hour at room temperature. The spectrophotometer was set to 595 nm. Zero the instrument with blank sample (not required for microplate readers). The absorbance of the standard and unknown samples was then measured.

3.12 Statistical Analysis

All data were expressed as Mean ± SEM and were analyzed by Statistical Package for Social Science software (version 20) using one-way analysis of variance (ANOVA) with

multiple comparisons. Tukey's *post-hoc* test was used to determine the difference between groups. Values of $P < 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Neurobehavioural Studies

4.1.1 Effect of taurine on learning and memory (Step-down avoidance test) on Wistar rats subjected to acute and chronic restraint stress

The acute restraint stressed group that received taurine at 100 mg/kg showed significant ($P < 0.05$) decrease in number of shocks when compared with doses 200 and 400 mg/kg

taurine. However, there was no significant difference with 200 and 400 mg/kg taurine as compared with the control group. The step down latency showed significant ($P < 0.05$) increase at 100, 200 and 400 mg/kg taurine as compared with the control group. The chronic restraint stressed groups also showed significant ($P < 0.05$) decrease in number of shocks in groups that received 100, 200 and 400 mg/kg taurine as compared with the control group. There was significant ($P < 0.05$) increase in step down latency in the three chronic restraint stressed groups that received 100, 200 and 400 mg/kg taurine, when compared to the control group (Table 4.1).

Table 4.1: Effect of taurine on learning and memory using step-down avoidance test on Wistar rats subjected to acute and chronic restraint stress

	Acute groups		Chronic groups	
Treatments	**NS**	**SDL (Sec.)**	**NS**	**SDL (Sec.)**
Control (DW)	3.67 ± 0.40^{a}	207.17 ± 1.58^{a}	2.50 ± 0.34^{a}	206.33 ± 1.73^{a}
Taurine:				
100 mg/kg	1.67 ± 0.21^{b}	214.00 ± 4.76^{b}	1.83 ± 0.40^{a}	228.00 ± 7.03^{b}
200 mg/kg	3.50 ± 0.76^{a}	216.67 ± 8.73^{b}	1.17 ± 0.17^{c}	230.67 ± 10.72^{b}

400 mg/kg	2.17 ± 0.65^{b}	228.50 ± 9.98^{c}	1.83 ± 0.31^{b}	223.17 ± 7.84^{b}

Note: values are mean ± SEM, n = 6. DW (distilled water), NS (number of shocks) and SDL (step down latency). a,b,c, = values with different superscript differ significantly ($P < 0.05$).

4.1.2 Effects of taurine on learning and memory (elevated plus-maze) on mice subjected to acute and chronic restraint stress

In acute group, there was significant ($P < 0.05$) decrease in the duration of acquisition and retention in all the three restraint stressed groups that were administered 100, 200 and 400 mg/kg taurine when compared to the control group. Also in the chronic restraint-stressed groups, there was significant ($P < 0.05$) decrease in the duration of acquisition and retention in all the three restraint stressed groups that were administered 100, 200 and 400 mg/kg taurine when compared with the control group (Table 4.2).

Table 4.2: Effects of taurine on learning and memory using elevated plus-maze on mice subjected to acute and chronic restraint stress

	Acute Restraint Stress		Chronic Restraint Stress	
Treatments	**Acquisition (Sec.)**	**Retention (Sec.)**	**Acquisition (Sec.)**	**Retention (Sec.)**
Control (DW)	48.83 ± 10.03^{b}	22.00 ± 3.79^{b}	77.17 ± 8.13^{b}	47.00 ± 10.12^{b}
Taurine:				
100 mg/kg	43.00 ± 10.20^{a}	21.83 ± 10.99^{b}	71.67 ± 12.95^{a}	22.33 ± 2.47^{a}
200 mg/kg	37.50 ± 10.79^{c}	17.33 ± 3.72^{a}	54.17 ± 7.35^{c}	22.50 ± 7.45^{a}

400 mg/kg	34.67 ± 4.52^{d}	19.50 ± 7.29^{c}	47.50 ± 12.50^{d}	21.83 ± 15.28^{a}

Note: values are mean ± SEM, n = 6, DW (distilled water) a,b,c,d = values with different superscript differ significantly ($P < 0.05$).

4.1.3 Effects of taurine on exploratory activity in mice (hole-board test) subjected to acute and chronic restraint stress

The acute restraint stressed group showed significant ($P < 0.05$) increase in number of head dips at 200 mg/kg taurine when compared with doses of 100 and 400 mg/kg taurine, and the control group. The chronic restraint stressed group showed significant ($P < 0.05$) increase in number of head dips at 200 mg/kg taurine when compared with the control group (Table 4.3).

Table 4.3: Effects of taurine on exploratory activity in mice subjected to acute and chronic restraint stress using hole-board test

	Acute Restraint Stress	Chronic Restraint Stress
Treatments	**Number of head dips**	**Number of head deeps**
Control (DW)	15.33 ± 2.95^{a}	15.33 ± 2.17^{a}
Taurine:		
100 mg/kg	14.00 ± 0.58^{a}	17.00 ± 2.71^{a}
200 mg/kg	22.00 ± 1.24^{b}	18.17 ± 1.49^{b}
400 mg/kg	12.33 ± 1.33^{a}	16.67 ± 1.17^{a}

Note: values are mean ± SEM, n = 6, DW (distilled water) a,b = values with different superscript differ

significantly ($P < 0.05$).

4.1.4 Effects of taurine on motor coordination in mice (beam-walk assay) subjected to acute and chronic restraint stress

In the acute restraint stressed group, there was significant ($P < 0.05$) decrease in time spent on the beam in groups that recieved 100, 200 and 400 mg/kg taurine when compared to the control group. There was also a significant ($P < 0.05$) decrease in number of foot slips at 200 and 400 mg/kg taurine when compared with the control group. In the chronic restraint stressed group, there was significant ($P < 0.05$) decrease in duration spent on the beam in the group that received 100, 200 and 400 mg/kg taurine when compared with the control group. There was significant ($P < 0.05$) decrease in number of foot slips at 200 and 400 mg/kg taurine when compared to control group. However, there was significant ($P < 0.05$) increase in number of foot slips at dose of 100 mg/kg taurine in both acute and chronic restraint stressed groups (Table 4.4).

Table 4.4: Effects of taurine on motor coordination in mice (Beam-walk assay) subjected to acute and chronic restraint stress

	Acute Restraint Stress		Chronic Restraint Stress	
Treatments	**Latency (Sec.)**	**Number of foot slips**	**Latency (Sec.)**	**Number of foot slips**
Control (DW)	54.16 ± 3.27^{a}	09.67 ± 1.28^{a}	50.00 ± 4.28^{a}	4.83 ± 0.31^{a}
Taurine:				
100 mg/kg	41.67 ± 3.07^{b}	13.67 ± 0.56^{b}	40.83 ± 5.23^{a}	8.33 ± 1.12^{b}
200 mg/kg	30.33 ± 2.17^{c}	05.67 ± 1.67^{c}	$36.67 \pm 5.27^{a,b,d}$	3.50 ± 0.22^{c}

400 mg/kg	$38.83 \pm 7.33^{b,c}$	05.83 ± 0.75^{d}	08.33 ± 2.83^{e}	4.67 ± 0.42^{a}

Note: values are mean ± SEM, n = 6, DW (distilled water) a,b,c,d,e = values with different superscript differ significantly ($P< 0.05$).

4.2 Haematological Indices

4.2.1 Effects of taurine on red blod cells and erythrocytic indices of Wistar rats subjected to acute restraint stress

There was a significant ($P < 0.05$) increase in red blood cell counts at doses of 100, 200 and 400 mg/kg taurine compared to the control group (Figure 4.1). There was significant ($P < 0.05$) increase in haemoglobin concentration at doses of 100, 200 and 400 mg/kg taurine treated groups when compared to the control group (Figure 4.2). The result also revealed significant a ($P < 0.05$) increase in packed cell volume at doses of 100, 200 and 400 mg/kg taurine compared with control group (Figure 4.3). There was a significant ($P <0.05$) increase in mean corpuscular volume in 100 mg/kg taurine group, while at the 200 and 400 mg/kg taurine showed a significant decrease compared with control group (Figure 4.4). There was no significant difference in the mean corpuscular haemoglobin in taurine treated groups, when compared with the control group (Figure 4.5). There was significant ($P < 0.05$) decrease in mean corpuscular haemoglobin concentration in 100 mg/kg group when compared with 200 and 400 mg/kg taurine and control groups (Figure 4.6). However there was no significant difference in Erythrocyte osmotic fragility in taurine treated groups, when compared to the control group (Figure 4.7).

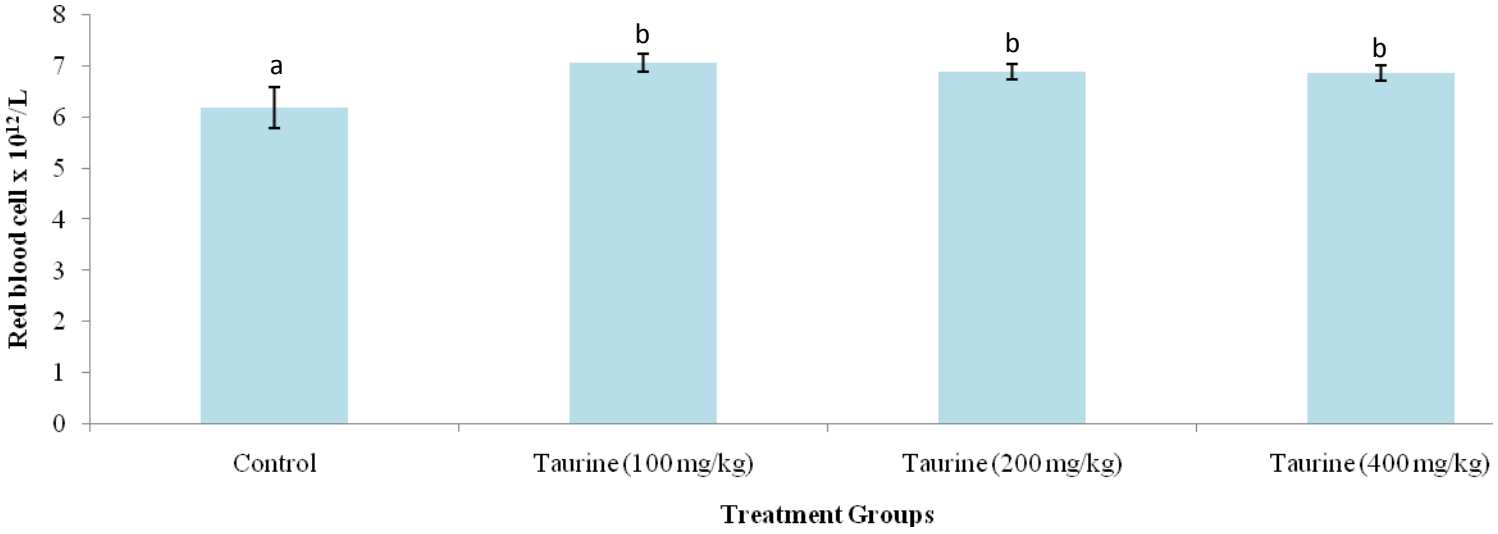


Figure 4.1: Effects of taurine on red blood cell counts of Wistar rats subjected to acute restraint-stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different ($P < 0.05$)

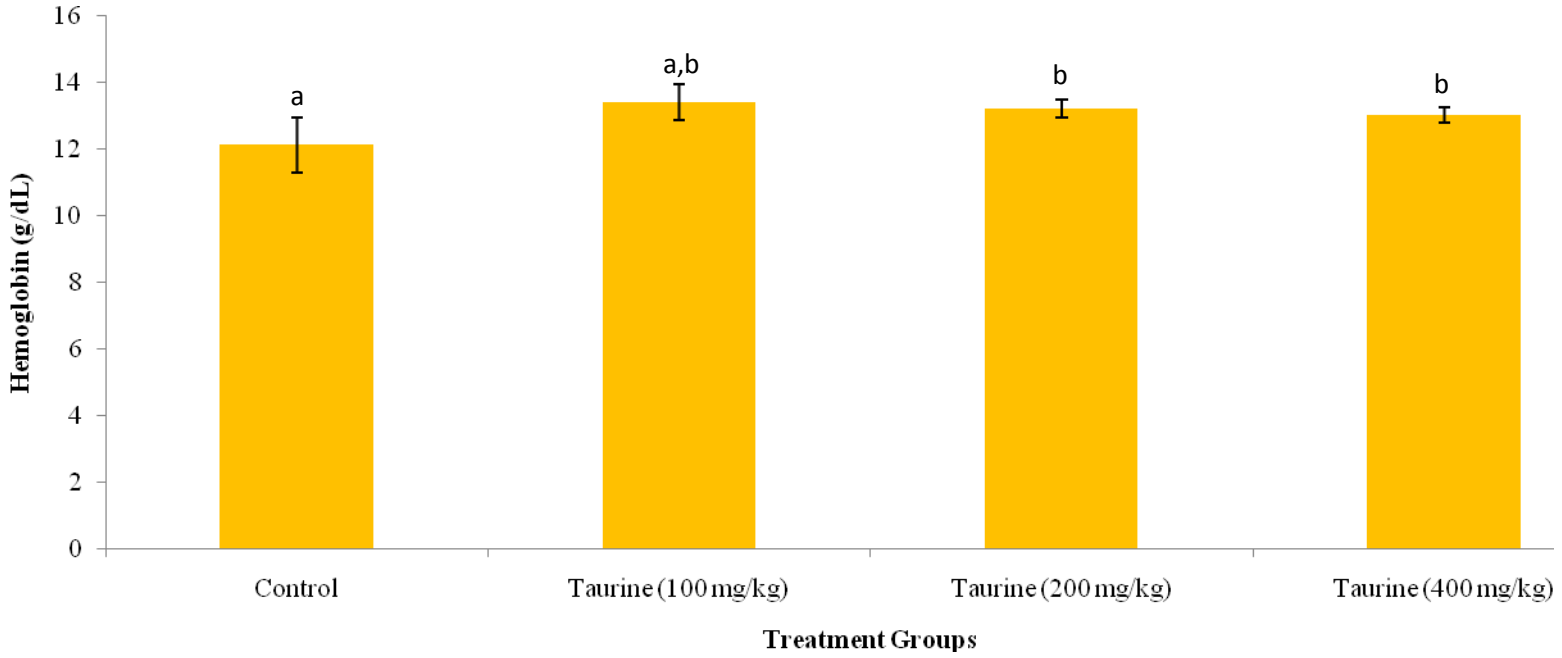


Figure 4.2: Effects of taurine on haemoglobin concentration of Wistar rats subjected to acute restraint stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different ($P < 0.05$)

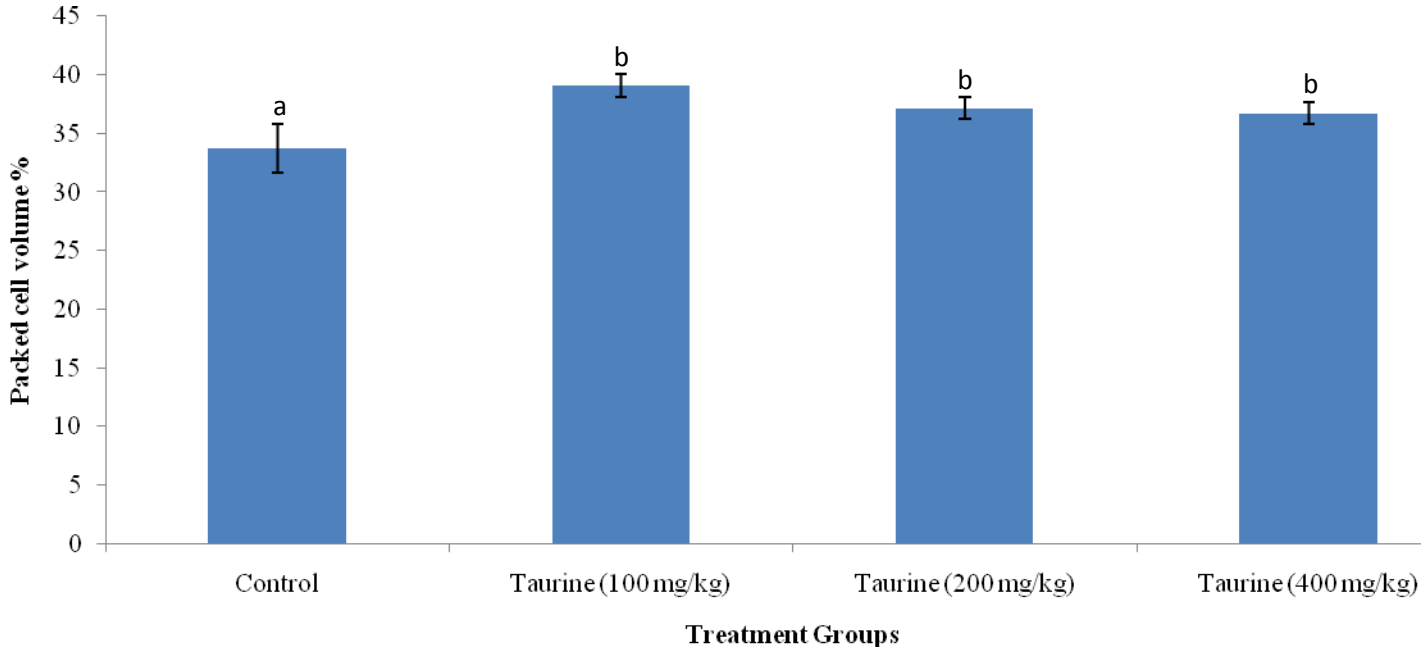


Figure 4.3: Effects of taurine on packed cell volume of Wistar rats subjected to acute restraint stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different ($P < 0.05$)

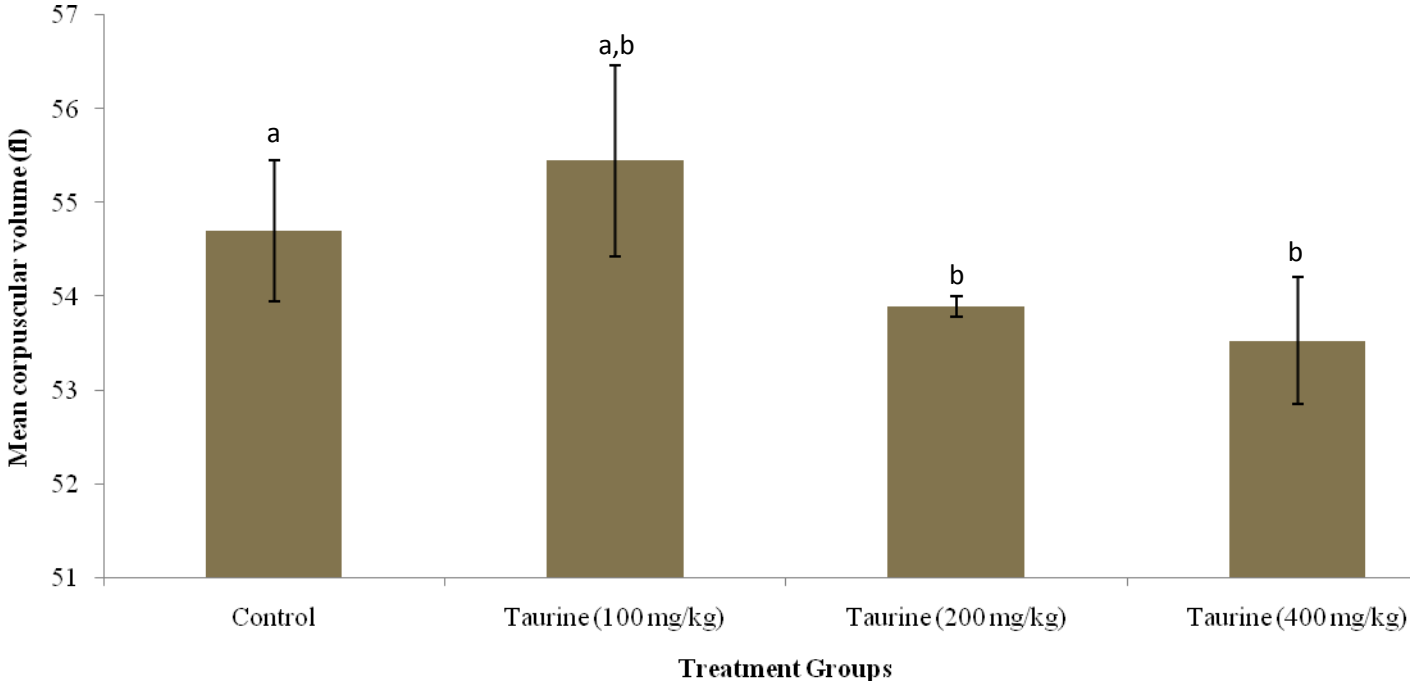


Figure 4.4: Effects of taurine on mean corpuscular volume of Wistar rats subjected to acute restraint-stress

Note: values are mean ± SEM, n = 6

a,b = means with different superscript letters are significantly different ($P < 0.05$).

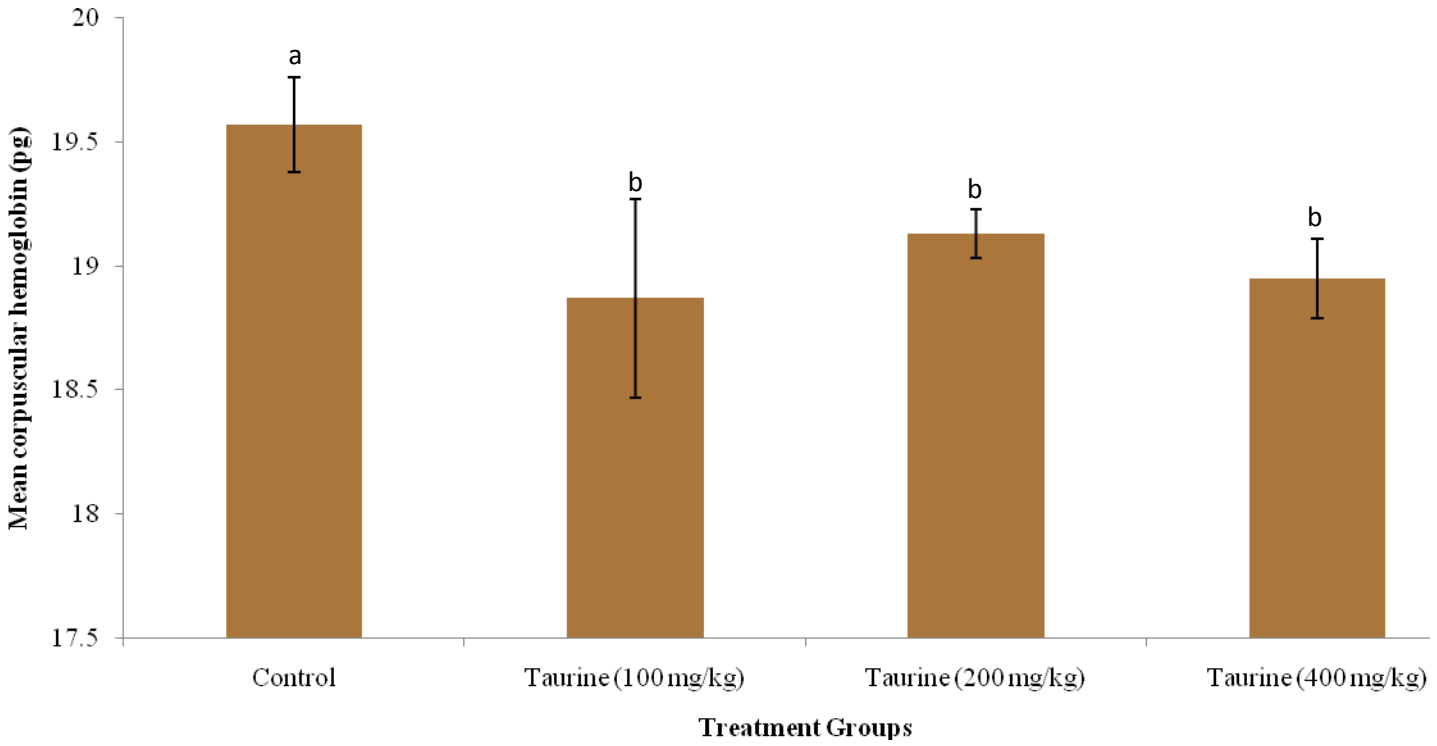


Figure 4.5: Effects of taurine on mean corpuscular haemoglobin of Wistar rats subjected to acute restraint stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different (P < 0.05).

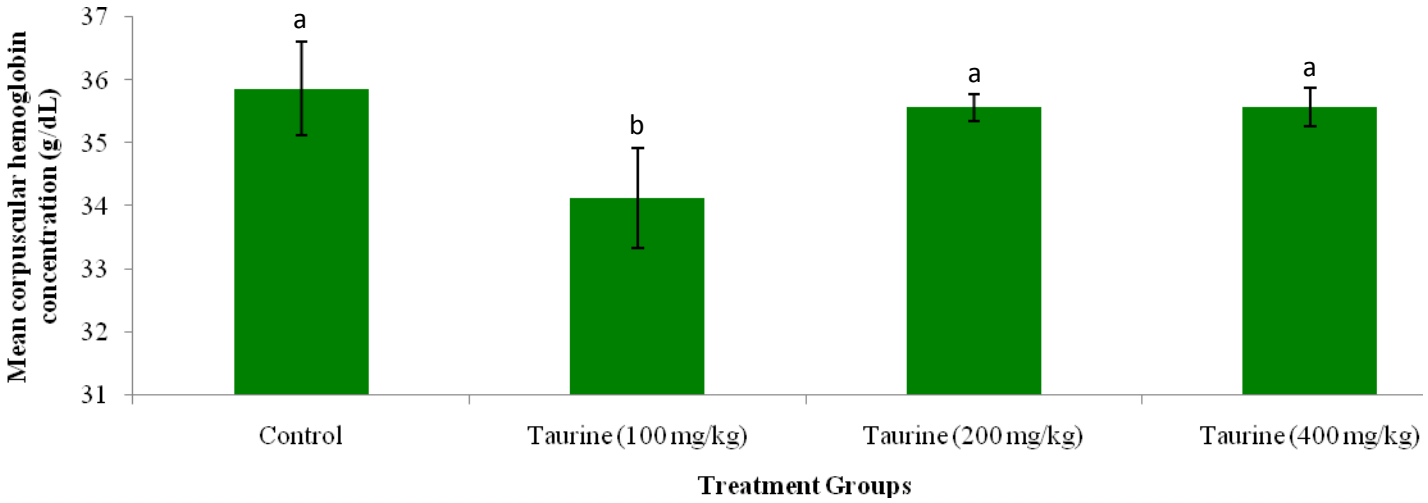


Figure 4.6: Effects of taurine on mean corpuscular haemoglobin concentration of Wistar rats subjected to acute restraint stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different (P < 0.05).

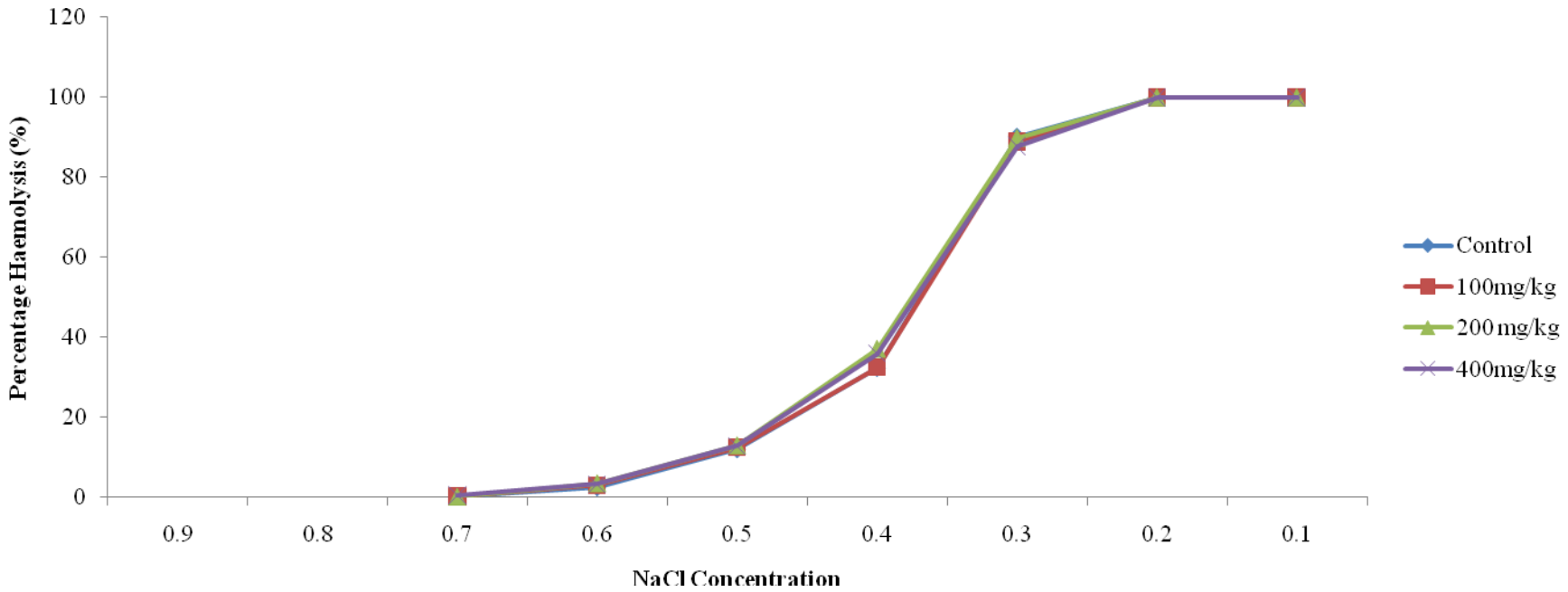


Figure 4.7: Effects of taurine on erythrocyte osmotic fragility of Wistar rats subjected to acute restraint stress

Note: values are mean ± SEM, n = 6

4.2.2 Effects of taurine on leucocyte and platelet counts of Wistar rats subjected to acute restraint stress

There was a significant ($P < 0.05$) increase in the platelet counts at dose of 200 mg/kg taurine when compared with dose of 100 and 200 mg/kg taurine and the control group (Figure 4.8). There was significant ($P < 0.05$) decrease in WBC count at dose of 100 mg/kg taurine when compared with dose of 200, 400 mg/kg and control group (Figure 4.9). There was significant ($P < 0.05$) decrease in neutrophil counts at doses of 100 and 200 mg/kg taurine when compared with 400 mg/kg and the control group (Figure 4.10). The lymphocyte count at dose of 100 mg/kg taurine was lower than that recorded at doses of 200 and 400 mg/kg taurine and control group ($P < 0.05$) (Figure 4.11). There was a significant ($P < 0.05$) decrease in neutrophil/lymphocyte ratio at dose of 200 mg/kg, when compared with the control group (Figure 4.12).

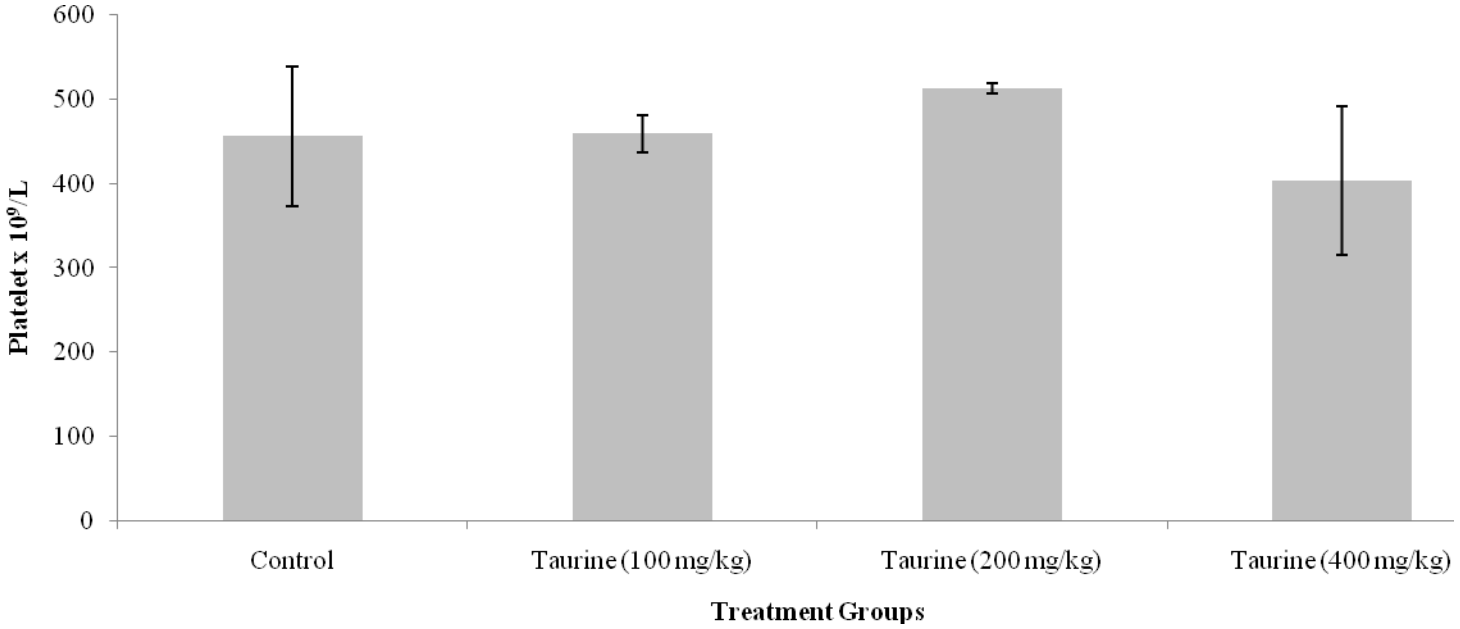


Figure 4.8: Effects of taurine on platelet counts of Wistar rats subjected to acute restraint stress

Note: values are mean ± SEM, n = 6
a,b,c = means with different superscript letters are significantly different ($P < 0.05$)

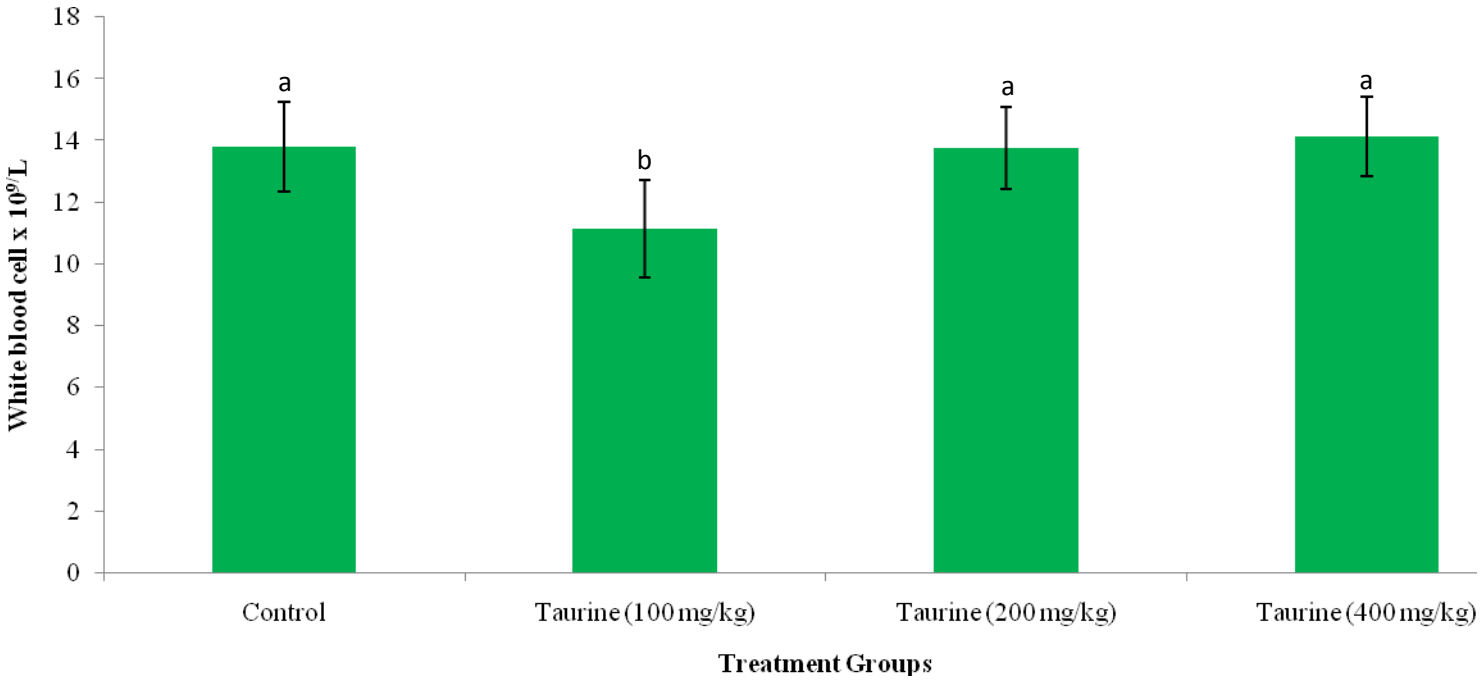


Figure 4.9: Effects of taurine on white blood cell counts of Wistar rats subjected to acute restraint-stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different ($P < 0.05$)

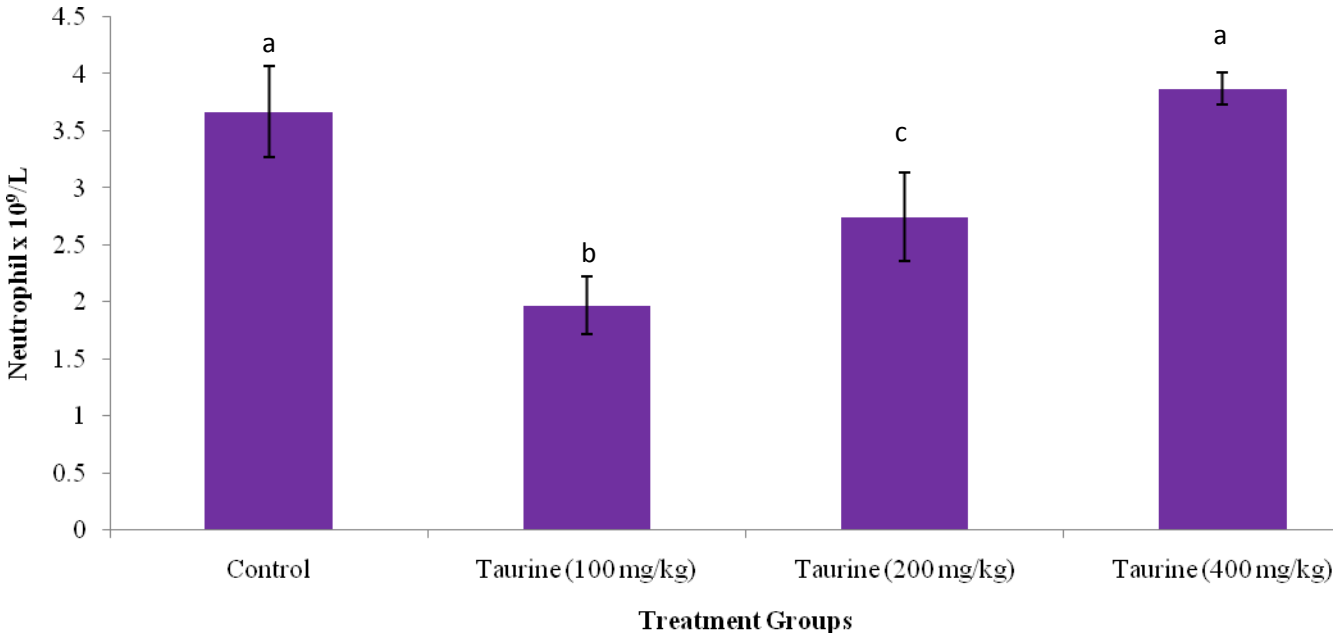


Figure 4.10: Effects of taurine on neutrophil counts of Wistar rats subjected to acute restraint stress

Note: values are mean ± SEM, n = 6
a,b,c = means with different superscript letters are significantly different (P < 0.05)

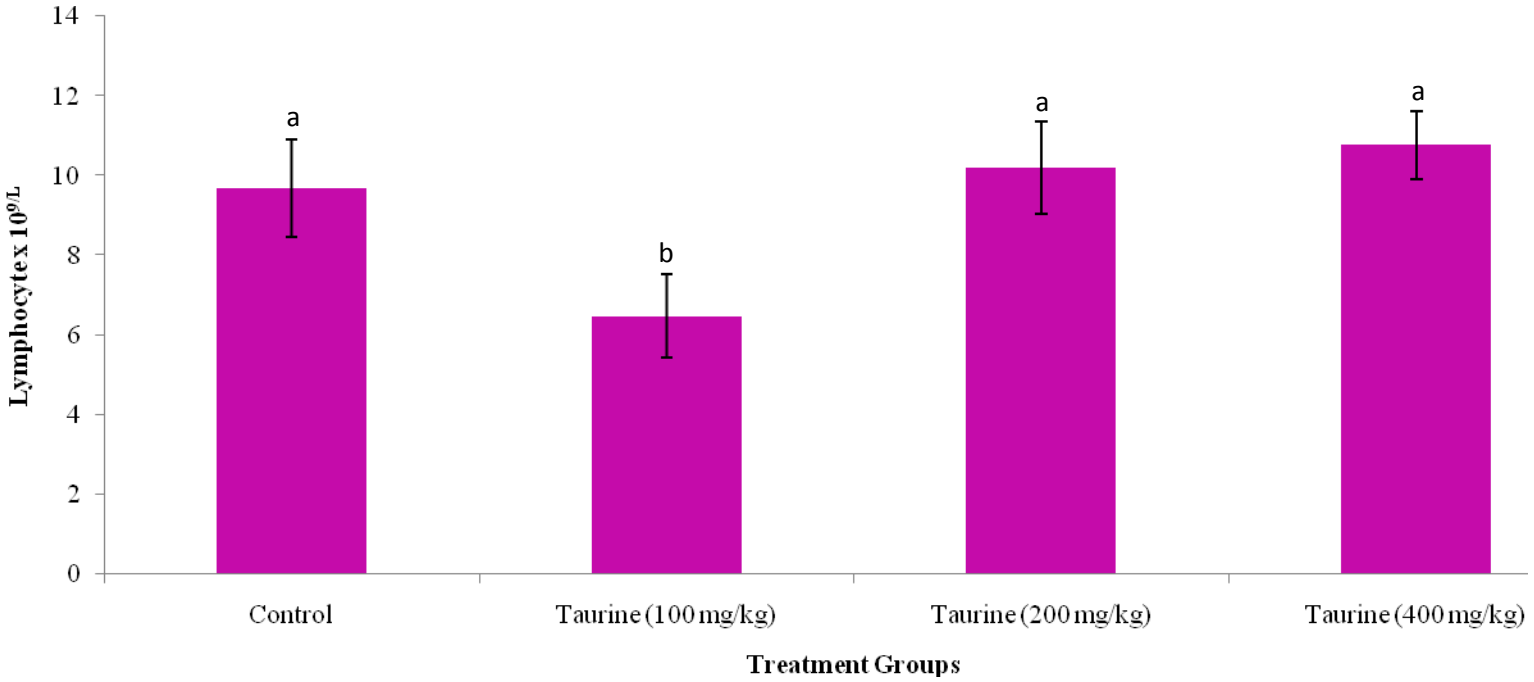


Figure 4.11: Effects of taurine on lymphocyte counts of Wistar rats subjected to acute restraint stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different (P < 0.05).

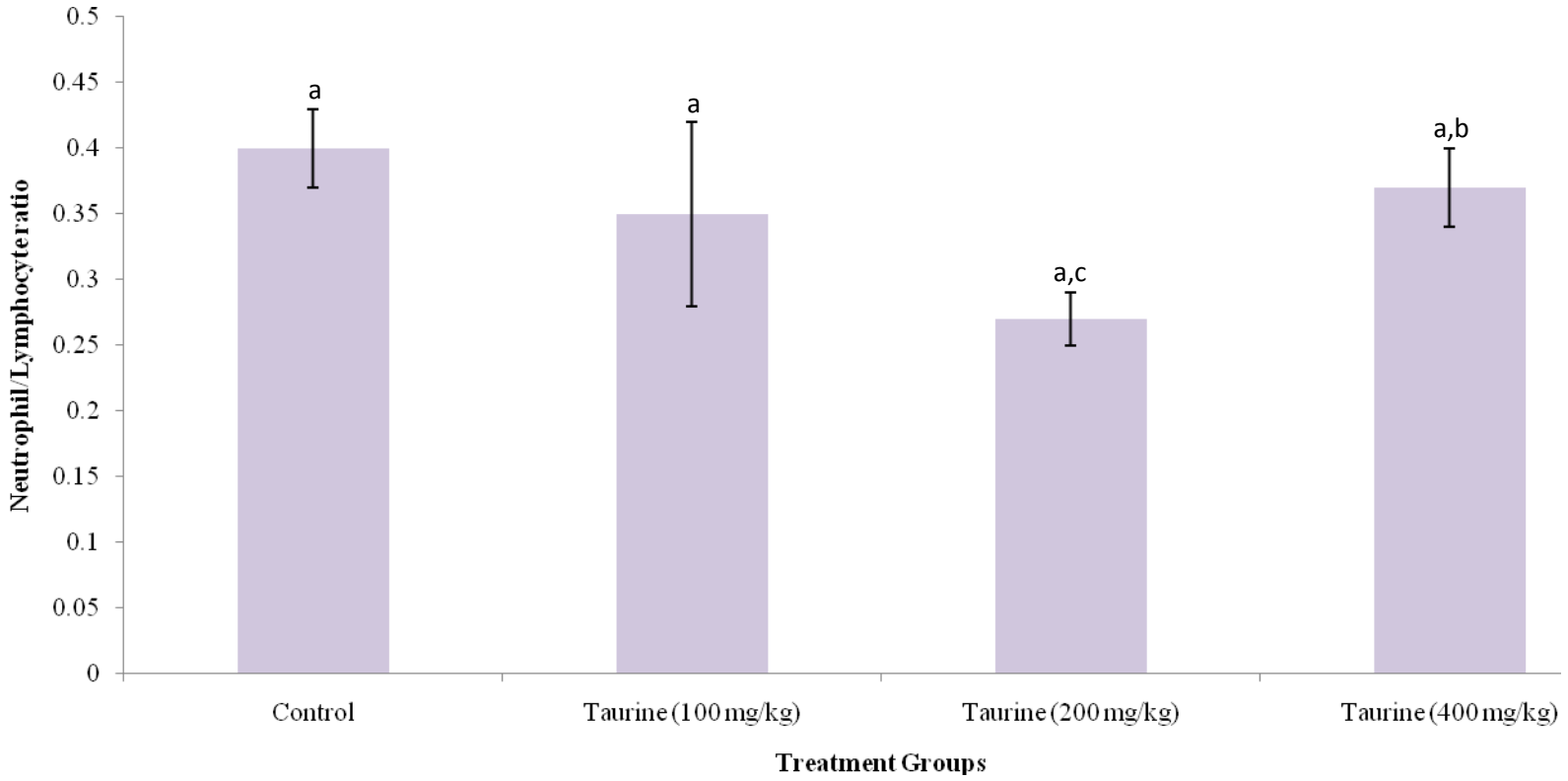


Figure 4.12: Effects of taurine on neutrophil/lymphocyte ratio of Wistar rats subjected to acute restraint stress

Note: values are mean ± SEM, n = 6

a,b,c = means with different superscript letters are significantly different ($P < 0.05$).

4.2.3 Effects of taurine on red blood cell counts and erythrocytic indices of Wistar rats subjected to chronic restraint stress

There was significant ($p < 0.05$) decrease in the red blood cell counts in group that recieved 400 mg/kg taurine, when compared to the control group. However, there was significant increase at 100 mg/kg taurine dose group (Figure 4.13). There was significant ($P < 0.05$) decrease in haemoglobin concentration at 100 and 200 mg/kg taurine when compared with 400 mg/kg and the control groups (Figure 4.14). There was significant ($P < 0.05$) decrease in packed cell volume at 100 and 200 mg/kg taurine, when compared with dose of 400 mg/kg and the control group (Figure 4.15). The results showed significant ($P < 0.05$) decrease in mean corpuscular volume at 400 mg/kg taurine, when compared with the control group (Figure 4.16). The results also revealed significant ($P < 0.05$) decrease in the mean corpuscular haemoglobin at dose of 400 mg/kg taurine, when compared with the control group (Figure 4.17). There was significant ($P < 0.05$) increase in Mean corpuscular haemoglobin concentration at 100, 200 and 400 mg/kg taurine, when compared with the control group (Figure 4.18). However, there was no significant difference in erythrocyte osmotic fragility in taurine treated groups, when compared with the control group (Figure 4.19).

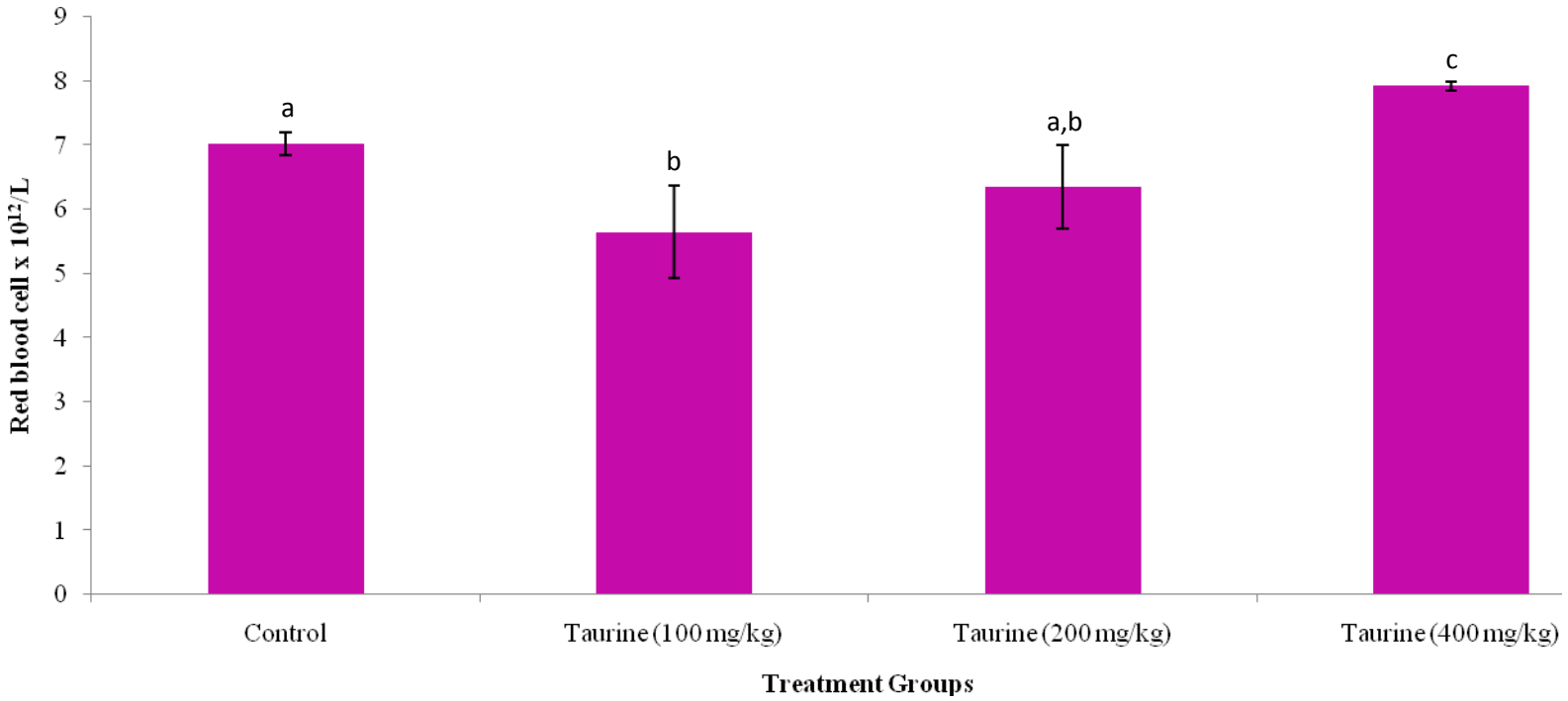


Figure 4.13: Effects of taurine on RBC counts of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b,c = means with different superscript letters are significantly different ($P < 0.05$).

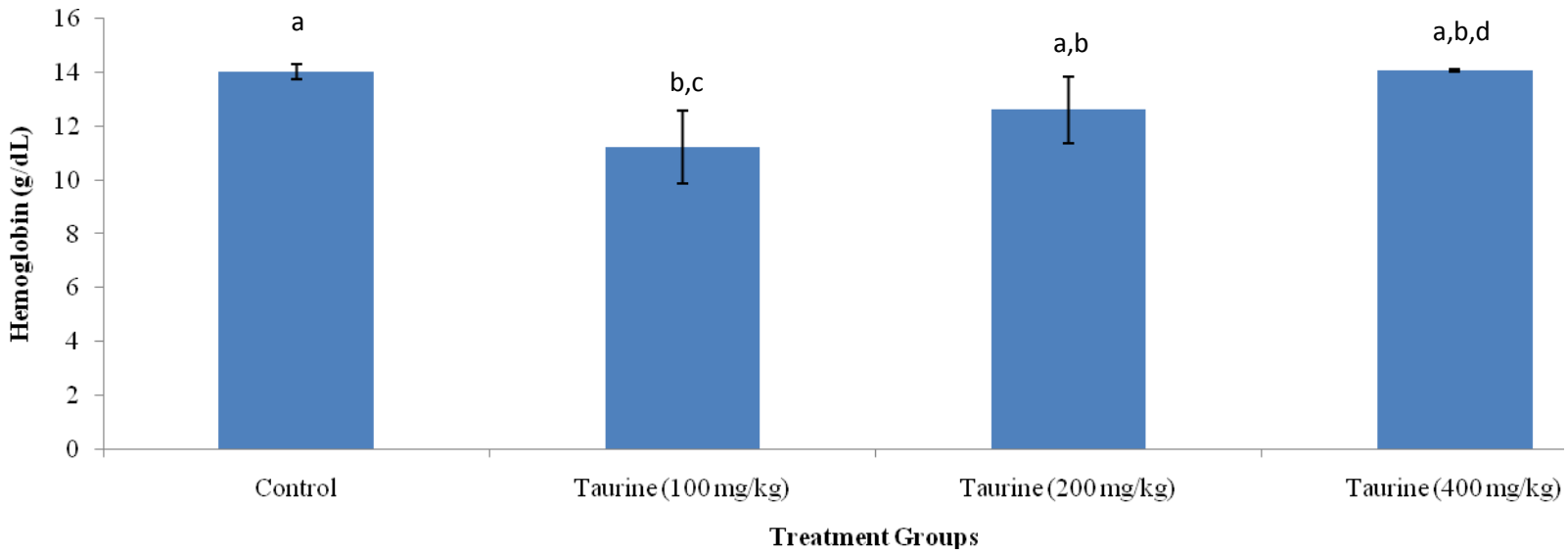


Figure 4.14: Effects of taurine on Hb concentration of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b,c,d = means with different superscript letters are significantly different ($P < 0.05$).

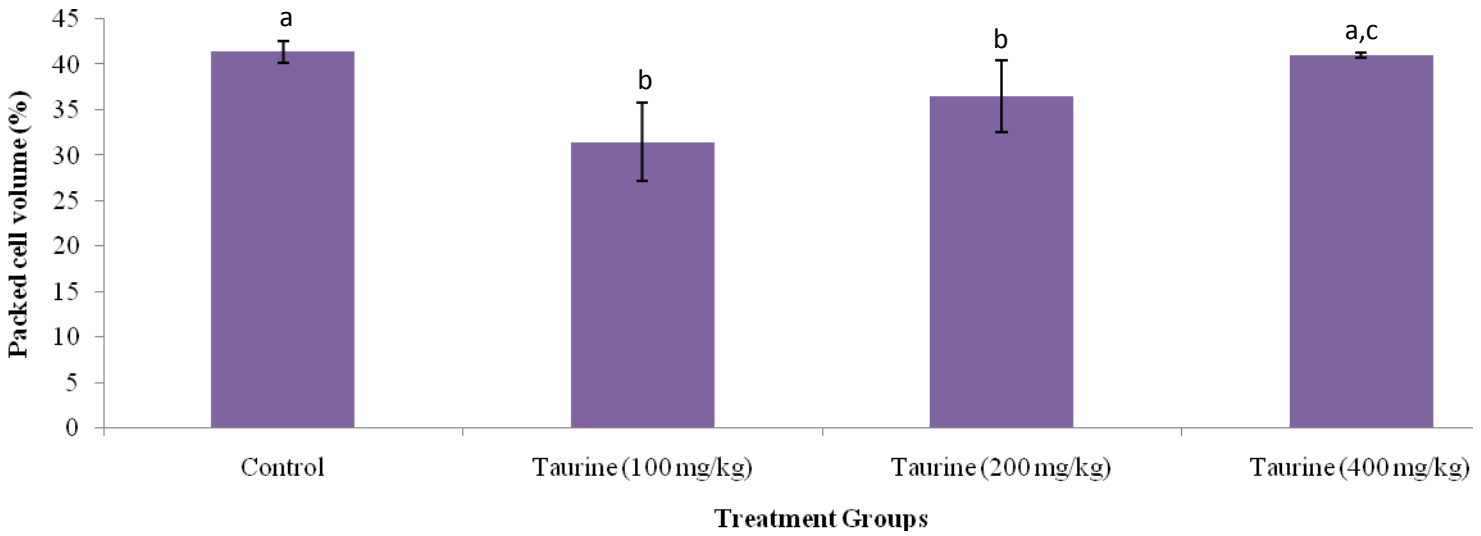


Figure 4.15: Effects of taurine on packed cell volume of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b,c,d = means with different superscript letters are significantly different ($P < 0.05$).

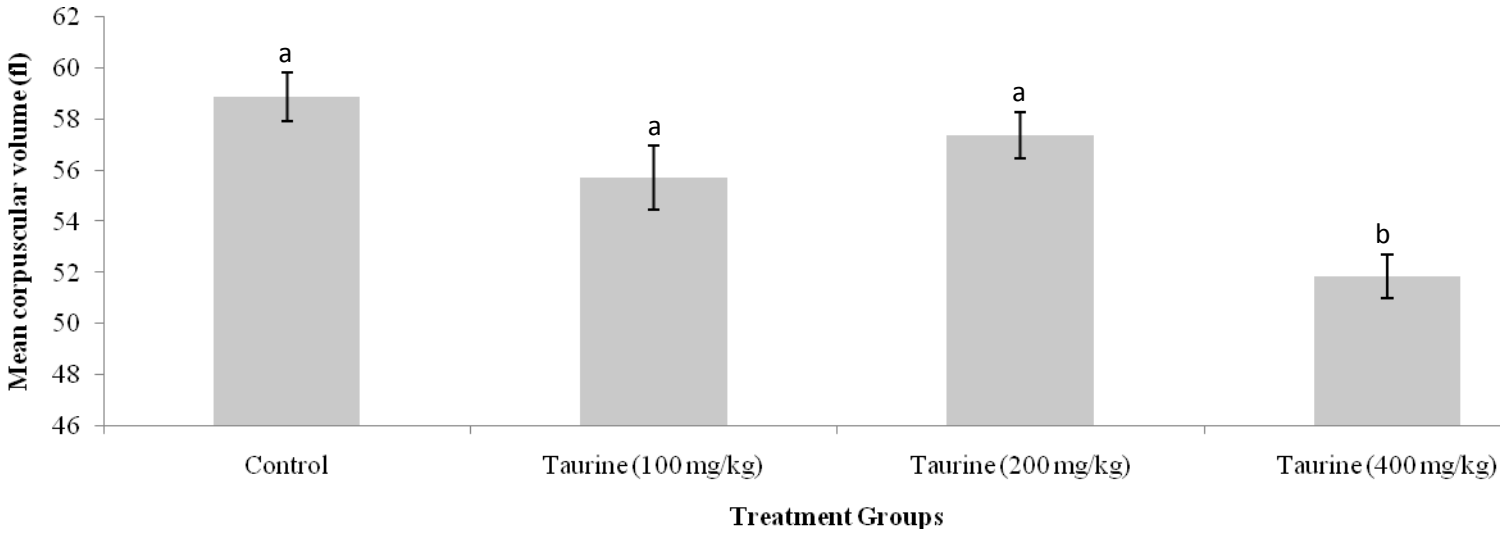


Figure 4.16: Effects of taurine on mean corpuscular volume of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different ($P < 0.05$).

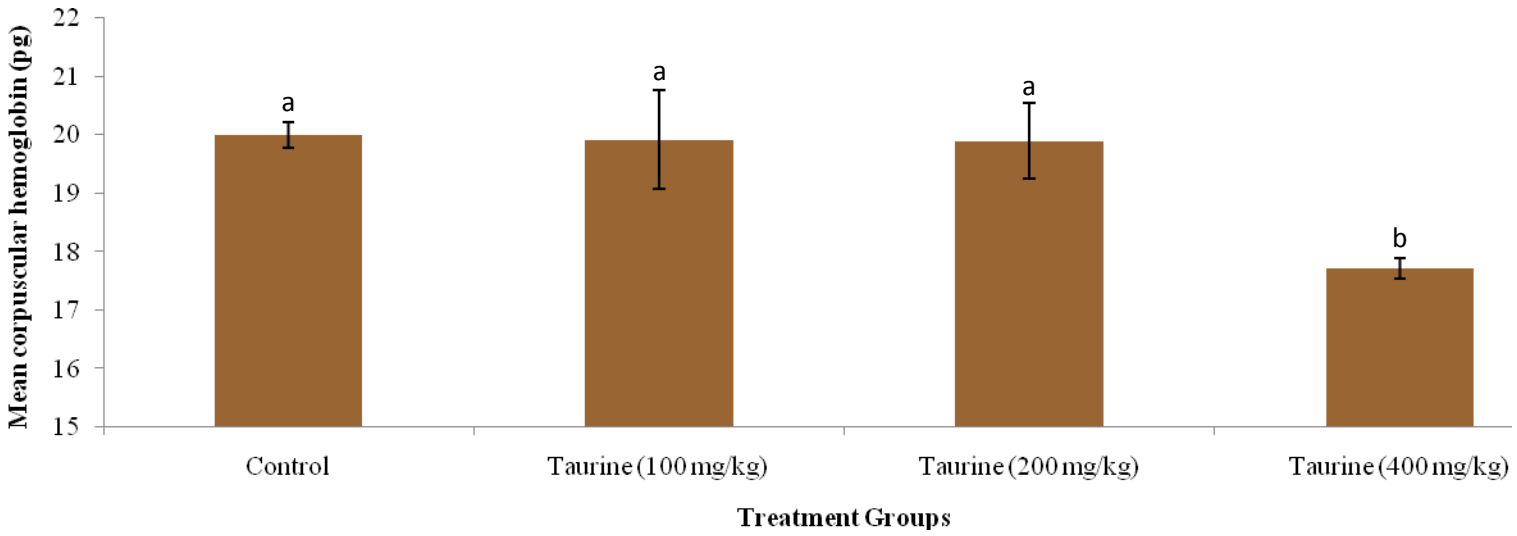


Figure 4.17: Effects of taurine on mean corpuscular haemoglobin of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different ($P < 0.05$).

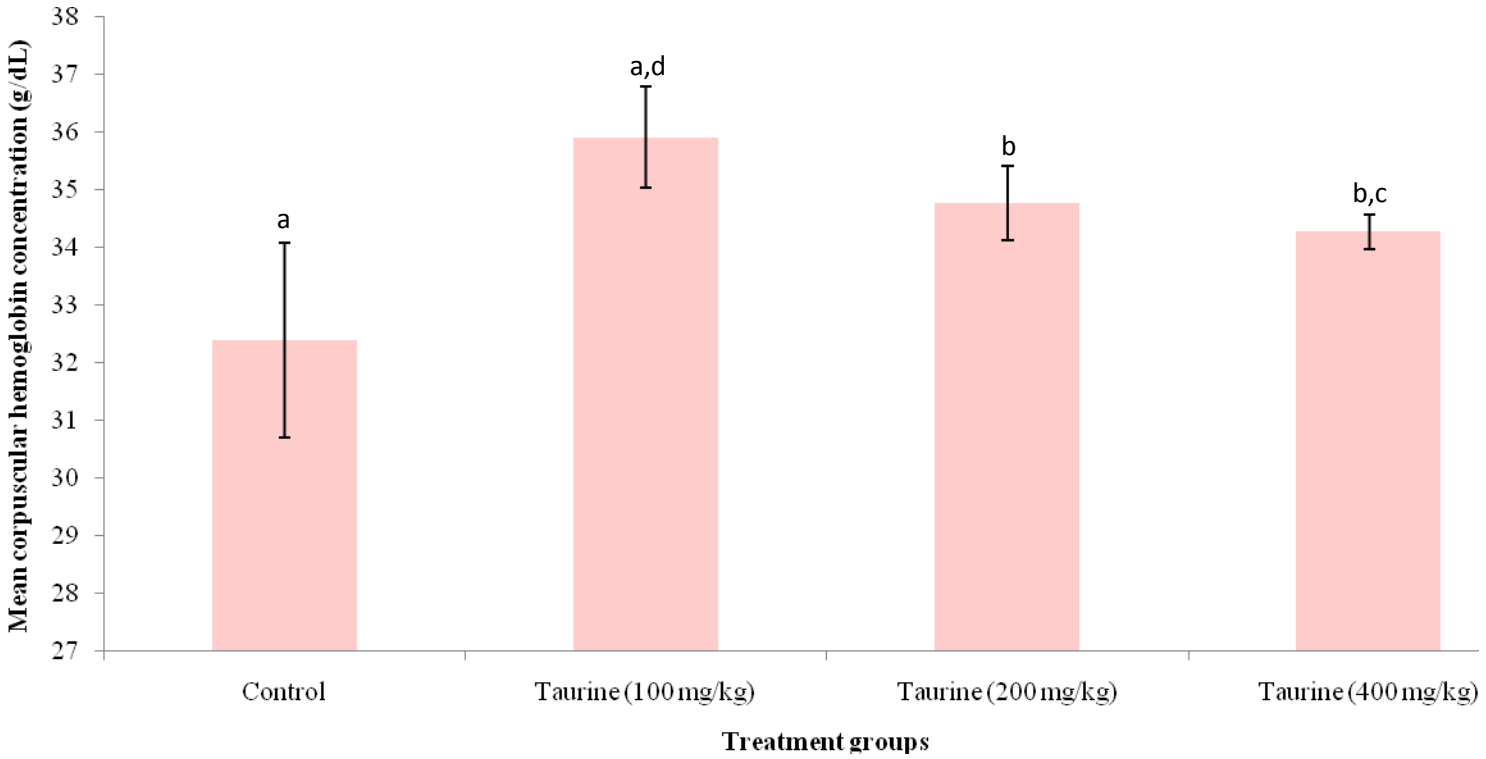


Figure 4.18: Effects of taurine on mean corpuscular haemoglobin concentration of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b,c,d = means with different superscript letters are significantly different ($P < 0.05$).

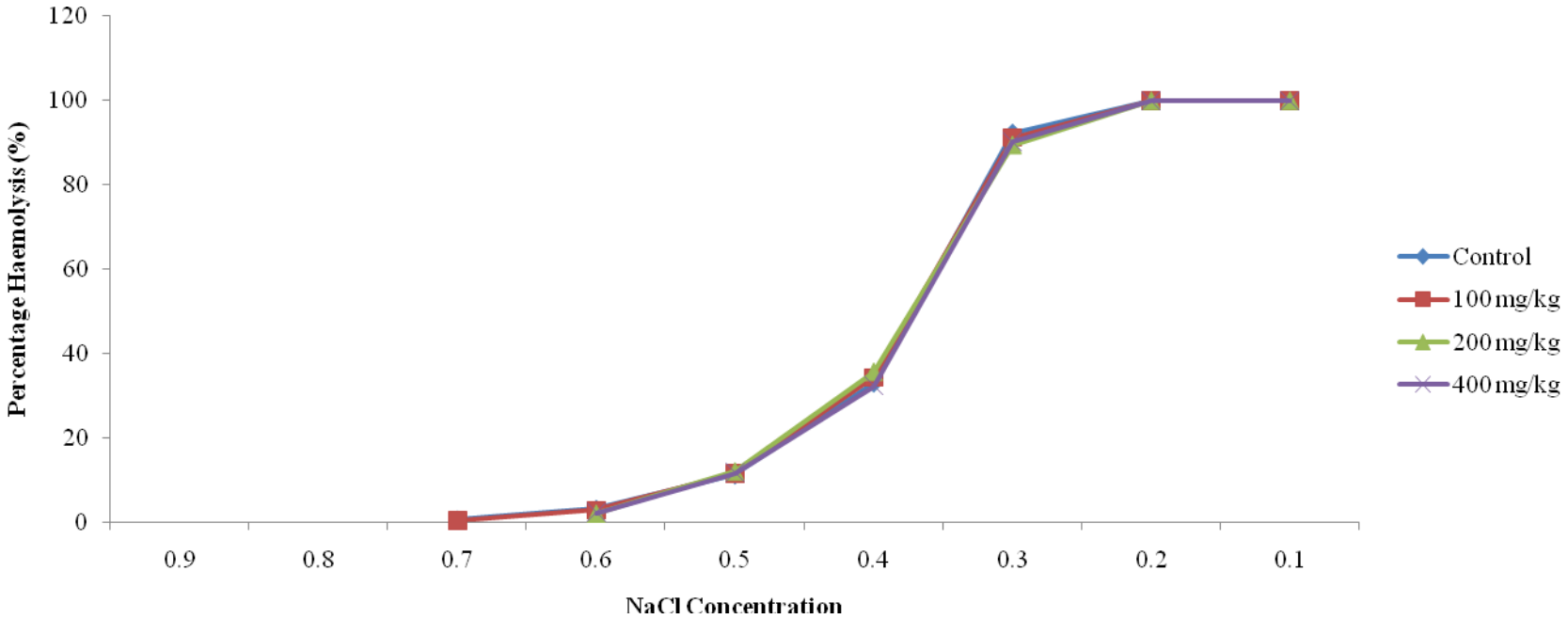


Figure 4.19: Effects of taurine on Erythrocyte osmotic fragility of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6

4.2.4 Effects of taurine on leucocyte and platelet counts of Wistar rats subjected to chronic restraint stress

There was a significant ($P < 0.05$) increase in platelet counts at 100 mg/kg taurine and significant decrease at 200 and 400 mg/kg dose group when compared to the control group (Figure 4.20). There was significant ($P < 0.05$) increase in white blood cell counts at dose of 100 mg/kg taurine, and significant decrease at 400 mg/kg taurine compared with othe treated and the control groups (Figure 4.21). There was significant ($P < 0.05$) decrease in neutrophil counts at doses of 100 and 200 mg/kg taurine, when compared with 400 mg/kg taurine and control group (Figure 4.22). There was significant ($P < 0.05$) decrease in lymphocyte counts at dose of 400 mg/kg taurine, when compared with 100 and 200 mg/kg taurine and the control groups (Figure 4.23). There was significant ($P < 0.05$) increase in the neutrophil/lymphocyte ratio at dose of 400 mg/kg taurine, when compared with 100 and 200 mg/kg taurine and control group (Figure 4.24).

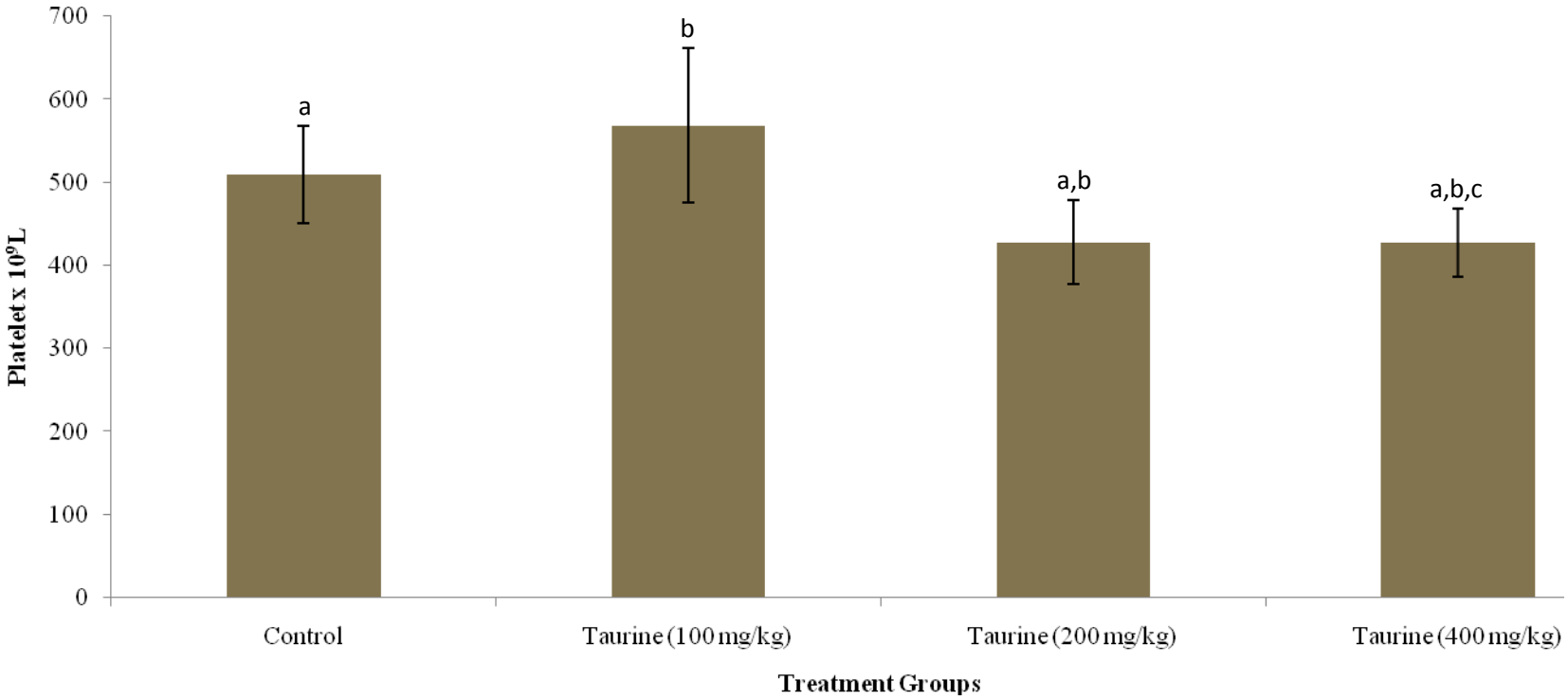


Figure 4.20: Effects of taurine on platelet counts of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b,c = means with different superscript letters are significantly different ($P < 0.05$).

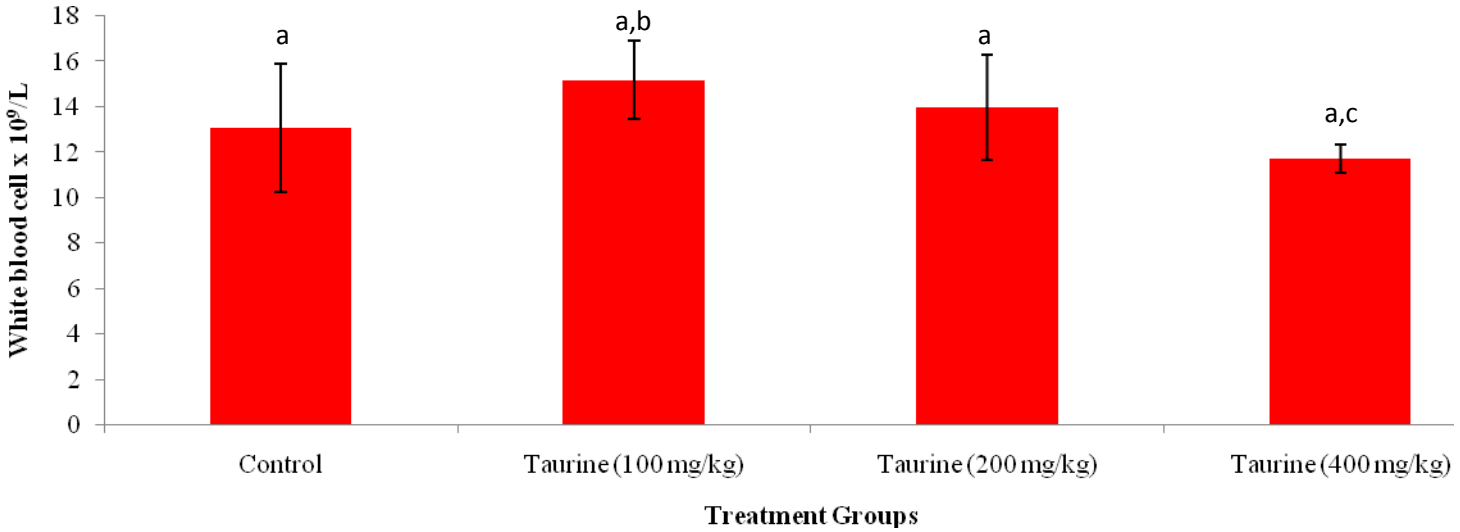


Figure 4.21: Effects of taurine on white blood cell counts of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b,c = means with different superscript letters are significantly different ($P < 0.05$).

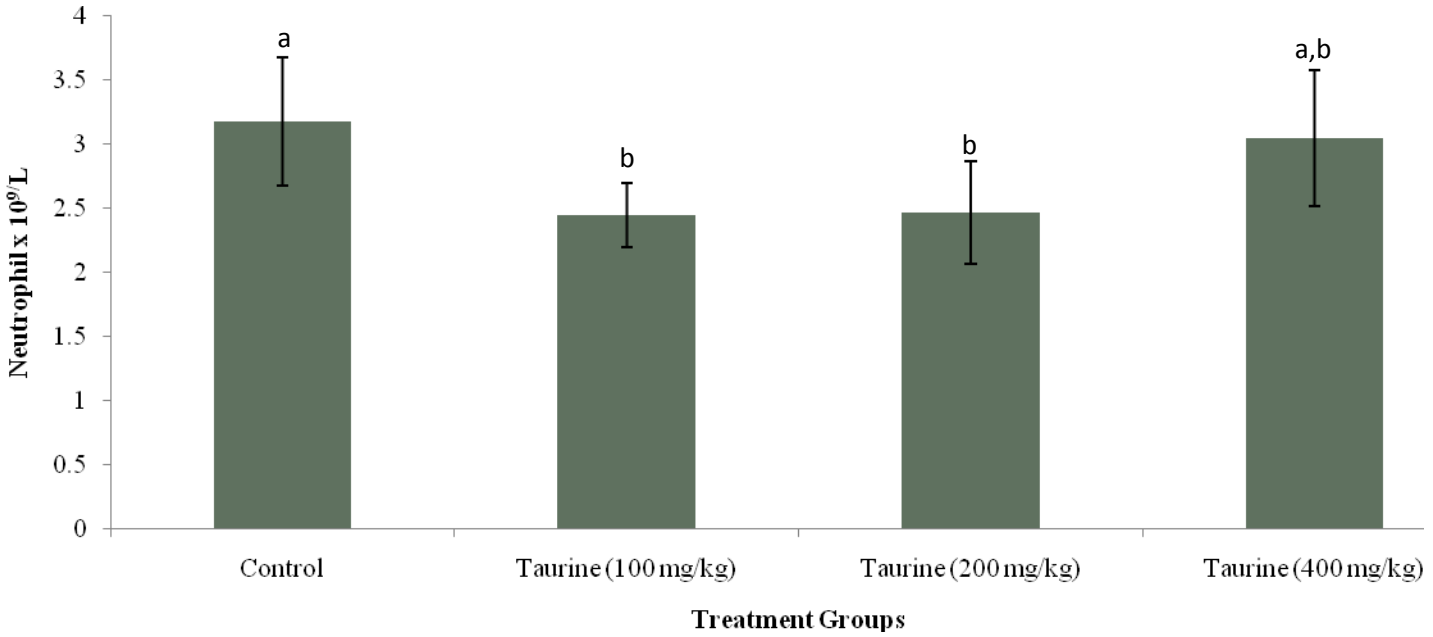


Figure 4.22: Effects of taurine on neutrophil counts of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different ($P < 0.05$).

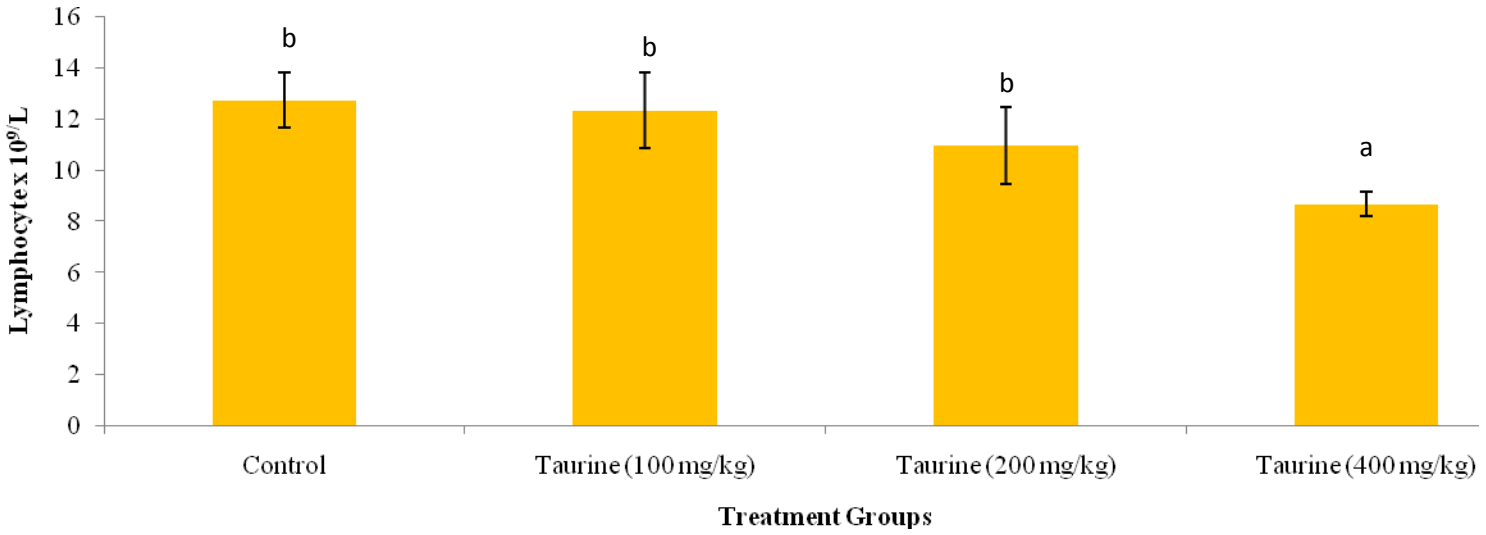


Figure 4.23: Effects of taurine on lymphocyte counts of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b = means with different superscript letters are significantly different ($P < 0.05$).

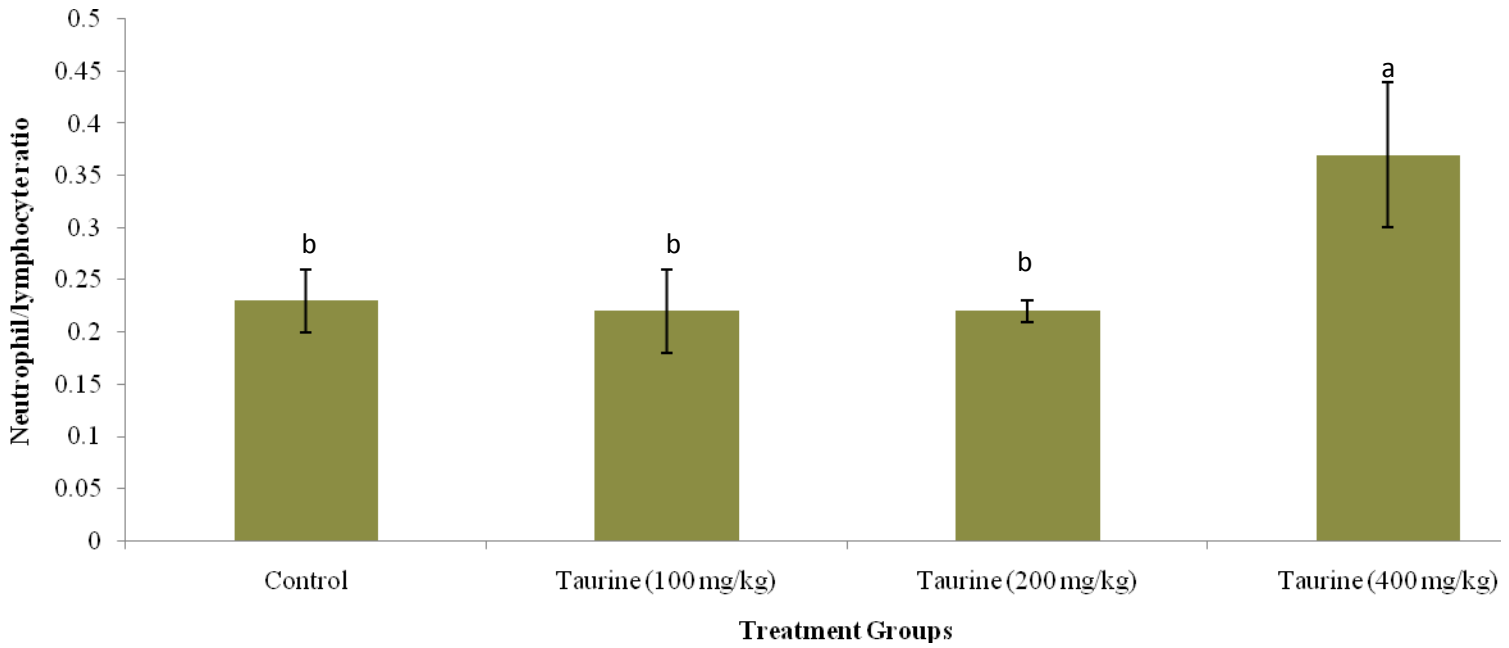


Figure 4.24: Effects of taurine on neutrophil/lymphocyte ratio of Wistar rats subjected to chronic restraint stress

Note: values are mean ± SEM, n = 6
a,b,c,d = means with different superscript letters are significantly different ($P < 0.05$).

4.3 Oxidative Stress Studies

4.3.1 Effects of taurine treatments on malondialdehyde, acetylcholinesterase and enzymatic antioxidant in the brain of acute restraint stressed Wistar rats

There was no significant difference in the malondialdehyde, acetylcholinesterase, superoxide dismutase, catalase and glutathione peroxidase in the taurine treated groups, when compared with their control groups (Table 4.5).

Table 4.5: Effects of taurine treatments on malondialdehyde concentration, acetylcholonesterase activity and other antioxidant enzymes in the brain of acute restraint stressed Wistar rats

Treatments	MDA (nmol/mg pr)	AChE (nmol/mg pr)	SOD (IU/L)	CAT (IU/L)	GPx (IU/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

4.3.2 Effects of taurine treatments on malondialdehyde, acetylcholinesterase and antioxidant enzymes in the brain of acte restraint Stressed Wistar rats.

There was no significant difference in malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase in the taurine treated groups when compared with their control groups. However, there was a significant ($P < 0.05$) increase in the activity of acetylcholinesterase in the group that received 100 mg/kg taurine when compared with the control group (Table 4.6).

Table 4.6: Effects of taurine treatments on malondialdehyde concentration, acetylcholinasterase activity and other antioxidant enzymes in the brain of chronic restraint-stressed Wistar rats

Treatments	MDA (nmol/mg pr)	AChE (nmol/ml pr)	SOD (IU/L)	CAT (IU/L)	GPx (IU/L)
Control (DW)	1.25 ± 0.10^{ns}	21.33 ± 0.71^{a}	2.30 ± 0.13^{ns}	51.83 ± 0.95^{ns}	47.00 ± 1.03^{ns}
Taurine:					
100 mg/kg	1.18 ± 0.09^{ns}	24.17 ± 1.17^{b}	2.33 ± 0.08^{ns}	52.67 ± 0.99^{ns}	47.67 ± 0.88^{ns}

200 mg/kg	1.13 ± 0.10^{ns}	22.17 ± 0.65^{a}	2.37 ± 0.10^{ns}	53.33 ± 1.15^{ns}	48.33 ± 1.31^{ns}
400 mg/kg	1.18 ± 0.09^{ns}	22.33 ± 1.17^{a}	2.22 ± 0.05^{ns}	53.00 ± 0.93^{ns}	49.83 ± 1.08^{ns}

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

4.3.3 Effects of taurine on serum protein concentration in acute restraint-stressed Wistar rats

There was no significant difference in the total protein, albumin, globulin and albumin/globulin ratio in the taurine treated groups, when compared with the control group (Table 4.7).

Table 4.7: Effects of taurine on serum protein concentration in acute restraint-stressed Wistar rats

Treatments	TP (g/dL)	ALB (g/dL)	GLB (g/dL)	A/G
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, distilled water; TP, Total protein; ALB, Albumin; GLB, Globulin; A/G, Albumin:Globulin ratio

4.3.4 Effects of taurine on serum total protein (TP) concentration in chronic restraint-stressed Wistar rats

There was a significant ($P < 0.05$) increase in total protein concentration in the restraint stressed groups that received 200 and 400 mg/kg taurine when compared with 100 mg/kg taurine and the control groups. However, there was a gradual decrease total protein in the restraint stressed group that received 100 mg/kg taurine but, the difference was not significant when compared with the control group. The albumin showed significant ($p < 0.05$) increase in albumin concentration in the restraint stressed groups that received 200 and 400 mg/kg taurine when compared with the 100 mg/kg taurine and the control groups. However, there was a gradual decrease in albumin concentration in the restraint stressed group that received 100 mg/kg taurine, but this difference was not significant when compared with the control group. The serum globulin concentration showed gradual increase in the restraint stressed group that received 400 mg/kg taurine, but the difference was not significant difference when compared with other restraint stressed groups and the control group. The albumin/globulin ratio revealed no significant difference in all the restraint stressed taurine treated groups when compared with the control group (Table 4.8).

Table 4.8: Effects of taurine on serum protein concentration in chronic restraint-stressed Wistar rats

Treatments	TP (g/dL)	ALB (g/dL)	GLB (g/dL)	A/G
Control (DW)	63.50 ± 1.11^{b}	35.50 ± 1.43^{b}	28.00 ± 0.32^{ns}	1.26 ± 0.46^{ns}
Taurine:				
100 mg/kg	62.17 ± 1.14^{b}	33.50 ± 0.85^{b}	28.67 ± 0.29^{ns}	1.16 ± 0.29^{ns}
200 mg/kg	66.00 ± 1.57^{a}	37.83 ± 1.76^{a}	28.17 ± 0.19^{ns}	1.34 ± 0.92^{ns}
400 mg/kg	66.33 ± 1.36^{a}	37.00 ± 2.07^{a}	29.33 ± 0.7^{ns}	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

4.3.5 Effects of taurine treatments on the activities of serum enzymes in acute and chronic restraint-stressed Wistar rats.

The acute restraint stressed group showed significant ($P < 0.05$) increase in activities of alanine aminotransferase in the group that recieved 200 mg/kg taurine. However, there was a significant ($P < 0.05$) decrease in the aspartate aminotransferase activity at 400 mg/kg taurine, when compared with control group. In the chronic restraint stressed group, there was gradual decrease in the aspartate aminotransferase activity in all the three restraint stressed groups that were administered taurine. However, there was no significant difference when the taurine treated groups were compared with the control group. The result for alanine aminotransferase showed significant ($P < 0.05$) increase in the restraint stressed group that received 400 mg/kg taurine and a gradual increase in alanine aminotransferase activity in the restraint stressed group that received 100 mg/kg taurine. However, there was no significant difference when compared to the control group. There was a significant ($P < 0.05$) increase in alkaline phosphatase activity in all the three chronic restraint stressed groups that were administered taurine (table 4.9).

Table 4.9: Effects of taurine treatments on activities of serum enzymes in acute and chronic restraint-stressed Wistar rats

	Acute restraint stress			Chronic restraint stress		
	AST	ALT	ALP	AST	ALT	ALP
Treatments	(IU/L)	(IU/L)	(IU/L)	(IU/L)	(IU/L)	(IU/L)
Control (DW)	42.00 ± 2.44^{a}	47.83 ± 1.74^{a}	68.17 ± 1.14^{ns}	42.50 ± 0.99^{ns}	46.67 ± 1.17^{ns}	65.00 ± 1.41^{a}
Taurine:						
100 mg/kg	42.50 ± 1.15^{a}	48.00 ± 0.82^{a}	68.17 ± 2.43^{ns}	42.33 ± 1.33^{ns}	47.17 ± 1.64^{ns}	71.00 ± 1.37^{b}
200 mg/kg	45.83 ± 1.25^{a}	51.17 ± 2.04^{b}	68.83 ± 2.36^{ns}	41.17 ± 1.40^{ns}	45.17 ± 1.30^{ns}	71.50 ± 3.51^{b}
400 mg/kg	38.17 ± 0.79^{b}	45.50 ± 3.78^{a}	64.83 ± 1.66^{ns}	41.17 ± 1.19^{ns}	44.83 ± 1.49^{ns}	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

CHAPTER FIVE

5.0 DISCUSSION

Taurine have been reported to ameliorate age-related cognitive decline in memory acquisition and retention. Following chronic taurine supplementation, age-dependent decline in spatial memory acquisition and retention is preserved (El-Idrissi *et al*., 2013). It is also known that taurine can reverse damages caused by various tissue assaults (Pan *et al*., 2010; Yalcinkaya *et al*., 2009), and can repair an already damaged nervous tissue and reverse impaired brain functions (El-Idrissi *et al*., 2009, 2013; Kim *et al*., 2014; Lu *et al*., 2014; Park *et al*., 2014).

The present study indicated that taurine, improved cognitive functions in rats. This agrees with the findings of Sanberg and Fibiger (1979), who reported that chronic treatment with taurine altered the impairment of acquisition and to a lesser extent, retention of a step-down passive avoidance task in rats. However, the present study did not agree with the study by Rivas-Arancibia *et al*. (2000), who suggested that taurine did not show any effect in young and mature rats, but it was helpful in improving memory in old rats. In their study, the effect of only one taurine treatment of 43 mg/kg was determined. In a different study by Vohra and Hui (2000), taurine did not show any effect on the escape latency or the step down latency of mice. The improved learning acquisition and memory retention observed in the present study was also exhibited by acute and chronic restraint-stressed mice after taurine administration in the elevated plus-maze test as evidenced by short acquisition and retention time observed in the 200 and 400 mg/kg taurine treated groups.

Taurine treated mice spent less time to reach the closed arm of the elevated plus-maze as compared with the control group. Researchers have shown that, taurine influences the activities of neurotransmitters in the brain and that taurine affects brain chemicals and also improves cognitive function (Sajid *et al*., 2013). Earlier studies revealed that taurine does affect learning by improving both memory acquisition and memory retention in rats (El-Idrissi, 2009). Previous reports have indicated that, taurine administration could suppresse and also delay learning and memory abilities in rats (Ito *et al*., 2012). However there are other studies which reported that taurine does not exert any effect on learning and memory (Ito *et al*., 2009). In the present study, it was shown that taurine improved learning and short-time memory in chronic restraint stressed Wistar rats and mice, probably by influencing the activity of neurotransmitters in the brains of mice and Wistar rats.

Hole-board experiment is a measure of exploratory behaviour in animals. A decrease in this parameter reveals a sedative behaviour, while an increase indicates stimulatory activity (File and Pellow, 1985), and it's accepted as a parameter for the evaluation of anxiety conditions in animals (Crawley, 1985). Hole-board model indicated that head-dipping behaviour was sensitive to changes in emotional state of the animal and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behaviour (Tanko *et al*., 2009). In this study, exploratory bahaviour in mice revealed that, the acute and chronic restraint-stressed groups that received taurine at 200 mg/kg showed significant increase in the number of head dips

when compared with taurine 400 mg/kg group, indicating that exploratory behaviour was more in the taurine 200 mg/kg group. The reason for significant decrease in the number of head dips in the taurine 400 mg/kg groups is not clear. However, decrease in exploratory activity by reduction in head dip is a measure of CNS depressant activity (Adzu *et al*., 2002), while an increase is a measure of CNS stimulatory activity. Earlier reports indicated that taurine has an antidepressant-like effect having the ability to change depression-related signaling cascades in the hippocampus (Iio *et al*., 2012). Other studies reported that taurine has a depressant effect on the CNS (Barbeau *et al*., 1975), and suggested that it may exert a generalized depressant activity on neural excitation. However later study have reported that, taurine administration significantly decreased depression like symptoms in rats only at the highest dose of taurine (Sajid *et al*., 2013). In the current study, taurine administration significantly decreased depression like symptoms in mice possibly through its antidepressant-like effect in changing depression-related signaling cascades in the hippocampus. This increase in exploratory behaviour of taurine at the dose of 200 mg/kg shows neuroactive property of taurine and its possible application in anxiety condition.

Oxidative stress has been identified as a possible mechanism for the induction of cognitive impairment (Liu *et al*., 2014). Oxidative stress occurs when there is imbalance between the antioxidant defence and the production of ROS culminating in oxidative damage to biomolecules such as DNA, lipid and proteins (Halliwell and Gutteridge, 2007). The brain is very susceptible to oxidative damage because of its high oxygen content, low level of antioxidant activity and its abundant level of polyunsaturated fatty

acids (Butterfield *et al*., 2001; Moreira *et al*., 2007). Taurine administration in acute restraint stress group revealed significant decrease in the time spent on the beam in group administered taurine 200 mg/kg when compared to the control group. The time taken to reach goal-box is correlated with neuroactivity, and suggests that taurine did not exhibit sedative effect. Taurine also showed significant decrease in the number of foot slips in the 200 and 400 mg/kg taurine treated groups when compared with 100 mg/kg taurine group. In the chronic groups, the three doses of taurine showed significant decrease time spent on the beam. However, there was a significant increase in the number of foot slips in the group that received taurine at 100 mg/kg. This indicates that taurine seems not to possess a sedative property. Restraint stress is a well-known method for the production of acute or chronic stress. The result showed that, taurine which is an antioxidant, ameliorated cognitive performance of mice in the restraint stressed groups. In this study, taurine may have imrporved motor coordination through its neuroactivity action.

In the current study, the significant increase in WBC at dose of 200 and 400 mg/kg taurine compared to 100 mg/kg taurine group in the acute restraint stressed group might be considered as a defensive mechanism by the immune system. This finding corroborates with work of Patrick-Iwuanyanwu *et al*. (2007) 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. In the chronic restraint stressed group, the reason for the significant decrease in WBC 400 mg/kg taurine dose group is not clear. However, it could be due to difference in duration of the restraint stress. The significant increase in

the platelet count in the 200 mg/kg taurine group subjected to acute restraint and significant increase in 100 mg/kg taurine group subjected to chronic restraint, could be attributed to different mechanisms such as stabilization of the platelet membrane, antithrombotic effect, decreased platelet aggregation and the reduction of lipid peroxidation within the platelet membrane (Akande *et al*., 2014c).

The significant increase in neutrophils count at 400 mg/kg taurine dose in both acute and chronic restraint stressed groups was a clear indication that taurine protected the neutrophils from destruction. Circulating neutrophils are committed to apoptosis by ROS, which was produced by activated cells. It was recognized that taurine may protect neutrophils from apoptosis by its antioxidant activity. In this study taurine might have protected the neutrophils by its antioxidant role. The significant decrease in lymphocyte counts in the chronic restraint stressed rats following taurine administration could be that, taurine did not confer any protection on the lymphocytes in this group. However the acute restraint stressed group showed significant increase in lymphocyte at taurine doses of 200 and 400 mg/kg compared with the control group. Previous studies have reported that exogenous taurine maintains human lymphocyte viability, through its membrane-stabilizing capacity (Frick *et al*., 2009). Other 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).

The significant ($P < 0.05$) decrease in neutrophils/lymphocytes (N/L) ratio in the acute restraint stressed group at dose 200 mg/kg and significant increase in 400 mg/kg taurine

group was an indication of typical stress reaction. Neutrophil/lymphocyte ratio has recently been reported as a predictor of hepatocellular carcinoma (Xiao *et al*., 2014). It was 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 was known that taurine protects the leukocyte from destruction by inhibiting lipid peroxidation and neutrophil activation (Kim *et al*., 1998). The increase in neutrophil/lymphocyte ratio observed in the chronic restraint stressed group treated with 400 mg/kg taurine in this study was in agreement with earlier findings (Minka and Ayo, 2007; Minka *et al*. 2009). That is, the ratio increases in stress situations, especially in those induced via free-radical mechanism. Thus, the administration of taurine decreased the N/L ratio in the present study 100 and 200 mg/kg taurine doses. This study might therefore, postulates that taurine prevented the risk of adverse effects due to ROS-induced cell damages and destruction in Wistar rats.

The significant increase in RBC count at 200 and 400 mg/kg taurine doses in both the acute and chronic group, could mean that taurine through it antioxidant role protected the RBC from oxidative stress due to restraint stress. There was significant increase in haemoglobin (Hb) concentration when doses 200 and 400 mg/kg taurine were compared with dose of 100 mg/kg taurine in the acute and chronic restraint stressed groups. The significant increase in PCV at 200 and 400 mg/kg taurine groups in both acute and chronic restraint stressed groups, respectively indicate that taurine improved PCV in restraint stressed Wistar rats. In a different study, they found that immobilization may induce appearance of stored erythrocytes and reticulocytes in circulation and they

suggested that immobilisation-induced stress leads to release of stored erythrocytes from the spleen depot. Other effects of taurine on red blood cell might be attributed to its antioxidant role by scavenging ROS that stimulate membrane lipid peroxidation and hence, stabilize the red blood cell membrane membrane (Hedberg *et al*., 2009). In this study, the increased values of red blood cell and associated parameters Hb, PCV at doses of 200 and 400 mg/kg taurine are suggestive of antioxidant role of taurine. Therefore, taurine may have protected the bone marrow, liver and haematological metabolism (Oyelami *et al*., 2003). This study showed that taurine protected red blood cell through its antioxidant role and argumented Hb, PCV concentration in the restraint stress groups. This result agrees with the findings of Anand *et al*. (2010) and Akande *et al*. (2014a) who reported that taurine improved PCV, Hb concentration and red blood cell counts in Wistar rats.

The reason for the significant decrease in MCV and MCH at 400 mg/kg taurine dose in acute and chronic restraint stressed groups is not clear. However, taurine at doses 100 and 200 mg/kg attenuated the anaemic response produced by restraint stress in the Wistar rats. This suggests that chronic restraint stress provoked microcytic hypochromic anaemia in rats (Akande *et al*., 2014a). Taurine attenuates the anaemic response produced by restraint stress in the rats. This may be due to taurine's ability to improve the erythrocyte membrane integrity by mitigation of oxidative damage to the erythrocyte membrane. Taurine may suppress haemolysis through osmoregulation and biomembrane stabilisation (Takagi *et al*., 2011). It was therefore, hypothesised that taurine improved erythrocyte parameters in the restraint-stressed groups using these mechanisms. The

erythrocyte osmotic fragility did not show any significant difference when the taurine treated groups were compared with the control group. Studies have shown that oxidative stress increases haemolysis (Bernabucci *et al*., 2002). 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 (Redmond *et al*., 1998; Pendav *et al*., 2009). The current study showed that, taurine have prevented erythrocyte osmotic fragility in the treated groups through its action as an antioxidant. We therefore hypothesized that taurine have improved the erythrocyte parameters in the restraint-stressed groups using this mechanism.

Oxidative stress occurs when there was imbalance between the antioxidant defence, and the production of ROS culminating in oxidative damage to biomolecules such as DNA, lipid and proteins (Halliwell and Gutteridge, 2007). In the present study, there was a gradual decrease in MDA concentration in all the three chronic restraint stressed groups that received taurine, but there was no significant difference in the MDA concentration when compared with the control group in both acute and chronic restraint stressed Wistar rats. One of the major oxidation products of peroxidised polyunsaturated fatty acids is MDA. Increased MDA content is an indication of oxidative stress and lipid peroxidation (Demir *et al*., 2011). Lipid peroxidation leads 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 has been shown to attenuate tissue lipid peroxidation, either by inhibition of ROS formation or by binding Fe^{2+} like a chelator (Wu *et al.*, 2011).

Studies have also shown that taurine abolishes 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 taurine may contribute to the mitigation of lipid peroxidation in the brain of the restraint-stressed Wistar rats. This study is similar to work done by (Parildar-Karpuzoðlu *et al.*, 2008) who reported that no significant changes were detected in lipid peroxidation and antioxidant system in brains of old rats following taurine treatment and suggested that in old rats the liver seems more susceptible to age-related lipid peroxidation increases and taurine level changes than the brain.

The brain AChE activity showed significant increase in the 100 mg/kg taurine group in the chronic restraint stressed group only as compared to other taurine groups and the control group. AChE is a crucial enzyme involved in the degradation of acetylcholine. It plays an important role in the regulation of numerous vital functions, including neurobehavioural processes. AChE inhibition induces oxidative stress, resulting in neurotoxicity that may be manifested as cognitive impairment in rodents (Kazi and Oommen, 2012). The finding agrees with the findings that taurine enhance AChE activity in rats (Das *et al.*, 2009; Akande *et al.*, 2014b).

The results of the current study did not show any significant difference in the activities of the brain antioxidant enzymes (SOD, CAT and GPx) in both acute and chronic taurine treated restraint-stressed groups as compared with the control group. Antioxidant enzymes are involved in the antioxidant defence 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 activity by enhancing the antioxidant system through the formation of chloramines with hypochlorous acid and replacing glutathione GSH) in biological systems during oxidative stress (Devi and Anuradha, 2010). 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*., 2014b). Taurine may also inhibit lipid peroxidation by inducing GPx and SOD. It also protects tissues against reduced glutathione pool depletion by preventing a decrease of glutathione reductase activity. The result of the presnt study showed that the antioxidant, taurine prevented oxidative stress and loss of cellular antioxidants, suggested that taurine protected the brain from restrain stress-induced oxidative damage.

There was significant increase in total protein and albumin concentrations in the group that received 200 and 400 mg/kg taurine 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 ROS in biological systems (El-

Nekeety *et al*., 2009). Taurine significantly increased total protein and albumin concentrations. There was no significant 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). This might have contributed to its ability to normalise 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; thus contributing to the normalisation 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 the acute and chronic restraint-stressed groups. In the acute group, the decrease in AST in the 400 mg/kg taurine group is not well understood. On the other hand the significant increase in ALT 200 mg/kg taurine dose in the acute restraint stressed group could be attributed to hepatoprotective effect of taurine. The ALP in the chronic restraint stressed group showed significant increase in all the three taurine treated groups when compared to the control group. It is notable that the hepatoprotective property of taurine was 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 are not well understood. However, it may be due to differences in duration of exposure to stress. Changes in AST activity is less specific for liver disease, and it may also signal damage to cardiac and skeletal muscles, red blood cell, kidney and brain tissue (Rochling, 2001).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The present studies have shown that treatment with taurine improved neurobehavioural and cognitive changes in acute and chronic restraint stress. Taurine improved PCV, Hb, RBC and WBC in restraint-stressed Wistar rats. To the best of our knowledge, the study has for the first time, revealed the ability of taurine to protect the integrity of RBC membrane as evidenced in its ability to ameliorate the restraint stressed-induced increase in erythrocyte osmotic fragility. Taurine alleviated the alterations in the activities of the brain antioxidant enzymes: SOD, CAT and GPx. It also alleviated alterations in the activities of the enzymes: AST, ALT, ALP and AChE. Taurine also alleviated alterations in the concentrations of total protein and albumin in plasma of both the acute and chronic restraint-stressed groups. The studies have further reaffirmed the ability of taurine to protect the brain against restraint stress-induced lipid peroxidation demonstrated by reduced MDAconcentration in taurine treated groups**.**

6.2 Conclusion

Taurine improved learning and short-time memory in both acute and chronic restraint-stressed Wistar rats and mice. It also improved haematological parameters of restraint stressed Wistar rats. Taurine counteracted the restraint stress-induced lipid peroxidation and maintained the antioxidant defence system. It alleviated alterations in activities of some serum enzymes (AST, ALT, ALP) and plasma proteins in the acute and chronic

restraint-stressed rats. It was concluded that taurine has beneficial on neurobehavioural, biochemical and haematological alterations in individuals, constantly exposed to stress.

6.3 Recommendations and Future Research

i. Stress is invariably part of man. Therefore, this study recommended that individuals passing through stress be advised to use taurine as a potent anti-stress agent.

ii. High risk populations (vegetarians) should be encouraged to supplement their diets with antioxidants such as taurine.

iii. Animals such as sniffer dogs, African Giant rat, geese and ostriches, whose cognitive function is been put to task should be pretreated regularly with antioxidants, such as taurine.

iv. Other methods of stress models should be explored to determine the effect of taurine on such stress models.

v. Further work should be done to discover taurine receptor as this receptor is yet to be identified.

6.4 Contributions to Knowledge

i. Taurine possesses anti-stress activity, especially at the dose of 200 (51.67 ± 1.33) and 400 mg/kg (47. 50 ± 1.38).

ii. Exploratory behaviour in mice was significantly higher in the acute restraint-stressed group which received taurine at 200 mg/kg (22.00 ± 1.24), evidenced

by increased number of head dips when compared with taurine 400 mg/kg (18.17 ± 1.49) group.

iii. Taurine administration in the elevated plus-maze showed decreased acquisition time (34.67 ± 4.52) and short retention time (19.50 ± 7.29) in the highest dose of taurine (400 mg/kg) in the acute restraint-stressed group. Short acquisition (47.50 ± 12.50) and retention (21.83 ± 15.28) time were observed in the highest dose of taurine (400 mg/kg) in chronic restraint-stressed groups, when compared with the control group.

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APPENDIX 1

Effect of taurine on leukocyte and platelet counts in acute restraint stress Wistar rats.

Treatment	WBC $x10^9/l$	PTL $x\ 10^9/l$	Neutrophil %	Lymphocyte %	N/L
Control (DW)	13.80±1.45	456.33±82.40	26.59±0.40 [b]	70.14±1.23 [b]	0.38±0.03
Taurine: 100 mg/kg	11.15±1.59	459.17±22.22	17.67± 0.25 [a]	58.12±1.06 [a]	0.30±0.07
200 mg/kg	13.75±1.33	512.83±6.00	20.00± 0.39 [b]	74.18±1.15 [b]	0.27±0.02
400 mg/kg	14.13±1.28	403.42±87.90	27.39± 0.14 [b]	76.22±0.85 [b]	0.36±0.03

Note: values are means ± SEM, n = 6
a,b = means with different superscript letters are significantly different ($P < 0.05$)
DW, distil water; WBC, white blood cell; PLT, platelet; N/L, neutrophil/lymphocyte

APPENDIX 2

Effect of taurine on erythrocytic indices in acute restraint stress wistar rats.

Treatment	Hb g/dl	PCV %	RBC x 10^{12}/l	MCV fl/cell	MCH pg	MCHC g/dl
Control (DW)	12.13±0.82	33.73±2.09	6.19±0.40	54.70±0.75^{b}	19.57±0.19^{b}	35.87±0.75
Taurine: 100 mg/kg	13.40±0.54	39.12±0.97	7.07±0.18	55.45±1.02^{b}	18.87±0.40^{b}	34.13±0.79
200 mg/kg	13.23±0.27	37.17±0.89	6.90±0.15	53.90±0.11^{b}	19.13±0.10^{b}	35.57±0.21
400 mg/kg	13.03±0.24	36.73±0.97	6.87±0.15	53.53±0.68^{a}	18.95±0.16^{a}	35.58±0.30

Note: values are means ± SEM, n = 6
a,b = means with different superscript letters are significantly different ($P < 0.05$)
Hb, haemoglobin; PCV, pack cell volume; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration

APPENDIX 3

Effect of taurine on leukocyte and platelet counts in chronic restraint stress Wistar rats.

Treatments	WBC x 10^9/L	PTL x 10^9/L	Neutrophil %	Lymphocyte %	N/L
Control (DW)	13.08±2.82[a]	509.67±58.83[a]	24.31±0.50[a]	97.48±1.09[a]	0.25±0.03[a]
Taurine: 100 mg/kg	15.20±1.73[b]	568.67±92.86[b]	16.12±0.25[b]	81.25±1.49[b]	0.20±0.04[a]
200 mg/kg	14.00±2.32[a]	427.67±50.34[c]	17.64±0.40[b]	78.43±1.49[a]	0.22±0.01[a]
400 mg/kg	11.72±0.64[c]	427.33±41.21[c]	26.02±0.53[a]	74.06±0.47[a]	0.35±0.69[b]

Note: values are means ± SEM, n = 6
a,b,c,d = means with different superscript letters are significantly different ($P < 0.05$)
DW, distil water; WBC, white blood cell; PLT, platelet; N/L, neutrophil/lymphocyte

APPENDIX 4

Effect of taurine on erythrocytic indices in chronic restraint stress Wistar rats

Treatments	HB g/dL	PCV %	RBC x 10^{12}/L	MCV fl/cell	MCH pg	MCHC g/dL
Control (DW)	14.03±0.27[a]	41.43±1.20[a]	7.02±0.18[a]	58.90±0.96[a]	20.00±0.21[a]	32.40±1.69
Taurine: 100 mg/kg	11.22±1.37[b]	31.52±4.26[b]	5.65±0.72[b]	55.73±1.24[b]	19.92±0.84[a]	35.92±0.87
200 mg/kg	12.62±1.24[b]	36.52±3.91[c]	6.35±0.65[a]	57.380.88 [a]	19.90±0.64[a]	34.77±0.65
400 mg/kg	14.08±0.04[a]	41.05±0.33[a]	7.93±0.07[a]	51.85±0.85[c]	17.72±0.17[b]	34.28±0.30

Note: values are means ± SEM, n = 6

a,b = means with different superscript letters are significantly different ($P < 0.05$)

Hb, haemoglobin; PCV, pack cell volume; RBC, red blood cell; MCV, mean corpuscular volume; MCH,

mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration

APPENDIX 5

Effect of taurine on brain malondialdyhide, achetylcholinesterase, brain antioxidants and serum liver enzymes, total protein and albumin in acute restraint-stressed rats

Treatments	MDA	AChE	SOD	CAT	GPx	AST	ALT	ALP	TP	ALB
Control (DW)	1.30±0.08	24.17±1.17	2.23±0.08	52.33±1.05	48.33±1.12	42.00±2.44	47.83±1.74	68.17±1.14	63.67±1.28	35.00±1.29
Taurine: 100 mg/kg	1.27±0.10	22.67±1.02	2.23±0.11	52.67±0.95	47.17±3.06	42.50±1.15	48.00±0.82	68.17±2.43	64.00±1.00	35.33±1.12
200 mg/kg	1.150.08	24.17±0.75	2.35±0.19	51.67±1.33	47.50±0.62	45.83±1.25	51.17±2.04	68.83±2.36	65.00±1.21	35.33±1.12
400 mg/kg	1.12±0.11	23.00±1.37	2.25±0.08	51.671.36	47.50±1.38	38.17±0.79	45.50±3.78	64.83±1.66	65.17±1.42	37.67±1.05

Note: values are means ± SEM, n = 6
MDA, malondialdehyde; AChE, acetylcholinesterase; SOD, superoxide dismutase; CAT, catalase; GPx, glutathion peroxidase; AST, aspartate aminotransferase; ALT, alanine aminitransferase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin

APPENDIX 6

Effect of taurine on brain malondialdyhide, achetylcholinesterase, brain antioxidants, serum liver enzymes, total protein and albumin in chronic restraint-stressed rats

Treatments	MDA	AChE	SOD	CAT	GPx	AST	ALT	ALP	TP	ALB
Control (DW)	1.25±0.10	21.33±0.71	2.30±0.13	51.83±0.95	47.00±1.03	42.50±0.99	46.67±1.17	65.00±1.41	63.50±1.11	35.50±1.43
Taurine: 100 mg/kg	1.18±0.09	24.17±1.17	2.33±0.08	52.67±0.99	47.67±0.88	42.33±1.33	47.17±1.64	71.00±1.37	62.17±1.14	33.50±0.85
200 mg/kg	1.13±0.10	22.17±0.65	2.37±0.10	53.33±1.15	48.33±1.31	41.17±1.40	45.17±1.30	71.50±3.51	66.00±1.57	37.83±1.76
400 mg/kg	1.18±0.09	22.33±1.17	2.22±0.05	53.00±0.93	49.83±1.08	41.17±1.19	44.83±1.49	71.50±1.52	66.33±1.36	37.00±2.07

Note: values are means ± SEM, n = 6

MDA, malondialdehyde; AChE, acetylcholinesterase; SOD, superoxide dismutase; CAT, catalase; GPx, glutathion peroxidase; AST, aspartate aminotransferase; ALT, alanine aminitransferase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin

APPENDIX 7

Effect of Taurine on neurobehavioural responses of rodents subjected to acute restraint-stressed Wistat rats.

Treatment	ACQUISITION	RETENTION	SDL	NS	DSB	HEAD DIPS	LATENCY	FOOT SLIPS
Control (DW)	48.83±10.03	22.00±3.79	7.17±1.58	3.67±0.49	2.28±0.10	15.33±2.95	54.16±3.27	9.67±1.28
Taurine: 100 mg/kg	43.00±10.20	27.83±10.99	14.00±4.76	1.67±0.21	2.13±0.03	14.00±0.58	41.67±3.07	13.67±0.56
200 mg/kg	57.50±10.79	17.33±3.72	16.67±8.73	3.50±0.76	2.05±0.02	22.00±1.24	30.33±2.17	5.67±1.67
400 mg/kg	34.67±4.52	19.50±7.29	28.50±9.98	2.17±0.65	2.40±0.13	12.33±1.33	38.83±7.33	5.83±0.75

Note: values are means ± SEM, n = 6

SDL, step-down latency; NS, number of shock; DSB, duration of stay on bench

APPENDIX 8

Effect of Taurine on neurobehavioural responses of rodents subjected to chronic restraint stress.

Treatment Taurine	ACQUISITION	RETENTION	SDL	NS	DSB	HEAD DIPS	LATENCY	FOOT SLIPS
Control (DW)	77.17±8.13	47.00±10.12	6.33±1.73	2.50±0.34	2.00±0.00	15.33±2.17	50.00±4.28	4.83±0.31
100 mg/kg	71.67±12.95	22.33±2.47	28.00±7.03	1.83±0.40	2.00±0.00	17.00±2.71	40.83±5.23	8.33±1.12
200 mg/kg	54.17±7.35	20.50±7.45	30.67±10.72	1.17±0.17	2.12±0.07	18.17±1.49	36.67±5.27	3.50±0.22
400 mg/kg	47.50±12.50	41.83±15.28	23.17±7.84	1.83±0.31	2.15±0.02	16.67±1.17	28.33±2.83	4.67±0.42

APPENDIX 9



Restraint cage for rats

APPENDIX 10



Restraint cage for mice

APPENDIX 11



Elevated plus-maze apparatus for mice

APPENDIX 12



Beam-walk apparatus for mice